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PART 1 OF 2

NIOSH MANUAL OF ANALYTICAL METHODS

SECOND EDITION

Part I

NIOSH Monitoring Methods

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VOLUME 1

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Mr. John V. Crable and Dr. David G. Taylor, respectively Chief and Assistant Chief of the Measurements Research Branch, were responsible for the development and preparation of this Manual.

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FOREWORD

The National Institute for Occupational Safety and Health (NIOSH) is engaged in research to identify and evaluate hazards to the American working population. The results of this research are used to develop recommended criteria for the protection of all workers.

An important tool for evaluating the exposure of workers to toxic substances is the sampling and analytical method. This Manual, first published in 1974, is a compendium of such methods for use by the industrial hygienist and the occupational health chemist. Prior to the publication of the first edition, there was no comprehensive manual for such chemical exposure evaluations. That edition has become one of the most popular NIOSH publications.

I am pleased to present the second edition of this important Manual. It contains more than 300 sampling and analytical methods. Many of these procedures have been recommended to the Occupational Safety and Health Administration (OSHA) for use in their compliance monitoring program. All of NIOSH's analytical methods are made available in this publication for public use.



John F. Finklea, M.D.
Director, National Institute for
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PREFACE TO THE SECOND EDITION

In 1971, when occupational safety and health standards for nearly 400 chemical substances were adopted by the U.S. Department of Labor (29 CFR 1910.1000), there were few analytical procedures for accurately monitoring the exposure of workers to these toxic substances. The National Institute for Occupational Safety and Health (NIOSH) published the first edition of this Manual in 1974. The need for the publication was immediately apparent. Currently in its fourth printing, the first edition contains 39 analytical methods for measuring 130 toxic substances. The immediate need for these monitoring methods is in the OSHA compliance program and in NIOSH field studies. The methods are also useful for anyone interested in analyzing air samples for the chemical substances.

The first edition has been expanded to 110 methods and a second part added. Part I (Volume 1) of this second edition contains many of the methods in the earlier edition. A few of the older methods were dropped in favor of newer procedures, while some of the sampling and analytical methods have been revised based upon new research information. In addition, a number of new procedures that were developed or evaluated by NIOSH or its contractors have been included. Each of the methods in Part I of the new edition is classified according to the NIOSH Classification of Analytical Methods (Crable, J. V., and R. G. Smith, American Industrial Hygiene Association Journal, 26:149, 1975).

> Class A — Recommended: A method which has been fully evaluated and successfully collaboratively tested by a selected group of laboratories.
>
> Class B — Accepted: A method which has been subjected to a thorough evaluation procedure in the NIOSH laboratory and found to be acceptable.
>
> Class C — Tentative: A method which is in wide use and which has been adopted as a standard method or recommended by another Government agency or one of several professional societies such as ACGIH, AOAC, AIHA, ASTM, or ISC.
>
> Class D — Operational: A method in general use or approved by most professional industrial hygiene analysts but which has not been thoroughly evaluated by NIOSH or any professional society.
>
> Class E — Proposed: A new, unproved, or suggested method not previously used by industrial hygiene analysts but which gives promise of being suitable for the determination of a given substance.

The classification of a method is a guide to the usefulness of that analytical procedure.

A new section has been included in the second edition. Part II (Volumes 2, 3) includes 227 personal sampling and analytical methods that were evaluated and validated for accuracy and precision in the joint NIOSH/OSHA Standards Completion Program (SCP) during 1974-1976. These are Class B — Accepted methods, but have not been field tested.

The analytical procedures in Part I are assigned a permanent method number preceded by the letters "P&CAM" after the first draft is written. The methods in Part I are ordered by this P&CAM number. This pagination scheme allows for the introduction of methods into the Manual which were assigned

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numbers but were not ready for the earlier edition. The number also distinguishes between methods developed for the same chemical. Similarly, each of the substances in the Standards Completion Program was assigned a number preceded by the letter "S" at the beginning of that program. After a successful validation, that method is put into Part II of the Manual according to its assigned S-number.

The alphabetical index in each volume allows the user to locate each method by chemical name and method number.

ABSTRACT

This Manual contains three hundred and thirty-seven (337) sampling and analytical methods for use in industrial hygiene environmental monitoring. Some of the procedures are useful for measuring toxic constituents in blood, urine, or body tissues, but the majority are intended for use in personal air exposure monitoring.

Some of the methods were developed by NIOSH through contracts or interagency agreements. Others were developed and evaluated by NIOSH researchers or adapted from procedures found in the literature. Part I (Volume 1) contains 110 methods developed by NIOSH or its contractors. They are classified according to their reliability. Part II (Volumes 2, 3) contains 227 methods validated under the joint NIOSH/OSHA Standards Completion Program.

CONTENTS OF VOLUME 1

Foreword iii
Preface v
Abstract vii
Acknowledgments xiii

PART I
NIOSH Monitoring Methods

	P&CAM No.
Lead in Blood and Urine	102
Colorimetric Method for Free Silica	106
Antimony in Urine	107
Nitrogen Dioxide in Air	108
Free Silica (Quartz, Cristobalite, Tridymite) in Atmospheric Dust	109
Quartz in Coal Dust by Infrared Spectroscopy	110
Carbon Monoxide in Air	112
Carbon Monoxide in Blood	113
Fluoride in Urine	114
Chloride in Air	115
Cyanide in Air	116
Fluoride and Hydrogen Fluoride in Air	117
Acrolein in Air	118
Beryllium in Air	121
Selenium in Urine	124
Formaldehyde in Air	125
Hydrogen Sulfide in Air	126
Organic Solvents in Air	127
Arsenic in Urine and Air	139
Arsenic in Urine and Air	140
2,4-Toluenediisocyanate (TDI) in Air	141
p,p-Diphenylmethanediisocyanate (MDI) in Air	142
Mercury in Urine	145
Sulfur Dioxide in Air	146
Total Particulate Chromium in Air	152
Ozone in Air	153
Ozone in Air	154
Parathion in Air	158
Oil Mist in Air	159
Sulfur Dioxide in Air	160
Sulfur Dioxide in Air	163
Mercury in Urine	165
Mercury in Blood	167
Aromatic Amines in Air	168
Chromic Acid Mist in Air	169
General Procedure for Metals	173
Mercury in Air	175
Tin in Atmospheric Particulate Matter and Biologic Materials	176
Gallium in Air	177
Vinyl Chloride in Air	178
Carbon Disulfide in Air	179
Arsenic in Air	180

Preceding page blank

Selenium in Air 181
Chromium in Air 182
Polynuclear Aromatic Compounds in Air 183
Polynuclear Aromatic Hydrocarbons in Air 184
Benzo(a)pyrene in Air 186
Sulfuric Acid Mist in Air 187
Arsenic in Air 188
Antimony in Air 189
Indium in Air 190
Lead and Cadmium in Air 191
Arsenic in Blood 192
Antimony in Blood 193
Indium in Blood 194
Lead in Blood 195
Arsenic in Urine 196
Antimony in Urine 197
Gallium in Urine 198
Indium in Urine 199
Lead in Urine 200
Methyl Chloride in Air 201
Acrylonitrile in Air 202
Nitroglycerin and Ethylene Glycol Dinitrate (Nitroglycol) in Air 203
Sulfur Dioxide in Air 204
Ammonia in Air 205
Total Particulate Aromatic Hydrocarbons (TPAH) in Air 206
Lead in Blood and Urine 208
Free Chlorine in Air 209
Acrolein in Air 211
Gaseous and Particulate Fluorides in Air 212
Bis(chloromethyl) Ether in Air 213
Lead in Air or Blood 214
Inorganic Phosphate in Urine 215
Phosphoric Acid in Air 216
Benzene-Soluble Compounds in Air 217
Phosgene in Air 219
Chloromethyl Methyl Ether and Bis(chloromethyl) Ether in Air 220
Aliphatic Amines in Air 221
Zinc Oxide in Air 222
Cadmium in Blood 223
Cadmium in Urine 224
Kepone in Air 225
2,6-Di-t-Butyl-p-Cresol (DBPC) in Air 226
Polymethylsiloxane Mist in Air 227
Thiram in Air 228
Pentachlorophenol in Urine 230
Nitrogen Dioxide and Nitric Oxide in Air 231
Formic Acid in Air 232
Diazonium Salts and Azo Dyes in Air 234
Formaldehyde in Air 235
4.4'-Methylenebis(2-chloroaniline) in Air 236
Procedure for the Preparation of Tissue for Analysis 237

Asbestos Fiber in Air 239
Sodium Hydroxide in Air 241
Phosphorous in Air 242
Benzidine in Air 243
Polychlorinated Biphenyls (PCB) in Air 244
Chrysotile Asbestos in Bulk Samples 245
3,3'-Dichlorobenzidine in Air 246
Methyl Alcohol in Air 247
Hydrazine Compounds in Air 248
Benzo(a)Pyrene in Particulates 251
N-Nitrosodimethylamine in Air 252
Polychlorinated Biphenyls (PCB) in Air 253
Thiophene in Air 255
Azelaic Acid in Air 256
Phosphorous (white) in Air 257
Free Silica (Quartz, Crystobalite, Tridymite) in Airborne Dust 259
Lead in Blood and Urine 262

Index

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LEAD IN BLOOD AND URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Lead	**Method No:**	P&CAM 102
Matrix:	Blood, Urine	**Range, Blood:**	3-> 250μg/100 mℓ
Procedure:	Dithizone	**Range, Urine:**	12-> 1000μg/100 mℓ
Date Issued:	3/23/72	**Precision:**	Average Coefficient of variation is 6%
Date Revised:	1/15/74	**Classification:**	A (Recommended)

1. **Principle of the Method**

   1.1 Whole blood or urine samples are ashed with nitric acid to destroy the organic matrix.

   1.2 The sample ash is dissolved in concentrated nitric acid and the solution diluted by the addition of double-distilled water.

   1.3 Diphenylthiocarbazone, which reacts with lead at pH 6 to 11.5, is added and lead dithizonate is formed. The dithizonate is then quantitatively extracted into chloroform at a pH of 9 to 11.

   1.4 The color of the lead dithizonate follows Beer's Law. Its absorbance is measured in a spectrophotometer at 510 nanometers.

2. **Range and Sensitivity**

   2.1 For a 10-mℓ blood sample, the analytical range extends from 3 μg to 250 μg per 100 mℓ of blood.

   2.2 For a 25-mℓ urine sample, the analytical range extends from 12 μg to 1000 μg per liter of urine.

   2.3 If the amount of lead in a given sample is found to exceed these ranges, a smaller aliquot may be taken for analysis.

3. Interferences

This procedure is subject to interference from stannous tin, bismuth, and thallium. However, these elements are found in biological samples so infrequently, that they do not present a significant problem.

4. Precision and Accuracy

This method has a reported accuracy of 97 ± 2% and coefficient of variation of 6% (Reference 11.1). These values are attainable by an experienced analyst.

5. Advantages and Disadvantages of the Method

5.1 The principal advantage of this method is that it has been thoroughly tested and evaluated. There are no other significant advantages.

5.2 On the other hand there are several significant disadvantages. Large quantities of glassware must be cleaned and kept clean. Large quantities of difficult-to-make reagents are required. This procedure is highly susceptible to contamination from outside sources. Finally, the procedure is tedious and time consuming and requires an analyst with a relatively high level of proficiency.

6. Apparatus

6.1 125-mℓ or 250-mℓ Phillips borosilicate glass beakers.

6.2 Squibb-type 125-mℓ separatory funnels with Teflon stopcocks.

6.3 A spectrophotometer capable of absorbance measurements at 510 nanometers and the appropriate sample cells.

6.4 Burettes with Teflon stopcocks for dispersing reagents.

6.5 Electric shakers capable of holding 8 separatory funnels.

7. Reagents

Analytical grade reagents are used. Purification is essential when biological tissues and fluids are being analyzed, because of the very low levels of lead in these materials. A reagent blank sample is carried through the entire procedure with each set of unknown samples and its analyzed lead content is subtracted from each analytical result to calculate the net quantity of lead in each unknown sample.

A boiling rod is used to prevent bumping in the flasks when the reagents are distilled. This rod is prepared by cutting 3- or 4-mm O.D. glass tubing to a length 1 cm greater than the height of the flask. The tubing is sealed at a spot about 1 cm above the bottom end which is firepolished but left open. Before each use, the liquid is shaken

out of the bottom section and the rod inserted in the flask. As the flask is heated a steady stream of air and vapor bubbles issues from the open space, thus providing nuclei for smooth boiling.

7.1 **Double-distilled Water.** To distilled water in an all-borosilicate glass still add a crystal each of potassium permanganate and barium hydroxide and redistill. Use for reagent and biological sample solutions unless tests indicate that single-distilled water is satisfactory; single-distilled water is usually adequate for air sample determinations.

7.2 **Nitric Acid, Concentrated.** Redistill in an all borosilicate-glass still the ACS reagent grade acid, 69.0% minimum, specific gravity 1.42. Use an electric heating jacket on the boiling flask to minimize danger of its breakage, and a boiling rod to prevent bumping, which otherwise would be severe. Discard the first 50 mℓ of distillate; this may be combined with the acid allowed to remain in the flask at the end of the distillation and used for washing glassware. The reagent is conveniently dispensed from a small automatic burette. No grease should be used on the stopcock.

7.3 **Nitric Acid, 1:99.** Dilute 10 mℓ of the redistilled, concentrated acid to 1 liter with double-distilled water.

7.4 **Ammonium Hydroxide, Concentrated.** Distill, in an all borosilicate-glass still, 3 liters of the ACS reagent grade, 28.0% minimum, specific gravity, 0.8957 at 60°F, into 1.5 liters of double-distilled water, contained in a 2-liter reagent bottle which is chilled in an ice bath. Continue the distillation until the bottle is filled to the previously marked 2-liter level. Submerge the condenser tube deeply in the water in the receiver, but withdraw it before discontinuing the heat to avoid siphoning back of distillate. This reagent may be prepared more conveniently from tank ammonia, using a small wash bottle to scrub the gas and a sintered glass delivery tube which extends to the bottom of the reagent bottle. The ammonia gas is absorbed in double-distilled water until the solution reaches the desired specific gravity.

7.5 **Chloroform.** Use a brand with a statement on the label that the chloroform passes the American Chemical Society test for suitability for use in dithizone procedures. In addition, each batch of chloroform should be purchased in glass containers only and should be tested as follows in the laboratory to make sure it is satisfactory for preparing the dithizone solutions: Add a minute quantity of dithizone to a portion of the chloroform in a test tube; shake gently, and then stopper with a cork. The faint green color should be stable for one day. Our experience has indicated that the procedures for reclaiming used chloroform are tedious, time-consuming, sometimes unsuccessful, and no longer warranted in view of the commercial availability of acceptable reagent grades.

7.6 **Extraction Dithizone.** Dissolve 16 mg of diphenylthiocarbazone (dithizone), Eastman Kodak Co. No. 3092, or equivalent, in 1 liter of chloroform. Store in a brown bottle in the refrigerator.

7.7 **Standard Dithizone.** Dissolve 8 mg of diphenylthiocarbazone in 1 liter of chloroform. Store in a brown bottle in the refrigerator BUT ALLOW TO WARM TO ROOM TEMPERATURE BEFORE USING. Age for at least one day; then standardize as described in the procedure. Restandardize every few months.

7.8 **Sodium Citrate.** Dissolve 125 g of the 2 $Na_3C_6H_5O_7.11\ H_2O$ salt in sufficient distilled water to provide a solution nearly 500 mℓ in volume. Adjust the pH to 9-10, using a very small quantity of phenol red indicator solution (strong red color) and fresh, pHydrion test paper to check the pH. Extract in a large separatory funnel with a 100 mg per liter chloroform solution of dithizone and finally with the extraction dithizone reagent until a green extract is obtained with the latter reagent. Add a small volume of lead-free citric acid until an orange color (pH 7) appears. Extract the excess dithizone repeatedly with chloroform until a colorless extract is obtained. Remove the last traces of chloroform.

7.9 **Hydroxylamine Hydrochloride.** Dissolve 20 g of the salt in distilled water to provide a volume of 65 mℓ. Add a few drops of m-cresol purple indicator, and then add ammonia until the indicator turns yellow (pH 3). Add a sufficient quantity of a 4% solution of sodium diethyldithiocarbamate to combine with metallic impurities. Mix. After a few minutes extract repeatedly with chloroform until the excess carbamate has been removed, as indicated by the absence of a yellow color in the final chloroform extract tested with a dilute copper solution. To the aqueous solution of the hydroxylamine hydrochloride add redistilled, 6N hydrochloric acid until the indicator turns pink, and adjust the volume to 100 mℓ with double-distilled water.

7.10 **Potassium Cyanide.** (DANGER! HIGHLY POISONOUS!!) To 50 g of potassium cyanide in a beaker, add sufficient distilled water to make a sludge. Transfer the sludge to a separatory funnel previously marked to show a 100-mℓ volume. Add a small amount of distilled water to the beaker and warm. (Potassium cyanide cools the solution as it dissolves, thus retarding the solution process.) Add this warm water to the separatory funnel but do not permit contents to exceed the 100-mℓ mark. Shake. Let stand until the contents come to room temperature. A practically saturated solution results.

Extract the lead by shaking repeatedly with portions of the extraction dithizone solution until the lead has been removed. Part of the dithizone dissolves in the aqueous phase but enough remains in the chloroform to color it. A green extract indicates that the lead has been completely extracted. Most of the dithizone in the aqueous phase is then removed by repeated extractions with pure chloroform.

20

Dilute the concentrated solution of potassium cyanide to 500 mℓ with double-distilled water. It should not be necessary to filter the solution, if the directions are followed precisely. Extraction is carried out before dilution because the higher pH of the dilute solution is less favorable.

Note: A colorless solution usually results if the above directions are followed. Occasionally aging results in a brown color or precipitate because of polymerization of hydrogen cyanide. This does not interfere with use of the reagent if it is carefully decanted. Old potassium cyanide reagent may lose enough strength to cause insufficient complexing of large amounts of zinc.

7.11 **Ammonia-Cyanide Mixture.** Mix 200 mℓ of the purified 10% potassium cyanide reagent with 150 mℓ of distilled ammonium hydroxide (specific gravity 0.9, corresponding to 28.4% NH_3) and dilute to 1 liter with double distilled water. If the measured specific gravity of the ammonia is not 0.9, use the equivalent volume as calculated from a table of specific gravity vs percent ammonia.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware must be rendered lead-free before use. This can be accomplished by using the following three-step procedure.

8.1.1 Wash in a detergent-tap water and follow with tap water and distilled water rinses.

8.1.2 Soak in 1:1 nitric acid and follow with tap and distilled water rinses.

8.1.3 Soak in 1:1 or concentrated nitric acid for 30 minutes and follow with tap, distilled, and double distilled water rinses. This entire procedure must be used only when one is initially preparing glassware for use in the lead analysis. For each following successive use of equipment which has previously been subjected to the entire cleaning procedure, it is necessary only to use the nitric cleaning procedure described in step 8.1.3. Polyethylene equipment can be effectively cleaned by subjecting it to steps 8.1.1 and 8.1.3. Chromic acid cannot be used on polyethylene.

8.2 **Collection and Shipping of Samples**

8.2.1 **Blood** must be collected in lead-free, heparinized vacutainers equipped with sterilized stainless steel needles. The vacutainers should be heparinized before the collection of the blood. Heparinized vacutainers are available commercially from most laboratory supply houses. The addition of a preservative after the collection of the blood can result in error because of volume changes. Caution should be exercised during handling

to prevent any external contamination. The samples should be kept as cool as possible (do not freeze) during storage and shipment and should be shipped to the laboratory as quickly as possible. Heparinized, refrigerated blood samples are stable for two weeks. Two milliliters (2 mℓ) of whole blood are adequate for this determination.

8.2.2 Urine samples must be collected in lead-free borosilicate or polyethylene bottles. At least 25 mℓ should be collected. The sample must be preserved by the addition of 5 mg of thymol per 100 mℓ of urine. The urine should be kept cool during shipment and storage. Urine treated with thymol and refrigerated is stable for one week.

8.3 **Analysis of Sample**

8.3.1 Transfer 10 mℓ of heparinized blood or 25 mℓ of urine to a 125-mℓ Phillips borosilicate glass beaker and add 7 mℓ nitric acid.

8.3.2 Place the samples on a hotplate operating at about 130°C and evaporate just to dryness. After the water is driven off in the initial evaporation to dryness, keep the beaker covered with a lead-free watchglass to increase the reflux action of the concentrated acid.

8.3.3 Cool the beaker briefly and then add successive portions of nitric acid ranging from 2 mℓ down to 0.5 mℓ as the ashing proceeds. Do not remove the watchglass at any time but merely slide it back sufficiently to facilitate each new addition of the acid.

8.3.4 Each time, as soon as the residue becomes light colored, heat on a 400°C hotplate just long enough to blacken the residue; then remove and cool the sample. Throughout the remainder of the ashing procedure, alternately heat the sample with a few drops of nitric acid on the 130°C hotplate and bake the residue on the 400°C hotplate for the few minutes required to darken it. Finally, the residue will remain pale yellow or light brown (due to iron content) after it has been heated for 5-10 minutes at the high temperature. Avoid excess baking at this stage because the ash will become decomposed to a difficultly soluble form. It is now ready for solution and analysis.

8.3.5 Warm the sample ash with 2 mℓ of concentrated nitric acid for a few minutes. Then add 25 mℓ of distilled water and heat on the hotplate until a clear solution is obtained.

8.3.6 Cool to room temperature. Add to the solution in the beaker 1 mℓ of hydroxylamine hydrochloride, 4 mℓ of sodium citrate (10 mℓ is required

for a urine sample), and 1 drop of phenol red indicator, and titrate to a strong red color with concentrated ammonia reagent. Add a few drops excess of ammonia to make sure that the pH is between 9 and 10, using fresh pHydrion test paper to check the pH.

Note: Phenol red has a weak orange-red color in strong acid, yellow in weak acid, and a red color in alkaline solution. Do not mistake the first color for that produced in alkaline medium.

8.3.7 Transfer the sample quantitatively with double-distilled water rinsings to a 125-mℓ Squibb separatory funnel containing 5 mℓ of the potassium cyanide reagent.

8.3.8 Add 5 mℓ of the extraction dithizone and shake two minutes, after releasing the initial pressure by momentarily opening the stopcock of the inverted separatory funnel. Allow the chloroform layer to settle.

8.3.9 Draw off most of the extraction dithizone into a second funnel containing exactly 30 mℓ of 1:99 nitric acid.

8.3.10 Add a second 5-mℓ portion of extraction dithizone to the first funnel and shake as before. Allow the layers to separate and combine the extracts in the second funnel. Continue the process until the extraction dithizone reagent remains green. A rough estimate of the lead present in the sample may be made on the basis of 20 μg for each cherry red 5 mℓ extract.

8.3.11 Shake the second funnel for two minutes to transfer the lead to the 1:99 nitric acid layer. Allow the layers to separate. Discard the chloroform layer.

8.3.12 Shake the nitric acid solution with approximately 5 mℓ of reagent chloroform and let settle. Drain the settled chloroform through the stopcock bore as completely as possible without loss of the aqueous layer. Evaporate the last drop of chloroform clinging to the upper surface of the liquid.

Note 1: Start a zero lead standard at the beginning of this step by placing 30 mℓ of 1:99 nitric acid in a separatory funnel. This zero lead standard will be used to set the spectrophotometer at zero absorbance for each series of samples being analyzed.

Note 2: If the quantity of lead estimated for any sample exceeds the 25-μg range of the colorimetric determination, pipet an appropriate aliquot of the nitric acid solution at the end of Step 8.3.11 into a clean

separatory funnel that contains 5 mℓ of 1:99 nitric acid (to minimize errors by possible leakage of the stopcock). Add sufficient 1:99 nitric acid to make 30 mℓ total volume, and continue from the beginning of 8.3.12.

Note 3: Start lead standards at this point if required. Add 5 mℓ portions of 1:99 nitric acid to each of four separatory funnels, then 2.5-, 5.0-, 7.5-, and 12.5-mℓ portions of dilute standard lead solution (2 μg Pb/mℓ) from a burette, one to each separatory funnel. Finally, add the proper quantity of 1:99 nitric acid to make a total volume of 30 mℓ. Continue from the beginning of 8.3.12.

8.3.13 Add 6.0 mℓ of the ammonia-cyanide mixture, exactly 15.0 mℓ of the standard dithizone, and shake two minutes. Allow the layers to separate. Drain the chloroform layer containing the lead dithizonate through a clean cotton plug into a clean, dry test tube, and cork the tube immediately.

8.3.14 Decant this solution carefully into a dry photometer tube leaving the water behind. If any water spots are visible in the optical light path, transfer again to another photometer tube.

8.3.15 Set the spectrophotometer at a wavelength of 510 mμ.

8.3.16 Set the instrument at zero absorbance using the zero lead standard solution.

8.3.17 Read the absorbances of the samples and of the reagent blank.

9. **Calibration and Standards**

9.1 **Standard Lead Solution.** Dissolve 1.5984 g of pure lead nitrate in 1 liter of 1:99 nitric acid to provide a strong stock solution that contains 1 mg Pb per mℓ. Pipet exactly 20-mℓ into a 500-mℓ volumetric flask and make to mark with 1:99 nitric acid to give a dilute stock solution containing 40 μg Pb per mℓ. (A standard lead solution, 10 μg Pb/mℓ, was stable in 1:99 nitric acid for three years.) Prepare a working solution, containing 2 μg Pb per mℓ, just before it is needed by pipetting 5 mℓ into a 100-mℓ volumetric flask and making to mark with 1:99 nitric acid.

9.2 **Standard Curve.** A standard curve is constructed (see 8.3.12, Note 3) using 5-, 10-, 15-, and 25-μg quantities of lead.

10. **Calculations**

Calculate the lead content of each sample by multiplying its absorbance by the standardization factor (the slope of the standard curve in micrograms of lead per unit

of absorbance). Subtract the blank value from the gross lead content of each sample to obtain the net amount of lead expressed in micrograms. Divide this value by the original sample volume to obtain the concentration of lead in the original sample.

11. References

11.1 Keenan, R.G., et al., "The 'USPHS' Method for Determining Lead in Air and in Biological Materials," *Amer. Ind. Hyg. Assoc. J., 24:* 481 (1963).

COLORIMETRIC METHOD FOR FREE SILICA

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Free Silica (Quartz)	Method No:	P&CAM 106
Matrix:	Biologic Tissue, Airborne Particulates, Dusts	Range:	10 μg to 2.5 mg
Procedure:	Acid Digestion, Filtration, Color Development		
Date Issued:	3/31/72	Precision:	Relative Stand. Dev. = 9.25%
Date Revised:	1/15/74	Classification:	B (Accepted)

1. Principle of the Method

   1.1 The sample is digested in phosphoric acid to remove the silicates.

   1.2 The remaining crystalline material is dissolved in hydrofluoric acid.

   1.3 Silica is determined colorimetrically as silicomolybdate (420 mμ) or as molybdenum blue (820 mμ).

2. Range and Sensitivity

   2.1 In the silicomolybdate range, concentrations from 0.1 mg to 2.5 mg can be detected.

   2.2 In the molybdenum blue range, concentrations from < 5 μg to 140 μg can be detected.

   2.3 The range can be extended by varying the 0.50-g sample size.

3. Interferences

   3.1 The phosphate ion reacts with molybdic acid to form a yellow phosphomolybdate complex. It can be eliminated by lowering the pH to 1.2-1.3 with 10N H_2SO_4.

3.2 The ferric ion may consume the reducing agent and cause low results. As much as 1.0 mg will not interfere. Iron in excess of 1.0 mg per sample must be removed by preliminary treatment with 10:1 HCl-HNO_3.

4. Precision and Accuracy

Relative standard deviation on a run of 10 replicate samples was 9.25%. The wide deviation is due to error introduced by solution of some of the quartz in the phosphoric acid or the resistance of some silicates to dissolve. The same 10 samples reflected a mean free silica analysis of 3.36% on a sample with a concentration of 3.33% as determined gravimetrically. The error is less than 1%.

5. Advantages and Disadvantages of the Method

5.1 The major advantage lies in the versatility and wide range of the method. With facile pretreatment, dusts, airborne particulates and tissues can be analyzed by this method. If the SiO_2 concentration falls below the silicomolybdate detection limit, the simple addition of another reagent increases the sensitivity twentyfold.

5.2 The major disadvantage is the loss of free silica by solution during the phosphoric acid digestion. Quartz solubility is a function of the particle size, so the small, fine samples are vulnerable to this source of error. Some silicates may be resistant to phosphoric acid.

6. Apparatus

6.1 Sampling Equipment. The sampling unit for the filter-collection method has the following components:

6.1.1 The filter unit, consisting of the filter media and appropriate cassette filter holder, either a 2 or 3 piece filter cassette.

6.1.2 A vacuum pump such as a personal sampling pump. This pump must be properly calibrated so the volume of air sampled can be measured as accurately as possible. The pump must be calibrated with a representative filter unit in the line. Flow rate, time, and/or volume must be known.

6.1.3 Thermometer.

6.1.4 Manometer.

6.1.5 Stopwatch.

6.2 Membrane filters, 47 mm diameter, mean pore size, 0.45μ.

6.3 550-watt, 115-volt, Type RH, Precision heater (Precision Scientific Company).

6.4 750-watt variable transformer with built-in voltmeter.

6.5 Serological Rotator.

6.6 250-mℓ borosilicate glass Phillips beakers.

6.7 Short-stemmed glass funnels, with stems bent.

6.8 Crucible tongs padded with rubber or Tygon tubing.

6.9 Spectrophotometer with capacity of readings at 420 mμ and 820 mμ.

6.10 Filter funnel, membrane mount, and flask assembly.

6.11 125 mℓ polyethylene beakers, polyethylene watch glasses, stirring rods, 50 mm discs.

6.12 Polyethylene reagent bottles to store water, boric acid and standard solutions.

6.13 Bakelite or Nalgene buret (10 mℓ).

6.14 Constant temperature water bath.

Reagents

All reagents used should be ACS Reagent Grade or better.

7.1 Silica-free water for all solutions and dilutions. Use distilled deionized water and store in polyethylene carboy.

7.2 Hydrofluoric acid, 48%.

7.3 Orthophosphoric acid, 85%.

7.4 Hydrochloric acid, 1:10.

7.5 **Boric Acid Solution, 5%.** Dissolve 200 grams of boric acid crystals in 4 liters of warm, silica-free water. Cool. Filter with vacuum through a 0.45 micron membrane filter. Store in a polyethylene container.

7.6 **Molybdate Reagent.** Dissolve 50 g of ammonium molybdate tetrahydrate in about 400 mℓ of silica-free water. Acidify with 40 mℓ of concentrated sulfuric acid. Cool. Dilute to 500 mℓ. Store in dark.

7.7 **Sulfuric Acid, 10N.** Cautiously add 555 mℓ of concentrated H_2SO_4 to about 1.3 liters of water. Cool. Dilute to 2 liters.

7.8 **Reducing Solution.**

(A) Dissolve 9 grams of sodium bisulfite in 80 mℓ of water.

(B) In 10 mℓ of water dissolve 0.7 gram of anhydrous sodium sulfite and 0.15 gram of 1-amino-2-naphthol-4-sulfonic acid, in that order.

Combine solutions A and B and dilute to 100 mℓ. Reagent stored in refrigerator is stable for about one month.

7.9 Quartz – finely ground and acid-washed.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glass and polyethylene ware used should be washed thoroughly and subjected to a final rinse in silica-free water.

8.2 **Collection and Shipping of Samples**

8.2.1 **Tissue samples** are usually those collected at the time of autopsy; 500 mg or more should be submitted. They should be frozen, if possible and placed in plastic containers. Whirlpacks for small specimens are convenient. Bags, bottles or jars of plastic materials may be used.

8.2.2 **Air samples** should be collected on standard 37 or 47 mm cellulose membrane filters as follows:

(1) Assemble the filter unit by mounting the filter disc in the filter casette.

(2) Connect the exit end of the filter unit to the pump with a short piece of flexible tubing.

(3) Turn on pump to begin sample collection. The flow rate, times, and/or volume must be measured as accurately as possible. The sample should be taken at a flow rate of 2 liters per minute. A minimum sample of 100 liters should be collected. Larger sample

volumes are encouraged provided the filters do not become loaded with dust to the point that loose material might fall off or the filter become plugged.

(4) The sample cassettes should be shipped in a suitable container designed by NIOSH to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample is dislodged from the filter nor that the sample surface be disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the cassettes on top of the sample filter.

(5) With each batch of samples, one filter, labelled as a blank, must be submitted. No air should be drawn through this filter.

8.2.3 Bulk or rafter samples should be submitted in quantities equaling or exceeding 0.5 gram.

8.3 **Analysis of Samples**

8.3.1 A weighed sample containing no more than 2.5 mg SiO_2 or a filter membrane on which airborne particulates have been collected is placed in a clean 250-mℓ Phillips borosilicate glass beaker. Redistilled nitric acid (3 to 4 mℓ) is added and the sample heated to absence of brown fumes and dryness. The process is repeated until a white residue remains. (The filter should be consumed.) A blank should be carried through all steps of the analysis. If the sample is received on a membrane filter, an untreated membrane of the same type should be ashed. If a bulk sample is used, a beaker containing only the nitric or perchloric acid is started.

8.3.2 When polyvinyl chloride membranes have been used for sampling, nitric acid ashing is inadequate. Two mℓ of $HClO_4$ are added and heated slowly to just short of dryness. Ashing should be complete at this point. If necessary a second portion of $HClO_4$ can be added.

8.3.3 Add 25 mℓ of 85% phosphoric acid to the beaker. Start a reagent blank from this point, using only phosphoric acid. Cover the beaker with a bent-stem funnel.

8.3.4 In a hood preheat the Precision Heater for at least 45 minutes at 70 volts (about 240°C). Heat the sample for exactly 8 minutes, swirling it by the action of the serological rotator.

8.3.5 Remove the beaker from the heater by grasping it with padded crucible tongs and swirl it vigorously for one minute.

8.3.6 Allow the beaker to cool and add approximately 125 mℓ of water at 60° to 70°C. Swirl to mix the syrupy phosphoric mixture with the water.

8.3.7 Filter the sample with suction through a membrane filter. A Millipore disc, 47 mm in diameter with mean pore size of 0.45μ, is suitable. Wash thoroughly with 1:10 HCl.

8.3.8 Place the membrane flat in the bottom of a 150-mℓ polyethylene beaker and add 0.5 mℓ of 48% HF to the membrane surface. Float a thin polyethylene disc of about 50 mm diameter over the membrane and cover the beaker. Allow it to stand for 30 minutes.

8.3.9 Add 25 mℓ of water and 50 mℓ of boric acid solution. Stir well, cover.

8.3.10 Heat the solution in a 40°C water bath for at least 10 minutes.

8.3.11 Add 4 mℓ of molybdic acid reagent while stirring, staggering the addition at 2 minute intervals between samples. Time with a stopwatch from the beginning of the addition to the first sample.

8.3.12 Twenty minutes after the first addition, add 20 mℓ of 10N sulfuric acid, and stir thoroughly.

8.3.13 If any yellow color persists, read within two minutes after acidification in a spectrophotometer at 420 mμ against distilled water. Subtract blank.

8.3.14 If a colorless solution results, allow it to stand for 2 to 5 minutes and add 1 mℓ of 1-amino-2-naphthol-4-sulfonic acid reagent.

8.3.15 Mix and read after 20 minutes at 820 mμ against distilled water. Subtract blank. This color is stable for several hours.

9. Calibration and Standards

9.1 A 0.5 mg/mℓ SiO_2 stock standard is made by dissolving 250 mg of finely ground, acid-washed quartz in 10 mℓ of 48% hydrofluoric acid. Solution rate is slow and it may need to stand overnight. Dilute to 500 mℓ. This standard is stable indefinitely if stored in a polyethylene container.

9.2 A calibration curve in the silicomolybdate color range is made by diluting 1-, 2-, 3-, 4-, 5-, and 6-mℓ aliquots of the stock standard to 25 mℓ in polyethylene beakers and proceeding from step 8.3.9. Absorbance against mg of SiO_2 is plotted.

9.3 A standard usable in the molybdenum blue range is made by preparing a 1:25 dilution of the stock standard described in 9.1. This standard will contain 20μg SiO_2 per mℓ.

9.4 Dilute standards similar to those described in 9.2 are prepared for use in plotting the calibration curve. The upper limit for this curve is 140 μg.

10. Calculations

10.1 Milligrams of SiO_2 per sample is read from the appropriate calibration curve.

$$\text{mg } SiO_2/\text{gram of sample} = \frac{\text{mg } SiO_2 \text{ in sample}}{\text{sample weight in grams}}$$

$$\%\ SiO_2 = \frac{\text{mg } SiO_2}{\text{g of sample}} \times 0.1$$

10.2 Micrograms SiO_2 per sample is read from the appropriate calibration curve.

$$\mu\text{g } SiO_2/\text{gram of sample} = \frac{\mu\text{g } SiO_2 \text{ in sample}}{\text{sample weight in grams}}$$

$$\%\ SiO_2 = \frac{\mu\text{g } SiO_2}{\text{g of sample}} \times 0.0001$$

11. References

11.1 Talvitie, N.A., "Determination of Quartz in the presence of Silicates Using Phosphoric Acid," *Anal. Chem.*, 23, 623 (1951).

11.2 Talvitie, N.A., and Frances Hyslop, "Colorimetric Determination of Siliceous Atmospheric Contaminants," *Amer. Ind. Hyg. Assoc. J.*, 19:54 (1958).

11.3 Talvitie, N.A., "Determination of Free Silica: Gravimetric and Spectrophotometric Procedure Applicable to Air-Borne and Settled Dust," *Amer. Ind. Hyg. J.*, 25, 169, March-April, 1964.

ANTIMONY IN URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Antimony	**Method No:**	P&CAM 107
Matrix:	Urine	**Range:**	1-10μg/samples
Procedure:	Colorimetric – Rhodamine B	**Precision:**	+5%
		Classification:	A(Recommended)
Date Issued:	4/13/73		
Date Revised:	11/8/76		

1. **Principle of the Method**

   1.1 Urine samples are ashed with a mixture of sulfuric and nitric acids to obtain a clear digest.

   1.2 The sample ash is treated with perchloric acid to oxidize any antimony present to the pentavalent state.

   1.3 Rhodamine B dye is added to form a colored complex with the pentavalent antimony.

   1.4 The colored complex is extracted with benzene and its absorbance at 565 nanometers is measured in a spectrophotometer or colorimeter.

2. **Range and Sensitivity**

   2.1 One microgram of antimony is detectable per determination. For 50 mℓ of urine sample, this should correspond to 0.02 mg/liter of urine.

   2.2 No range is given.

3. **Interferences**

   3.1 Iron is the only commonly encountered ion likely to interfere. Iron interference can be eliminated by extracting with isopropyl ether instead of benzene but this solvent causes troublesome emulsion formation. In the presented method 750 μg Fe/mℓ showed no significant interference. This is well above the normal iron content in urine of 7 μg/Kg body weight per day.

3.2 Gallium and thallium can also interfere.

4. **Precision and Accuracy**

4.1 Three standard unknowns containing 3.0, 5.0 and 8.0 μg Sb/mℓ were analyzed in triplicate by ten collaborating laboratories plus a referee. Mean standard deviations from the amount of antimony present were ±0.113, ±0.237, and ±0.245 μg/mℓ on each of the three unknowns. This corresponds to mean coefficients of variation of 3.8, 4.7, and 3.1 per cent respectively.

4.2 For these same samples, the mean recoveries were 101, 104, and 107 per cent respectively.

5. **Advantages and Disadvantages of the Method**

5.1 This method requires no special equipment except a good spectrophotometer.

5.2 Unless all antimony present is pentavalent, recoveries cannot be complete. Digestions must be arranged to minimize the formation of antimony (IV), which is difficult to convert to antimony (V). Ten drops of perchloric acid is sufficient to oxidize the antimony, but less than this may give low results.

5.3 Also it is imperative to operate at low temperatures throughout the determinations. Samples, reagents, and apparatus should all be kept cold in a refrigerator prior to use.

5.4 Although benzene was used in this method, due to its toxicity, toluene or xylene might be better solvents for the extraction.

5.5 This method has not been performed in this laboratory.

6. **Apparatus**

6.1 125 mℓ Erlenmeyer flasks.

6.2 125 mℓ Squibb separatory funnels with Teflon stopcocks.

6.3 1 cm light path cuvettes for spectrophotometer.

6.4 Pipettes, test tubes, ice bath.

6.5 Polyethylene bottles with Teflon lined caps for sample collection.

6.6 Spectrophotometer

7. **Reagents**

The reagents described should be made up using C.P. grade or a better grade of chemical.

7.1 Double distilled water

7.2 Sulfuric acid, sp. gr. 1.84

7.3 Nitric acid, sp. gr. 1.42

7.4 Perchloric acid, redistilled 70%

7.5 Benzene or toluene

7.6 **Hydrochloric acid, 6N**: This reagent is prepared from concentrated hydrochloric acid (sp. gr. = 1.19) by mixing with an equal volume of double distilled water.

7.7 **Orthophosphoric acid, 3N**: Dilute 70 mℓ of 85% phosphoric acid to 1 liter with double distilled water.

7.8 **Rhodamine B**: 0.02% w/v in double distilled water.

7.9 **Antimony standard stock solution, 100 μg/mℓ**: Dissolve 0.1000 g antimony in 25 mℓ conc. sulfuric acid with heat and dilute to 1 liter with double distilled water.

7.10 Thymol – Reagent Grade

8. **Procedure**

8.1 **Cleaning of Equipment**: All glassware should be thoroughly cleaned; the following procedure is recommended:

8.1.1 Wash with a detergent and tap water solution followed by tap water and distilled water rinses.

8.1.2 Soak in chromic acid for 30 minutes and follow with tap and distilled water rinses.

8.1.3 Soak in 1:1 or concentrated nitric acid for 30 minutes and then follow with tap, distilled, and double distilled water rinses.

This entire procedure should be used only initially. Any glassware re-used need only be nitric acid cleaned as in Section 8.1.3. Polyethylene equipment can be cleaned by subjecting it to Sections 8.1.1 and 8.1.3 only.

8.2 **Collection and Shipping of Samples**

Urine samples should be collected in clear borosilicate or polyethylene bottles. At least 100 mℓ should be collected. The sample must be preserved by the addition of 5 mg of thymol per 100 mℓ of urine. The urine should be kept cool during shipment and storage. Urine samples treated with thymol and refrigerated, are stable for 1 week.

8.3 **Analysis of Samples**

8.3.1 Specific gravities of the urine samples are determined after the samples have been allowed to warm to room temperature. This may be done using a specific gravity meter or a *reliable* hydrometer.

8.3.2 Precool in an ice bath or refrigerate all reagents and glassware to be used for the analysis.

8.3.3 Transfer 50 mℓ of urine into a 125 mℓ Erlenmeyer flask. The sample is wet ashed with 5 mℓ concentrated sulfuric acid and 5 mℓ concentrated nitric acid. It may be found necessary to add nitric acid in 1 mℓ increments up to 5-10 mℓ in order to obtain a clear digest.

8.3.4 The sample is heated to white fumes of sulfur trioxide and then cooled slightly.

8.3.5 Ten drops of 70% perchloric acid are added and the sample is again heated to white fumes. The resulting digest is cooled in a melting ice bath until temperature equilibrium is reached (at least 30 minutes).

Note: All operations after the addition of phosphoric acid should be carried out quickly. Thus, it would seem best to handle the color development (Sections 8.3.6 to 8.3.9) of each sample individually and then read the absorbances collectively. Color development should be carried out in subdued light.

8.3.6 5 mℓ of *precooled* 6N hydrochloric acid is added *slowly* by pipette and the solution is allowed to stand in the ice bath an additional 15 minutes.

8.3.7 8 mℓ of *precooled* 3N phosphoric acid is added, followed immediately by the addition of 5 mℓ Rhodamine B solution.

8.3.8 *Without delay,* the flask is stoppered, shaken vigorously, and transferred to a *precooled* 125 mℓ, Squibb separatory funnel with Teflon stopcock.

8.3.9 10.0 mℓ of *precooled* benzene is added and the contents are shaken vigorously for one minute. The aqueous layer is discarded.

8.3.10 The benzene phase (red if antimony is present) is collected in a test tube and allowed to stand a few minutes for any water to settle out.

8.3.11 A 1 cm light path cuvette is rinsed 3 times with 1-2 mℓ of the extract, then filled and the absorbance read at 565 nanometers against a benzene blank.

9. **Calibration and Standards**

The calibration curve is prepared by carrying 1-10 micrograms of antimony through the complete oxidation, extraction, and analysis procedures (Sections 8.3.1 to 8.3.11) and then plotting absorbance vs. micrograms per total sample.

10. **Calculations**

10.1 Determine the concentration of antimony in the sample in μg Sb per mℓ or mg per liter of urine.

$$\text{mg Sb}/\ell = \mu\text{g Sb}/\text{m}\ell_{\mu}$$

where:

μg Sb = micrograms Sb determined from the calibration curve. (Section 9)

$\text{m}\ell_{\mu}$ = mℓ of urine sample ashed

10.2 The use of a specific gravity correction factor to normalize values to 1.024, the average specific gravity of urine, has been proposed. There are two ways of using this correction.

10.2.1 One way is to use a straightforward gravity correction, in which case the magnitude of the factor is small.

$$\text{Corrected (mg Sb/ℓ)} = \text{(mg Sb/ℓ)} \times \frac{1.024}{\text{specific gravity}}$$

10.2.2 The other method corrects for differences in total solids or salt content of various urine specimens, whereby the specific gravity of water is initially subtracted from the urine specific gravities. Because of the wide range of reported urine specific gravities, 1.010 to 1.040, the use of such a correction factor can lead to unusually or erroneously high results.

$$\text{Corrected (mg Sb/ℓ)} = \text{(mg Sb/ℓ)} \times \frac{(1.024 - 1.000)}{(\text{specific gravity} - 1.000)}$$

11. References

11.1 Amer. Conf. of Govt. Ind. Hyg.: "Recommended Method for Antimony in Air Samples and Biological Materials" (1963).

11.2 Maren, Thomas H.: "Colorimetric Microdetermination of Antimony with Rhodamine B." Anal. Chem 19, 487-91 (1947).

11.3 Elkins, H.B. "Concentration Adjustments in Urinalysis," Amer. Ind. Hyg. Assoc. J., 35, 559 (1974).

NITROGEN DIOXIDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Nitrogen Dioxide	**Method No.:**	P&CAM 108
Matrix:	Air	**Range:**	.01 to 10 μg/liter
Procedure:	Colorimetric	**Precision:**	< 5% RSD
Date Issued:	12/11/72		
Date Revised:	January 10, 1974	**Classification:**	C (Tentative)

1. **Principle of the Method**

The nitrogen dioxide is absorbed in an azo dye forming reagent (Reference 1). A stable pink color is produced within 15 minutes which may be read visually or in an appropriate instrument at 550 nm.

2. **Range and Sensitivity**

2.1 This method is intended for the manual determination of nitrogen dioxide in the atmosphere in the range of 0.005 to about 5 parts per million (ppm) by volume or 0.01 to 10 μg/liter, when sampling is conducted in fritted bubblers. The method is preferred when high sensitivity is needed.

2.2 Concentrations of 5 to 100 ppm in industrial atmospheres and in gas burner stacks also may be sampled by employing evacuated bottles or glass syringes. For higher concentrations, for automotive exhaust, and/or for samples relatively high in sulfur dioxide content, other methods should be applied.

3. **Interferences**

3.1 A tenfold ratio of sulfur dioxide to nitrogen dioxide produces no effect. A 30-fold ratio slowly bleaches the color to a slight extent. The addition of 1 percent acetone to the reagent before use retards the fading by forming another temporary product with sulfur dioxide. This permits reading within 4 to 5 hours (instead of the 45 minutes required when acetone is not added) without appreciable interferences. Interference from sulfur dioxide may be a problem in some stack gas samples (Section 2.2).

3.2 A fivefold ratio of ozone to nitrogen dioxide will cause a small interference, the maximal effect occurring in 3 hours. The reagent assumes a slightly orange tint.

3.3 Peroxyacylnitrate (PAN) can give a response of approximately 15 to 35 percent of an equivalent molar concentration of nitrogen dioxide (Reference 2). In ordinary ambient air the concentrations of PAN are too low to cause any significant error.

3.4 The interferences from other nitrogen oxides and other gases which might be found in polluted air are negligible. However, if the evacuated bottle or syringe method is used to sample concentrations above 5 ppm, interference from NO (due to oxidation to NO_2) is possible (Section 8.2.3).

3.5 If strong oxidizing or reducing agents are present, the colors should be determined within 1 hour, if possible, to minimize any loss.

4. Precision and Accuracy

4.1 A precision of 1 percent of the mean can be achieved with careful work (Reference 3); the limiting factors are the measurements of the volume of the sample and of the absorbance of the color.

4.2 At present, accuracy data are not available.

5. Advantages and Disadvantages of the Method

This is a simple method with direct coloration of absorbing reagent, which can be put directly into cuvettes and read, or diluted with absorbing reagent and read.

6. Apparatus

6.1 **Absorber.** The sample is absorbed in an all-glass bubbler with a 60 μ maximum pore diameter frit.

6.1.1 The porosity of the fritted bubbler, as well as the sampling flow rate, affect absorption efficiency. An efficiency of over 95 percent may be expected with a flow rate of 0.4 liter/minute or less and a maximum pore diameter of 60 μ. Frits having a maximum pore diameter less than 60 μ will have a higher efficiency but will require an inconvenient pressure drop for sampling (see formula in Section 6.1.2). Considerably lower efficiencies are obtained with coarser frits, but these may be utilized if the flow rate is reduced.

6.1.2 Since the quality control by some manufacturers is rather poor, it is desirable to measure periodically the porosity of an absorber as follows: Carefully clean the apparatus with dichromate-concentrated sulfuric acid solution and then rinse it thoroughly with distilled water. Assemble the bubbler, add sufficient distilled water to barely cover the fritted portion, and measure the vacuum required to draw the first perceptible stream of air bubbles through the frit. Then calculate the maximum pore diameter as follows:

$$\text{Maximum pore diameter } (\mu) = \frac{30\ s}{P}$$

where:

s = surface tension of water at the test temperature in dynes/cm (73 at 18°C, 72 at 25°C, and 71 at 31°C),

P = measured vacuum, mm Hg.

6.1.3 Rinse the bubbler thoroughly with water and allow to dry before using. A rinsed and reproducibly drained bubbler may be used if the volume (r) of retained water is added to that of the absorbing reagent for the calculation of results. This correction may be determined as follows: Pipet into a drained bubbler exactly 10 mℓ of a colored solution (such as previously exposed absorbing reagent) of absorbance A_1. Assemble the bubbler and rotate to rinse the inside with the solution. Rinse the fritted portion by pumping gently with a rubber bulb. Read the new absorbance, A_2, of the solution. Then:

$$10A_1 = (10 + r)\,A_2$$

or:

$$r = 10\frac{A_1}{A_2} - 1$$

6.2 **Air Metering Device.** A glass rotameter capable of accurately measuring a flow of 0.4 liter/minute is suitable. A wet test meter is convenient to check the calibration.

6.3 **Sampling Probe.** A glass or stainless steel tube 6 to 10 mm in diameter provided with a downward-facing intake (funnel or tip) is suitable. A small loosely fitting plug of glass wool may be inserted, when desirable, in the probe to exclude water droplets and particulate matter. The dead volume of the system should be kept minimal to permit rapid flushing during sampling to avoid losses of nitrogen dioxide on the surfaces.

6.4 **Grab-Sample Bottles.** Ordinary glass-stoppered borosilicate glass bottles of 30 to 250 mℓ sizes are suitable if provided with a mating ground joint attached to a stopcock for evacuation. Calibrate the volume by weighing with connecting piece, first empty, then filled to the stopcock with distilled water.

6.5 **Glass Syringes.** 50 or 100 mℓ syringes are convenient (although less accurate than bottles) for sampling.

6.6 **Air Pump.** A vacuum pump capable of drawing the required sample flow for intervals of up to 30 minutes is suitable. A tee connection at the intake is desirable. The inlet connected to the sampling train should have an appropriate trap and needle valve, preferably of stainless steel. The second inlet should have a valve for bleeding in a large excess flow of clean air to prevent condensation of acetic acid vapors from the absorbing reagent, with consequent corrosion of the pump. Alternatively, soda lime may be used in the trap. A filter and critical orifice may be substituted for the needle valve (Reference 4).

6.7 **Spectrophotometer or Colorimeter.** A laboratory instrument suitable for measuring the pink color at 550 nm, with stoppered tubes or cuvettes. The wavelength band width is not critical for this determination.

6.8 Assorted laboratory glassware.

7. Reagents

All chemicals used should be analytical reagent grade (Reference 5).

7.1 **Nitrite-Free Water.** All solutions are made in nitrite-free water. If available distilled or deionized water contains nitrite impurities (produces a pink color when added to absorbing reagent), redistill it in an all-glass still after adding a crystal each of potassium permanganate and of barium hydroxide.

7.2 **N-(1-Naphthyl) -Ethylenediamine Dihydrochloride, Stock Solution** (0.1%). Dissolve 0.1 g of the reagent in 100 mℓ of water. Solution is stable for several months if kept well stoppered in a brown bottle in the refrigerator. (Alternatively, weighed small amounts of the solid reagent may be stored.)

7.3 **Absorbing Reagent.** Dissolve 5 g of anhydrous sulfanilic acid (or 5.5 g of $NH_2C_6H_4SO_3H \cdot H_2O$) in almost a liter of water containing 140 mℓ of glacial acetic acid. Gentle heating is permissible to speed up the process. To the cooled mixture, add 20 mℓ of the 0.1% stock solution of N-(1-naphthyl) -ethylenediamine dihydrochloride, and dilute to 1 liter. Avoid lengthy contact with air during both preparation and use, since discoloration of reagent will result because of absorption of nitrogen dioxide. The solution is stable for several months if kept

well-stoppered in a brown bottle in the refrigerator. The absorbing reagent should be allowed to warm to room temperature before use.

7.4 **Standard Sodium Nitrite Solution** (0.0203 g/liter). One mℓ of this working solution of sodium nitrite ($NaNO_2$) produces a color equivalent to that of 10 $\mu\ell$ of nitrogen dioxide (10 ppm in 1 liter of air at 760 mm of mercury and 25°C: see Section 10.2.1). Prepare fresh just before use by dilution from a stronger stock solution containing 2.03 g of the reagent grade granular solid (calculated as 100 percent) per liter. It is desirable to assay (Reference 5), the solid reagent, especially if it is old. The stock solution is stable for 90 days at room temperature, and for a year in a brown bottle under refrigeration.

Procedure

8.1 **Cleaning of Glassware.** All washed glassware should be allowed to stand awhile in chromic acid solution, and then thoroughly rinsed with single and then double distilled water.

8.2 **Collection and Storage of Samples.** Three methods are described below. Concentrations below 5 ppm are sampled by the bubbler method. Higher concentrations may be sampled by the evacuated bottle method, or more conveniently (but less accurately) by the glass syringe method. The latter method is more useful when appreciable concentrations (e.g., 20 ppm) of nitric oxide are expected.

8.2.1 **Bubbler Method**

1. Assemble, *in order*, a sampling probe (optional), a glass rotameter, fritted absorber, and pump. Use ground-glass connections upstream from the absorber. Butt-to-butt glass connections with slightly greased vinyl or pure gum rubber tubing also may be used for connections without losses if lengths are kept minimal. The sampling rotameter may be used upstream from the bubbler provided occasional checks are made to show that no nitrogen dioxide is lost. The rotameter must be kept free from spray or dust.

2. Pipet 10.0 mℓ of absorbing reagent into a dry fritted bubbler (Section 6.1.3).

3. Draw an air sample through it at the rate of 0.4 liter/minute (or less) long enough to develop sufficient final color (about 10 to 30 minutes). Note the total volume of air sampled.

4. Measure and record the sample air temperature and pressure.

8.2.2 Evacuated Bottle Method

1. Sample in bottles of appropriate size containing 10.0 mℓ (or other convenient volume) of absorbing reagent. For 1 cm spectrophotometer cells, a 5:1 ratio of air sample volume to reagent volume will cover a concentration range up to 100 ppm; a 25:1 ratio suffices to measure down to 2 ppm.

2. Wrap a wire screen or glass-fiber-reinforced tape around the bottle for safety purposes.

3. Grease the joint lightly with silicone or fluorocarbon grease.

4. If a source of vacuum is available at the place of sampling, it is best to evacuate just before sampling to eliminate any uncertainty about loss of vacuum. A three-way Y stopcock connection is convenient. Connect one leg to the sample source, one to the vacuum pump, and the third to a tee attached to the bottle and to a mercury manometer or accurate gauge. In the first position of the Y stopcock, the bottle is evacuated to the vapor pressure of the absorbing reagent. In the second position of the Y stopcock the vacuum pump draws air through the sampling line to flush it thoroughly. The actual vacuum in the sample bottle is read on the manometer. In the third position of the Y stopcock the sampling line is connected to the evacuated bottle and the sample is collected.

5. The stopcock on the bottle is then closed. Allow 15 minutes with occasional shaking for complete absorption and color development.

6. For calculation of the standard volume of the sample, record the temperature and the pressure. The pressure is the difference between the filled and evacuated conditions. The uncorrected volume is that of the bottle plus that of the connection up to the stopcock minus the volume of absorbing reagent.

8.2.3 Glass Syringe Method

1. Ten mℓ of absorbing reagent is kept in a capped 50 (or 100) mℓ glass syringe, and 40 (or 90) mℓ of air is drawn in at the time of sampling.

2. The absorption of nitrogen dioxide is completed by capping and shaking vigorously for 1 minute, after which the air is expelled. (When appreciable concentrations – e.g., 20 ppm – of nitric oxide are suspected, interference caused by the oxidation of nitric oxide to nitrogen dioxide is minimized by expelling the air sample immediately after the absorption period.)

3. Additional air may be drawn in and the process repeated several times, if necessary, to develop sufficient final color.

8.2.4 **Effects of Storage.** Colors may be preserved, if well stoppered, with only 3 to 4% loss in absorbance per day; however, if strong oxidizing or reducing gases are present in the sample in concentrations considerably exceeding that of the nitrogen dioxide, the colors should be determined as soon as possible to minimize any loss. (See Section 3 for effects of interfering gases.)

8.3 **Analysis of Samples**

8.3.1 After collection or absorption of the sample, a red-violet color appears. Color development is complete within 15 minutes at room temperature.

8.3.2 Compare with standards visually or transfer to stoppered cuvettes and read in a spectrophotometer at 550 mμ, using unexposed reagent as a reference. Alternatively, distilled water may be used as a reference and the absorbance of the reagent blank deducted from that of the sample.

8.3.3 Colors too dark to read may be quantitatively diluted with unexposed absorbing reagent. The measured absorbance is then multiplied by the dilution factor.

Calibration and Standards

Either of two methods of calibration may be employed. The most convenient is standardizing with nitrite solution. Greater accuracy is achieved by standardizing with accurately known gas samples in a precision flow dilution system (References 3,6,7). The recently developed permeation tube technique (Reference 8) appears promising. If the gaseous method is used, the stoichiometric factor is eliminated from the calculations. Concentrations of the standards should cover the expected range of sample concentrations.

9.1 **Nitrite Solution Method**

9.1.1 Add graduated amounts of the $NaNO_2$ solution up to 1 mℓ (measured accurately in a graduated pipet or small buret) to a series of 25 mℓ volumetric flasks, and dilute to the mark with absorbing reagent.

9.1.2 Mix, allow 15 minutes for complete color development and read the colors (Section 8.3).

9.1.3 Good results can be obtained with these small volumes of standard solution if they are carefully measured. Making the calibration solutions up to 25 mℓ total volume, rather than the 10 mℓ volume used for samples, facilitates accuracy. If preferred, even larger volumes may be used with correspondingly larger volumetric flasks.

9.1.4 Plot the absorbances of the standard colors against the μℓ of nitrogen dioxide per mℓ of absorbing reagent. The latter values are equal to the corresponding mℓ of standard nitrite solution times 0.4 (Section 10.2.2). If preferred, transmittance may be plotted instead of absorbance, using semilogarithmic graph paper. The plot follows Beer's law. Draw the straight line through the origin giving the best fit, and determine the slope, K, which is μℓ of NO_2 intercepted at absorbance of exactly 1.0 or at 10 percent transmittance. For 1 cm cells, the value of K is about 0.73.

9.2 **Gaseous Standard Methods.** Two techniques are outlined below. Consult the original references for complete details, and Section 10.3 for calculations.

9.2.1 Method 1 (Reference 3) for gaseous standardization is as follows:

1. About 5 mℓ of pure liquid nitrogen dioxide is placed in a small glass bubbler (10 mm in diameter and 100 mm in length) provided with ground glass stopcocks and spherical joints on both intake and outlet tubes.

2. The bubbler is immersed in a thermos bottle ice bath and connected to an air line. A small pump with flowmeter delivers a steady stream of a few mℓ/min of air to the bubbler, thence through two flowmeters which permit the discarding of up to 90 percent of the NO_2 and finally to a large stream of carbon filtered air (1000 to 1500 liters/min) from a small blower. All this air passes through a 10 cm Biram anemometer mounted on the end of a pipe 10 cm in diameter. It has been found empirically with this arrangement that the anemometer reading in ft/min times 1.64 is equivalent to liters/min.

3. The bubbler is weighed to 0.1 mg at the beginning and end of an accurately measured time period. The stopcocks are closed each time before the bubbler is removed from the ice bath and wiped dry for weighing.

9.2.2 Method 2 (References 6,7) for preparing known dilutions of nitrogen dioxide consists in making a preliminary dilution (about 0.4%) of nitrogen dioxide in air in a stainless steel tank at 1000 lbs pressure. Subsequent dilution, by air in a flow system at atmospheric pressure, of the analyzed

tank mixture controlled by an asbestos plug and manometer yields concentrations of 0.1 to 10 ppm NO_2

9.2.3 Sample the gas mixtures by the bubbler method (Section 8.2.1), and read the colors (Section 8.3). Select concentrations and sample volumes to produce colors covering the accurate absorbance range of the spectrophotometer.

9.2.4 Standardization by gaseous samples can be based either upon a weight-volume relationship if the source of nitrogen dioxide is weighed, or a volume-volume relationship if the source is an analyzed tank mixture.

9.2.5 Calculate the concentration of the sample air stream, C, in ppm by volume ($\mu\ell$ NO_2/ℓ of air).

$$C = \frac{10^6\ (W_1 - W_2)}{t} \times \frac{0.532}{F_1} \times \frac{F_2}{F_3}$$

or: $$C = C_t \times \frac{F_2}{F_3}$$

where:

C	=	concentration of sample air stream in ppm.
$(W_1 - W_2)$	=	difference between initial and final weights, in grams, of nitrogen dioxide bubbler (or of permeation tube); weight loss is usually 0.01 to 0.05 g.
t	=	time interval in minutes, between the weighings.
0.532	=	(24.47/46.0), ideal volume in ℓ at 25°C and 760 mm Hg of 1.0 g of nitrogen dioxide.
F_1	=	flow rate, in ℓpm, of air passed through nitrogen dioxide bubbler (or over permeation tube), corrected to 25°C and 760 mm Hg.
F_2	=	flow rate, in ℓpm, of concentrated gas mixture injected into sample air stream.
F_3	=	total flow rate, in ℓpm, of sample air stream
C_t	=	analyzed concentration of the tank mixture, in ppm by volume on an ideal gas basis.

9.2.6 For each standard color, calculate the $\mu\ell$ of nitrogen dioxide/mℓ of absorbing reagent:

$$\mu\ell\ NO_2/m\ell = C\ V$$

where:

C = ppm concentration (Section 9.2.5)

V = volume of air sample at 25°C and 760 mm Hg, in ℓ/mℓ of absorbing reagent.

9.2.7 Plot the absorbances of the colors against the μℓ of gaseous nitrogen dioxide/mℓ of absorbing reagent. Draw the straight line through the origin giving the best fit, and determine the slope, K (the value of μℓ/mℓ intercepted at absorbance of exactly 1.0).

10. Calculations

10.1 For convenience, standard conditions are taken as 760 mm Hg and 25°C, at which the molar gas volume is 24.47 liters. (This is identical with standard conditions for Threshold Limit Values of the American Conference of Governmental Industrial Hygienists; it is very close to the standard conditions used (Reference 9) for air-handling equipment, of 29.92 in. Hg, 70°F, and 50 percent relative humidity, at which the molar gas volume is 24.76 liters, of 1.2 percent greater.)

Ordinarily the correction of the sample volume to these standard conditions is slight and may be omitted; however, for greater accuracy, it may be made by means of the perfect gas equation.

10.2 Standardization by nitrite solution is based upon the empirical observation (References 1,6) that 0.72 mole of sodium nitrite produces the same color as 1 mole of nitrogen dioxide.

10.2.1 This factor is applied to calculate the equivalence of the nitrite solution to the volume of NO_2 absorbed as follows: One mℓ of the working standard solution contains 2.03×10^{-5} g $NaNO_2$. Since the molecular weight of $NaNO_2$ is 69.00, this is equivalent to:

$$\frac{2.03 \times 10^{-5}}{69.00} \times \frac{24.47}{0.72} = 1 \times 10^{-5}\ \ell \text{ of } NO_2, \text{ or}$$

$$= 10\ \mu\ell \text{ of } NO_2$$

10.2.2 In Section 9.1, the calibration standard containing 1 mℓ of nitrite solution (10 μℓ NO_2) per 25 mℓ total volume is equivalent to 10/25 or 0.4 μℓ of NO_2 per mℓ.

10.3 Compute the concentration of nitrogen dioxide in the sample as follows:

$$\text{Nitrogen dioxide, ppm} = AK/V$$

where:

A = measured absorbance

K = standardization factor from Section 9.1.4 or 9.2.7

V = volume of air sample, at 25°C and 760 mm Hg, in liters/mℓ of absorbing reagent.

10.4 If preferred, the graph from Section 9.1.4 or 9.2.7 may be used instead as follows:

$$\text{Nitrogen dioxide, ppm} = \mu\ell\ NO_2 \text{ per } m\ell/V$$

10.4.1 If V is a simple multiple of K, calculations are simplified. Thus, for the K value of 0.73 previously cited, if exactly 7.3 liters of air are sampled through a bubbler containing 10 mℓ of absorbing reagent, K/V = 1, and the absorbance is also ppm directly.

10.4.2 For exact work, an allowance may be made in the calculations for sampling efficiency and for fading of the color using the following equation:

$$\text{Nitrogen dioxide, ppm} = A_c K/VE$$

where:

A_c = corrected absorbance; the absorbance is corrected for fading of the color as indicated in Section 8.2.4 when there is a prolonged interval between sampling and measurement of the absorbance.

E = sampling efficiency; for a bubbler, E is estimated from prior tests using two absorbers in series (Reference 7 and Section 6.1.1); for a bottle or syringe, E = 1.0.

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FREE SILICA (QUARTZ, CRISTOBALITE, TRIDYMITE) IN ATMOSPHERIC DUST

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Quartz, Cristobalite, Tridymite	Method No.:	P&CAM 109
Matrix:	Atmospheric Dust	Range:	5 – 200 $\mu g/cm^2$
Procedure:	X-ray Diffraction		
Date Issued:	4/7/72	Precision:	± 5 μg
Date Revised:	1/15/74	Classification:	D (Operational)

1. Principle of Method

   1.1 Atmospheric dust samples are collected on membrane filters.

   1.2 The filters are ashed and the residues along with an internal calibration standard are redistributed onto silver membrane filters.

   1.3 Each sample is scanned by X-ray diffraction to determine the presence of free silica (quartz, cristobalite, and/or tridymite).

   1.4 If present, the mass of each free silica species is determined by measuring the ratio of the diffraction peak intensities of the free silica species and the internal standard (fluorite) and comparing this ratio to a calibration curve.

2. Range and Sensitivity

   2.1 The analytical range extends from 5 $\mu g/cm^2$ to 200 $\mu g/cm^2$ for each free silica species; the total atmospheric dust loading on the filter must not exceed 1 mg/cm^2.

   2.2 The sensitivity is 5 μg for each free silica species.

3. Interferences

   3.1 Several minerals have diffraction peaks that correspond in position to the major peak for quartz; these include micas (biolite, muscovite), potash, feldspars (microcline, plagioclase), sillimanite, graphite, iron carbide (FeC), and zircon. The

presence of these interferences is usually encountered in specific, recognizable situations and can be verified by X-ray diffraction analysis. Analytical measurements can be carried out at a secondary quartz peak with a commensurate decrease in sensitivity.

3.2 Comparable interference may occur for cristobalite and tridymite. This can be determined by X-ray diffraction analysis. If interference occurs, secondary cristobalite and tridymite peaks must be used.

3.3 Diffraction peak interference may also occur for the fluorite (CaF_2) internal calibration standard. Compensation must be made for increased peak intensity from the interfering species or an alternate standard must be employed.

3.4 The presence of specific elements in the sample (iron, in particular) may result in appreciable X-ray fluorescence, leading to high background intensity. This situation may be circumvented by employing a diffracted beam monochromator.

4. **Precision and Accuracy**

4.1 Bumstead (Reference 11.1) has reported eleven replicate measurements of quartz in 2 mg of coal dust as having an average of 0.63% (12.6 μg), a range of 0.51-0.91% and a standard deviation of 0.114% (2.3μg); he reports an accuracy of ± 30% (3.8 μg) of the quartz present in the sample.

4.2 No information is available regarding the accuracy for cristobalite or tridymite.

5. **Advantages and Disadvantages**

5.1 The X-ray diffraction method offers sensitivity equivalent to or greater than other methods (infrared or colorimetric), is non-destructive to the sample, is rapidly performed and may be automated.

5.2 The X-ray diffraction method is limited to a sample size of a few milligrams. Application of the method requires a rather high degree of technical skill and expensive equipment.

6. **Apparatus**

6.1 **Sampling Equipment.** The sampling unit for the filter-collection method has the following components:

6.1.1 The filter unit consisting of the filter media (Section 6.1.4) and a two-piece filter cassette holder.

6.1.2 A personal sampling pump. This pump must be properly calibrated (with a representative filter unit in the line) so the volume of air sampled can be measured accurately.

6.1.3 Ten-mm nylon cyclone.

6.1.4 Polyvinyl chloride membrane filters (or equivalent), 37 mm and 5.0μm pore size. (Gelman VM-1 filters are unacceptable because of the high background produced by the ash.)

6.2 **X-ray diffraction equipment**, including copper and/or molybdenum target X-ray tubes.

6.3 **Low temperature radiofrequency asher**: Tracerlab Model LTA 600 or equivalent, or muffle furnace.

6.4 Ultrasonic bath.

6.5 Filtration apparatus.

6.6 **Silver membrane filters**, 25 mm diameter having an 0.45 μm pore size: Selas Flotronics.

6.7 Aluminum weighing pans.

6.8 Porcelain crucibles with covers.

6.9 100 mℓ Pyrex beakers.

6.10 Glass microscope slides.

6.11 Non-serrated, non-magnetic forceps.

6.12 Metal spatula.

7. **Reagents**

7.1 **Quartz, cristobalite and tridymite powders**: Acid-washed and wet-sieved through 10 μm sieve.

7.2 Fluorite (CaF_2): 400 mesh powder. Analytical Grade.

7.3 Wetting agent: Aerosol OT.

7.4 Petroleum jelly.

7.5 **Standard Solutions** of fluorite, quartz, cristobalite and tridymite. (Refer to Section 9.1.)

8. **Procedure**

8.1 **Cleaning of Equipment.** It is important that all equipment be kept as free of contaminant dust as possible.

8.1.1 The spatula, forceps, etc., may be satisfactorily cleaned using ethyl alcohol and disposable non-linting tissues.

8.1.2 The aluminum weighing pans are cleaned by rinsing twice with distilled water and twice with ethyl alcohol, and allowing to dry in a dust-free environment.

8.2 **Collection and Shipping of Samples**

8.2.1 Samples are collected on 5.0μm pore size, PVC (or equivalent) membrane filters. The quantity of dust collected on the filter should be 2 mg or less. The filters are held in plastic cassettes that permit the attachment of a 10 mm nylon cyclone to the inlet side of the cassette to collect a respirable sample. The filter is kept in the cassette for shipment.

8.2.2 Whenever an air sample or series of air samples is collected, a bulk sample of the suspected parent material of the atmospheric contaminant should be obtained and shipped back to the laboratory with the air samples.

8.3 **Analysis of Samples**

8.3.1 Either of these two described methods may be used to ash the sample.

1. Using forceps and spatula, place the filter samples in aluminum weighing pans and situate within the sample compartments of the low-temperature asher so that the sample exposure to the radiofrequency-excited oxygen plasma is optimized. The samples are ashed for one hour at 100 watts RF power and at an oxygen flow rate of 75 mℓ/min., using the techniques recommended in the instrument manual.

2. Using forceps and spatula, place the filter samples in porcelain crucibles, loosely cover and place in muffle furnace. Hold for two hours at 600°C (800°C if graphite is present in the sample).

51

8.3.2 Carefully scrape the ash residue into a 100 mℓ beaker. Rinse the weighing pan (or crucible) several times with about 5 mℓ of water and pour the rinse water into the beaker. Add 1 mℓ of the CaF_2 standard solution, a few drops of wetting agent, and distilled water to bring volume up to about 50 mℓ.

8.3.3 Ultrasonically agitate the beaker and its contents for 30 minutes at maximum setting.

8.3.4 Filter solution through a 25 mm diameter, 0.45 μm pore size silver membrane filter under suction. Thoroughly wash down the filter holder with distilled water to ensure that all dust particles have been transferred to the filter.

8.3.5 Using forceps, remove the filter, place in a Petri dish and dry at 105°C for 15 minutes.

8.3.6 The silver filter is then attached to a glass microscope slide with petroleum jelly, and inserted into the X-ray diffractometer. A portion of the filter should be inserted beneath the clamping surface of the diffractometer.

8.3.7 The diffractometer is then scanned over the 2 θ-range corresponding to d = 4.5 to 2.3Å (For a copper tube, 2 θ = 18° to 39° and for a molybdenum tube, 2 θ = 9° to 18°). The presence of crystalline forms of silica is determined by the occurrence of diffraction peak, as follows:

Mineral	d (Most Intense)	d (Second Most Intense)
Quartz	3.34 Å	4.26
Cristobalite	4.05	2.49
Tridymite	4.11	4.33
Fluorite Standard	3.15	

The presence of interfering compounds can be determined by the presence and identification of other X-ray diffraction peaks.

8.3.8 The intensity of the most intense diffraction peak for quartz, cristobalite and tridymite (as given in Section 8.3.7) is determined by measuring peak height or peak area from the diffraction scan or by scaler (fixed time or fixed count) measurement at peak position. All measurements must be corrected for background. Comparable measurements are made for the fluorite standard at d = 3.15Å. If diffraction peaks from other compounds interfere with the most intense quartz, cristobalite or tridymite peak, the second most intense peak for these silica compounds must be employed.

8.3.9 The free silica to fluorite intensity ratios are determined, and the mass of quartz, cristobalite and/or tridymite is determined from the appropriate calibration curve.

9. Calibration and Standards

9.1 Standards

9.1.1 A standard solution of fluorite is prepared by adding 20 mg of fluorite to 100 mℓ of distilled water containing a few drops of wetting agent and agitation.

9.1.2 Known amounts of quartz, cristobalite and tridymite are weighed to the nearest 0.1 mg and are added to 100 mℓ distilled water containing a few drops of wetting agent to provide five standard solutions of each mineral covering a concentration range of 0.01 – 0.3 mg/mℓ.

9.2 Standard Curve

9.2.1 One mℓ each of the standard fluorite solution (Section 9.1.1) and one of the crystalline free silica solutions (Section 9.1.2) are added to 50 mℓ distilled water and are analyzed according to steps 8.3.4 through 8.3.8. Similar data are collected for each of the free silica solutions (Section 9.1.2).

9.2.2 Standard curves are prepared for quartz, cristobalite and tridymite in which the intensity ratio of the free silica standard to fluorite is plotted against free silica mass in mg. This plot should give a nearly straight line that passes through the origin.

10. Calculations

The concentration of free silica in air can be expressed as milligrams silica per cubic meter of air sampled.

$$\text{mg } SiO_2/m^3 = \frac{\text{mg Q} + \text{mg C} + \text{mg T}}{V_s}$$

where:

mg SiO_2/m^3 = total milligrams of free silica per cubic meter of air sampled.

mg Q, mg C, mg T = milligrams of quartz, cristobalite and tridymite, respectively, determined from the appropriate calibration curve.

V_s = volume of air sampled in cubic meters (1000ℓ = 1m^3) at standard conditions of 25°C and 760 mm Hg.

11. References

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QUARTZ IN COAL DUST BY INFRARED SPECTROSCOPY

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Quartz	**Method No:**	P&CAM 110
Matrix:	Coal Dust	**Range:**	10-100 μg quartz
Procedure:	Infrared Spectroscopy	**Precision:**	Relative standard deviation < 15% at 30 μg quartz
Date Issued:	4/17/72	**Classification:**	B (Accepted)
Date Revised:	1/15/74		

1. **Principle of the Method**

   1.1 Airborne coal dust samples (respirable fraction) are collected on mixed cellulose ester membrane filters.

   1.2 The filter samples are ashed in a low-temperature radiofrequency asher to destroy the organic matrix.

   1.3 The ash is incorporated into a potassium bromide (KBr) pellet of which an infrared spectrum from 900 cm^{-1} to 700 cm^{-1} is obtained.

   1.4 The absorbance at 800 cm^{-1} is calculated and the weight of quartz is determined by reference to a standard curve.

2. **Range and Sensitivity**

   2.1 For a 1- to 2-mg sample of coal dust, the analytical range extends from 10 to 100 μg of quartz.

   2.2 The sensitivity is 5 μg of quartz.

3. **Interferences**

   3.1 Cristobalite and tridymite both absorb in the area of the 800 cm^{-1} quartz analytical peak of the spectrum. The presence of significant amounts of cristobalite and tridymite can be ascertained by x-ray diffraction on a bulk sample.

3.2 No interference in the 800 cm^{-1} area of the spectrum is produced by the low-temperature ashing of kaolin ($Al_2O_3 \cdot 2\ SiO_2 \cdot 2H_2O$), which may be present in the coal sample.

3.3 Infrared spectra of water, calcite or limestone ($CaCO_3$), lime (CaO), and slaked lime [$Ca(OH)_2$] show no interference at 800 cm^{-1}.

3.4 Infrared spectra of the low-temperature ash of blank Millipore MF filters show no interference at 800 cm^{-1}

3.5 Amorphous silica does not interfere in the infrared analysis.

4. Precision and Accuracy

4.1 The relative standard deviation (computed from the results of 23 samples) is less than 15% at 30 μg quartz.

4.2 The accuracy has not been determined. No collaborative tests have been performed on this method.

5. Advantages and Disadvantages of the Method

5.1 This method offers greater sensitivity than does the x-ray diffraction method (Reference 11.1). The differential thermal analysis method requires a larger sample size than the infrared method, and in addition is not well suited to analysis of respirable-size ($<10\mu$) particles because thermal response decreases with decreasing particle size (Reference 11.2). The colorimetric method of Talvitie (References 11.3 and 11.4), offers comparable sensitivity, but fails to distinguish between the crystalline forms of free silica and also requires more dexterity of the analyst. In addition, quartz may be lost during the colorimetric analysis, causing an erroneously low result, or silicates may be retained, causing an erroneously high result.

5.2 The infrared method thus offers good sensitivity, does not require a large sample size, is ideally suited to the analysis of the respirable fraction of coal dust, and requires less dexterity of the analyst than do some other methods. A trained technician can perform 5-10 analyses per day.

5.3 The method, however, does require some expensive equipment.

6. Apparatus

6.1 Double beam, grating infrared spectrophotometer: Perkin-Elmer Model 621/or equivalent.

6.2 Low-temperature, radiofrequency asher: Tracerlab Model LTA600/or equivalent.

6.3 Microbalance: Cahn Model G-2 Electrobalance/or equivalent.

6.4 Analytical balance: Mettler Model H20/or equivalent.

6.5 13-mm evacuable pellet die.

6.6 30-ton laboratory press.

6.7 2-inch diameter agate mortar and pestle.

6.8 Aluminum weighing pans: Fisher Scientific/or equivalent.

6.9 Membrane filters: Millipore MF filters, 5-micron pore size, 37-mm diameter, white, plain, with filter backing.

6.10 Non-serrated, non-magnetic forceps.

6.11 Metal spatula.

6.12 Sampling apparatus:

6.12.1 Dorr-Oliver cyclones, 10-cm/or equivalent.

6.12.2 Unico Micronair personal sampling pumps/or equivalent.

6.12.3 Plastic cassettes (sample holders for 37-mm filters).

6.12.4 Cabinet with tight-sealing door (desiccator).

7. Reagents

7.1 Potassium bromide: Harshaw Chemical Company, infrared quality/or equivalent.

7.2 Quartz: Acid-washed, sieved to 10-micron size.

7.3 Ethyl alcohol: U.S.P. grade, 95%.

8. Procedure

8.1 **Cleaning of Equipment.** It is important that all equipment be kept as free of contaminant dust as possible.

8.1.1 The spatula, forceps, LTA sample compartments, mortar and pestle, and pellet die may be satisfactorily cleaned using ethyl alcohol and disposable non-linting tissues.

8.1.2 The aluminum weighing pans are cleaned by rinsing twice with distilled water and twice with ethyl alcohol, and allowing to dry in a dust-free environment.

8.2 **Collection and Shipping of Samples.** The coal dust samples (1-2 mg) are obtained and shipped and the sample weights (to 0.01 mg) are obtained by the procedure outlined in "Coal Dust in Air," Tentative Method of the Physical and Chemical Analysis Branch and Engineering Branch. (See, also, References 11.1 and 11.7.)

8.3 **Analysis of Samples**

8.3.1 Using forceps and spatula, carefully place the weighed filter samples in aluminum weighing pans, and situate within the sample compartments of the low-temperature asher so that sample exposure to the radiofrequency-excited oxygen plasma is optimized.

8.3.2 The samples are ashed for one hour at 100 watts RF power and at an oxygen flow rate of 75 cc/min., using the techniques recommended in the instrument manual.

8.3.3 Approximately 300 mg of KBr, dried overnight at 110-120°C, are weighed to 0.1 mg and added directly to the weighing pan containing the ash. The KBr and the ash are thoroughly mixed in the aluminum pan with an agate pestle. The KBr is weighed and mixed in a 25% or less relative humidity environment to facilitate sample handling.

8.3.4 The sample mixture is transferred to a 13-mm evacuable pellet die, and a pellet is made using standard technique. The finished pellet, about 0.85 mm thick, is weighed to 0.1 mg. The value of (weight finished pellet ÷ weight KBr initially added) is computed. This value usually is about 0.98.

8.3.5 The pellet is scanned in a Perkin-Elmer Model 621 double-beam grating infrared spectrophotometer. The standard settings described in the instrument manual are used. No mechanical reference beam attenuation is used.

Before each scan, the 100% transmittance and scale position adjustments are made at the 800 cm^{-1} frequency.

The pellet is scanned from 900 cm^{-1} to 700 cm^{-1} (11 to 14 microns) over a period of about 2 minutes. When the quartz peak at 800 cm^{-1} is small, an ordinate expansion of 5X is used to enhance the peak height. The pellet is scanned through each of 4 pellet diameters spaced 45° apart. The resulting 4 absorbance values at 800 cm^{-1} are averaged to obtain the value for the pellet. The range of absorbance values for a single pellet is usually 0.005 absorbance units or less, which corresponds to a range of 5 μg or less of quartz.

8.3.6 Absorbances are computed from the transmittance values for the peak and baseline, which are read to 0.1% transmittance.

The baseline is chosen to be the horizontal tangent to the maximum of the trace on the higher-frequency side of the quartz peak. Such a horizontal baseline is concentration-independent. The value of the baseline transittance is I_o. The value of the peak transmittance is I. The absorbance of the peak equals the common logarithm of I_o/I. When the absorbance value at the 800 cm^{-1} quartz peak has been determined for the pellet, the weight in micrograms of quartz is obtained by reference to the standard curve.

9. Calibration and Standards

9.1 **Standards.** Known amounts of quartz, between 10 and 100 μg, are weighed out to 0.001 mg, as a 0.5% dilution in KBr, on a microelectrobalance in a 25% or less relative humidity environment. Approximately 300 mg of KBr, dried overnight at 110-120°C, are weighed to 0.1 mg, added to the quartz mixture in an agate mortar, and ground with an agate pestle. Pellets are prepared as in 8.3.4 and weighed to 0.1 mg. The value of (weight of finished pellet ÷ weight of constituent solids) is computed. Again, this value usually is about 0.98.

9.2 **Standard Curve.** A plot is made of the pellet absorbances at 800 cm^{-1} (obtained as in 8.3.5) vs. weight in micrograms of quartz (corrected as in 10.2) in the pellets. This plot should give a straight line approximately passing through the origin.

10. Calculations

10.1 A correction factor is applied to the original weight of coal dust in the sample to account for loss of coal ash during the pellet-making process. The original weight of coal dust is multiplied by the value computed in 8.3.4. This corrected weight of coal dust is used when the percent quartz in the dust is computed.

10.2 A correction factor is applied to the amount of quartz weighed out in 9.1 to account for loss of quartz during the pellet-making process. The measured weight of quartz is multiplied by the value computed in 9.1. This corrected weight of quartz is used when the standard curve is plotted.

10.3 After the weight, in micrograms of quartz in the sample, has been determined as in 8.3.6, the percent quartz is computed:

$$\frac{\mu g \text{ of quartz in sample}}{\text{corrected wgt in mg of coal dust sample x } 1000} \times 100 = \% \text{ quartz}$$

11. **References**

11.1 Morse, K.M., H.E. Bumsted, and W.C. Janes, "The Validity of Gravimetric Measurements of Respirable Coal Mine Dust," *Amer. Ind. Hyg. Assoc. J.*, 32:104 (1971).

11.2 Weiss, B., E.A. Boettner, and M. Stenning, "Determination of Quartz: Evaluation of the Differential Thermal Analysis Method," *Arch. Environ. Health*, 20: 37 (1970).

11.3 Talvitie, N.A., "Determination of Free Silica: Gravimetric and Spectrophotometric Procedures Applicable to Air-Borne and Settled Dust," *Amer. Ind. Hyg. Assoc. J.*, 25:169 (1964).

11.4 Talvitie, N.A., and F. Hyslop, "Colorimetric Determination of Siliceous Atmospheric Contaminants," *Amer. Ind. Hyg. Assoc. J.*, 19:54 (1958).

11.5 Standard Method No. 19, United States Bureau of Mines, 4800 Forbes Avenue, Pittsburgh, Pennsylvania 15213.

11.6 Dixon, K., and T.S. Fretwell, "The Determination of Quartz in the Airborne Dust of Coal Mines and in Coal Measure Minerals by Infrared Spectroscopy," Report No. SNT/55/68, Scientific Department, South Nottinghamshire Area, National Coal Board, Great Britain.

11.7 Larsen, D.J., L.J. von Doenhoff, and J.V. Crable, "The Quantitative Determination of Quartz in Coal Dust by Infrared Spectroscopy," *Amer. Ind. Hyg. Assoc. J.*, 33:367 (1972).

CARBON MONOXIDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Carbon Monoxide	Method No:	P&CAM 112
Matrix:	Air	Range:	10-500 ppm
Procedure:	Infrared Absorption Spectrophotometry		
Date Issued:	4/7/72	Precision:	±10%
Date Revised:	1/12/74	Classification:	C (Tentative)

1. Principle of the Method

Air samples are obtained in 5-liter or larger inert plastic bags and analyzed by infrared spectrophotometry in a 10-meter-path-length gas cell. Compressed air tanks (scuba tanks) may also be analyzed.

2. Range and Sensitivity

The analytical range extends from 10 ppm to 500 ppm. The upper limit may be extended by using an aliquot of the sample.

3. Interferences

Any gas that absorbs infrared radiation at the analytical wavelength will interfere. Possible interferences include: acetylene, aldehyde, cyanogen, diazomethane, hydrogen sulfide, nitrosylchloride, nitrous oxide, olefins and propyne. These gases are not usually present in significant concentration in normal atmospheres, but they can readily be ruled out by reference to the complete spectrum of the gas sample scanned from 2-14 microns. Each of the interfering gases has additional absorption lines in other areas of the spectrum which readily denote its presence. Carbon dioxide in concentrations as high as 3% does not interfere. Water vapor is eliminated by the use of drying agent traps on the inlet of the IR cell.

4. Precision and Accuracy

4.1 The analytical method has a precision of ± 10%.

4.2 No collaborative testing has been done with this method.

5. Advantages and Disadvantages

5.1 The major advantage of the method over CO meters and detector tubes is that the analysis is more accurate. Approved detector tubes are accurate to only ± 25%.

5.2 The disadvantage is that samples must be returned to the laboratory for analysis whereas immediate results are available with CO meters and detector tubes.

6. Apparatus

6.1 Peristaltic sampling pump, diaphragm pump, or vacuum pump with filtered outlet to remove oil.

6.2 Tedlar bags (5-liter or larger).

6.3 Infrared spectrometer, 2- to 15-micron range, wavelength accuracy ± 0.015μ, resolving power 0.01μ, wavelength reproducibility 0.005μ, transmission accuracy ± 0.5%.

6.4 10-meter-path-length gas cell.

6.5 Manometer

6.6 Vacuum pump (capable of reducing pressure to 1 mm).

6.7 Gas-tight syringes (100 $\mu\ell$, 500 $\mu\ell$, 1000 $\mu\ell$).

6.8 Gas tank regulators, connections, and needle valves for introducing dilution gas, CO, and samples.

7. Reagents

7.1 Anhydrous calcium chloride or indicating drierite.

7.2 Carbon monoxide.

7.3 Tank or dry nitrogen or CO-free air. (CO-free air can be obtained by using cylinder air known to be free of CO, or by allowing dilution air to enter the cell via a Hopcalite absorption trap connected to the inlet of the cell.)

7.4 Hopcalite (used to remove CO if room air is used for dilution).

8. **Procedure**

8.1 **Cleaning of Equipment.** Equipments must be properly evacuated or flushed to minimize any background interference.

8.2 **Collection and Shipping of Samples**

8.2.1 The sampling containers, Tedlar and Mylar bags, are flushed or evacuated prior to use.

8.2.2 Samples are obtained by filling the sampling containers with air either by using a sampling pump to push air in or by simply opening the valve on an evacuated container and allowing it to reach atmospheric pressure.

8.2.3 After the sample has been taken, all parts are sealed to minimize leakage in or out of the containers.

8.2.4 Care must be taken in transporting the samples to the laboratory, so as not to damage or alter the existing sample.

8.2.5 CO samples do not deteriorate on standing. If samples collected for analysis are under reduced pressure, care should be taken to see that dilution does not occur because of leaks in the container. Atmospheric samples can generally be stored at atmospheric pressure to avoid dilution.

8.2.6 Compressed air tanks (scuba tanks) may also be analyzed for CO content.

8.3 **Analysis of Samples**

8.3.1 The 10-meter path-length gas cell is connected to a manometer via a T connection.

8.3.2 The cell is then evacuated to approximately 1.0 mm Hg.

8.3.3 The Tedlar bag sample is introduced into the cell through a calcium chloride or drierite tube. If a rigid sample container is used, the equilibrium pressure (Pe) must be noted and the cell filled to atmospheric pressure with CO-free air or dry nitrogen. A 15-minute waiting period before analysis is necessary for equilibrium to be established.

If the sample is a compressed air tank, fill the evacuated gas cell to atmospheric pressure with air from the tank.

8.3.4 The spectrum is scanned from 4-6μ (2500-1670 cm^{-1}) and the absorbance at 4.67μ (2143 cm^{-1}) is measured by the baseline technique.

9. **Calibration and Standards**

9.1 The volume of the 10-meter cell is determined by standard techniques. The simplest procedure is to evacuate the cell, and then bring to atmospheric pressure by permitting air to enter via a calibrated wet-test meter. The volume of the cell is the volume of air shown on the meter. After the volume has been ascertained, the cell is again evacuated, and known volumes of CO added with the aid of standard gas-tight syringes, through a rubber serum bottle cap attached to the inlet of the cell, or through the rubber tubing which connects the gas cell with the tank of dilution gas.[1] The pressure in the cell is then brought to atmospheric pressure with CO-free dry air or nitrogen. The absorbance at 4.67μ (2143 cm^{-1}) is measured as described in the procedure.

9.2 The CO concentration is calculated from the quantity of CO added, and the volume of the cell. For example, 1.0 mℓ CO in a 3.85-liter cell gives a concentration of 260 ppm CO. A calibration curve relating absorbance and concentration is prepared for a series of known volumes of CO introduced into the IR cell.

10. **Calculations**

10.1 The concentration of the unknown is read from the calibration curve. To calculate the standard curve concentrations in parts per million (ppm), one may use the following equation.

$$\text{Sample concentration (ppm)} = \frac{V_{std}}{V_c}$$

where:

V_{std} = Volume of CO added ($\mu\ell$)
V_c = Volume of IR cell (liter)

10.2 The observed concentration from the calibration curve is corrected for the volume of sample actually introduced into the cell. For samples introduced into the cell with syringes, the volume is readily known and the correction applied using the following equation:

$$\text{Sample concentration (ppm)} = C_{obs} \times \frac{V_c}{V_s}$$

1. An alternative is to use CO "span gases" of known concentration. These standards may be obtained from Mathieson.

where:

C_{obs} = Observed concentration from the calibration curve (ppm)

V_c = Volume of IR cell (liter)

V_s = Volume of sample (liter)

For samples introduced from non-rigid Tedlar bags with volumes greater than the volume of the IR cell, $V_s = V_c$ and the sample concentration = C_{obs}.

10.3 For samples introduced by pressure measurements, the sample concentration is calculated as follows:

$$\text{Sample concentration (ppm)} = C_{obs} \times \frac{(Pa)}{(Pa-Pe)}$$

where:

C_{obs} = Observed concentration from the calibration curve (ppm)

Pe = Equilibrium pressure after connecting the sample container to the IR cell.

Pa = Atmospheric pressure.

11. References

Intersociety Tentative Method, *Health Laboratory Science*, 7, 78 (January, Supplement, 1970).

CARBON MONOXIDE IN BLOOD

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Carbon Monoxide	Method No.: P&CAM 113
Matrix: Blood	Range: 1 to 50% carboxyhemoglobin in 0.5 to 2 mℓ of blood
Procedure: Gas chromatography	Precision (CV_T): 0.02 in the range 1 to 50% carboxyhemoglobin
Date Issued: 4/17/72	
Date Revised: 1/29/76	Classification: D (Operational)

1. Principle of the Method

1.1 Carbon monoxide bound to hemoglobin is released by reaction with potassium ferricyanide in a closed vessel containing helium. The amount of carbon monoxide released is measured by gas chromatography.

1.2 A sample of whole blood is diluted with water. An aliquot of the sample is analyzed for carbon monoxide. The hemoglobin in another aliquot is saturated with carbon monoxide and then analyzed. The resulting measurements are compared to determine the percent carboxyhemoglobin in the original blood sample. This percentage is an indicator of carbon monoxide exposure.

2. Range and Sensitivity

For a 0.5- to 2-mℓ blood sample, the analytical range extends from 1 to 50% carboxyhemoglobin.

3. Interferences

No interferences from methane, ethane, hydrogen sulfide, acetylene, hydrogen, argon, or carbon dioxide have been observed. Air does not interfere but its presence should be minimized.

4. Precision and Accuracy

4.1 Repeated analyses of whole blood samples containing 1 to 50% carboxyhemoglobin yielded a coefficient of variation of about 0.02. (See Reference 11.1.)

4.2 Whole blood samples containing concentrations of less than 10% carboxyhemoglobin show an analytical error of less than 10% of the known value, while samples containing greater than 10% carboxyhemoglobin have an analytical error of less than 5%.

5. Advantages and Disadvantages of the Method

5.1 The method is simple and rapid. No preparation of standards is required.

5.2 It is accurate and precise in the concentration range 1 to 50% carboxyhemoglobin.

5.3 There are no known interferences for this method.

6. Apparatus

6.1 Hypodermic syringes (30 and 100 mℓ)

6.2 Three-way surgical stopcocks.

6.3 An automatic shaker.

6.4 A gas chromatograph with a thermal conductivity detector and a suitable recorder.

6.5 A gas chromatographic column (0.25-in. x 6-ft stainless steel) packed with Molecular Sieve Type 5A (0.063-in. pellets).

6.6 A 25-mℓ gas sampling loop for the chromatograph.

6.7 A source of vacuum.

6.8 Assorted laboratory glassware including pipettes, volumetric flasks, and graduated cylinders of appropriate sizes.

7. Reagents

All reagents must be analytical reagent grade. Doubly distilled or distilled deionized water should be used in the preparation of reagent solutions and the dilution of samples.

7.1 Light petroleum oil.

7.2 Glacial acetic acid.

7.3 **Acetate Buffer Solution (pH=4.6)**. Dissolve 70 g of sodium acetate in 100 mℓ of water containing 15 mℓ of acetic acid.

7.4 Potassium Ferricyanide Solution. Dissolve 48 g of potassium ferricyanide and 1 g of saponin in 160 mℓ of water. Dilute this solution to 200 mℓ with acetate buffer. Prepare fresh daily.

7.5 Carbon monoxide gas.

7.6 Helium gas.

8. Procedure

8.1 Cleaning of Equipment. All glassware used should be detergent washed and thoroughly rinsed with tap water and distilled water.

8.2 Collection and Shipping of Samples

8.2.1 No special preparation is needed for the collection of blood samples. Oxalate, citrate, and heparin appear to cause no interference in the analytical method. (See Reference 11.2.)

8.2.2 Blood samples may be shipped or stored in stoppered test tubes at refrigerator temperatures for 10 days without significant loss of carbon monoxide. (See Reference 11.2.)

8.3 Preparation of Samples. Dilute the blood sample tenfold with doubly distilled water.

8.4 Procedure for Measuring the Carbon Monoxide Content of a Sample

8.4.1 Add 3 mℓ of petroleum oil to a 100-mℓ syringe and expel the air. The oil provides lubrication, minimizes foaming, and renders the syringe airtight.

8.4.2 Fit the syringe with a three-way surgical stopcock and draw into it a measured amount (5 to 20 mℓ) of a diluted blood sample. Expel the air.

8.4.3 Draw into the syringe exactly 10 mℓ of the ferricyanide solution. Take care not to introduce air.

8.4.4 Render a second 100-mℓ syringe airtight with a minimal amount of petroleum oil and fill it with helium. Attach a three-way stopcock to this syringe.

8.4.5 Connect the stopcocks of the two 100-mℓ syringes and deliver 40 mℓ of helium to the syringe containing the sample. Gently shake the syringe containing the sample and reagents for 1 min. Allow the syringe to stand in an upright position until any foam dissipates.

8.4.6 Lubricate a 30-mℓ syringe with a minimal amount of petroleum oil and draw into it 30 mℓ of the helium-blood gas mixture *via* the three-way stopcock of the sample syringe.

8.4.7 Inject this helium-blood gas mixture into the gas-sampling loop for subsequent gas chromatographic analysis.

8.5 **Procedure for Measuring the Total Carbon Monoxide Capacity of a Sample**

8.5.1 Add 3 mℓ of petroleum oil to a 100-mℓ syringe and expel the air.

8.5.2 Fit the syringe with a thee-way surgical stopcock and draw into it exactly 5 mℓ of the same diluted blood sample analyzed previously. Expel the air.

8.5.3 Draw into the syringe about 20 mℓ of pure carbon monoxide gas.

8.5.4 Place the syringe on an automatic shaker and shake for 10 min.

8.5.5 Expel the excess carbon monoxide and flush five times with helium.

8.5.6 Check the carbon monoxide content of the sixth helium flush by transferring 30 mℓ of the gas to the gas-sampling loop and analyzing it by gas chromatography.

8.5.7 If the carbon monoxide concentration is insignificant, then proceed as before in releasing and analyzing the carbon monoxide bound to hemoglobin. Follow steps 8.4.3 to 8.4.7. If the carbon monoxide concentration is significant, repeat the helium flushing procedure.

8.6 **Introduction of the Sample into the Gas Chromatograph**

8.6.1 A vacuum source is connected to the gas sampling loop and the loop is evacuated.

8.6.2 The 30-mℓ syringe containing helium and blood gases is attached to the inlet of the sampling-loop valve with a three-way surgical stopcock.

8.6.3 The 30-mℓ gas sample is introduced into the 25-mℓ evacuated sampling loop, and the excess gas is forced out through the vacuum valve.

8.6.4 The sampling-loop valve is rotated to introduce the sample into the gas chromatograph.

8.7 **Gas Chromatographic Operating Conditions.** The column temperature is typically maintained at 75°C. The helium carrier gas flow rate is 135 mℓ/min.

9. **Calibration and Standards**

A linear relation between gas chromatographic response and amount of carbon monoxide exists in the concentration range of interest. Since what is sought is only the carbon monoxide content of the sample relative to its total capacity for carbon monoxide, comparison of chromatographic peaks of a saturated sample and an unknown sample provides the needed information. Calibration and standards are unnecessary.

10. **Calculations**

The following formula is used to establish the relative amount of carboxyhemoglobin in a given sample:

$$\% \text{ Carboxyhemoglobin} = \frac{(A/V)}{(A_S/V_S)} \times 100$$

where: A = the peak height resulting from the analysis of the unsaturated sample.

V = the liquid volume (in mℓ) of the unsaturated sample.

A_S = the peak height resulting from the analysis of the saturated sample.

V_S = the liquid volume (in mℓ) of the sample which was saturated with carbon monoxide.

11. **References**

11.1 Dominquez, A.M., H.E. Christensen, L.R. Goldbaum, and V.A. Stembridge, "A Sensitive Procedure for Determining Carbon Monoxide in Blood or Tissue Utilizing Gas-Solid Chromatography," **Toxicol. Appl. Pharmacol.**, 1, 135 (1959).

11.2 Collison, H.A., F.L. Rodkey, and J.D. O'Neal, "Determination of Carbon Monoxide in Blood by Gas Chromatography," **Clin. Chem.**, 14, 162 (1968).

95

FLUORIDE IN URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Fluoride	**Method No.**	P&CAM 114
Matrix:	Urine	**Range:**	Lower Limit Urine 0.19 mg/liter
Procedure:	Ion Specific Electrode		
Date Issued:	8/28/72	**Precision:**	Unknown
Date Revised:	12/1/73	**Classification:**	D (Operational)

1. **Principle of the Method**

   1.1 Urine samples are diluted with an equal volume of a pH-activity coefficient buffer solution.

   1.2 A fluoride sensitive specific ion electrode and a reference electrode are inserted into the buffered sample. The observed potential difference between the electrodes can then be related to the fluoride concentration in the sample via calibration curves prepared with the same buffer and electrodes.

2. **Range and Sensitivity**

   The range and sensitivity of this method have not been established at this time. The electrode recommended range is 0.19-1900 mg/ℓ urine.

3. **Interferences**

   Hydroxide, the only positive interference in the measurement, is eliminated by use of the pH buffer. Negative interferences, which may result from complexion of fluoride by cations such as a calcium, are minimized by the buffer and EDTA added as a stabilizer. The buffer also yields a solution of high ionic strength in which the activity coefficient of fluoride is relatively constant.

4. **Precision and Accuracy**

   The accuracy and precision of this method have not been completely determined at this time. No collaborative tests have been performed on this method.

5. **Advantages and Disadvantages of the Method**

5.1 Advantages over previous methods include simplicity, accuracy, speed, specificity, and elimination of ashing, distillation or diffusion steps. Interfering ions are complexed.

5.2 No significant disadvantages are known.

6. **Apparatus**

6.1 Fluoride sensitive specific ion electrode, Model 94-09, Orion Research Corporation (Cambridge, Massachusetts) or equivalent.

6.2 Reference electrode, calomel or silver/silver chloride, sleeve junction preferable but not necessary.

6.3 pH/Millivolt meter, capable of measuring to within 0.5 millivolt.

6.4 Magnetic stirrer, equipped with small stirring bars which can spin freely in the sample beakers.

6.5 Plastic beakers (50 mℓ) and sample collection bottles (100-mℓ capacity), polyethylene or polypropylene.

6.6 Laboratory glassware.

7. **Reagents**

All chemicals used should be ACS Reagent grade or equivalent. After being prepared in clean glassware, all fluoride-containing solutions should be stored in plastic beakers and bottles.

7.1 Double Distilled Water.

7.2 Sodium Citrate.

7.3 Ethylenedinitrilotetracetic Acid (EDTA), disodium salt.

7.4 Glacial Acetic Acid.

7.5 Sodium Chloride

7.6 **Sodium Hydroxide, 5 M Solution.** Dissolve 20 g sodium hydroxide pellets in sufficient distilled water to give 100 mℓ of solution.

7.7 **Total Ionization Activity Buffer (TISAB).** Place 500 mℓ of double distilled water in a 1-liter beaker. Add 57 mℓ of glacial acetic acid, 58 g of sodium chloride, and 0.30 g of sodium citrate. Stir to dissolve. Place beaker in a water bath (for cooling). Slowly add 5M sodium hydroxide until the pH is between 5.0 and 5.5. Cool to room temperature and pour into a 1-liter volumetric flask and add double distilled water to the mark.

7.8 Sodium Fluoride, for preparation of standards.

7.9 **Standard Fluoride Solutions.** All solutions should be stored in plastic bottles. The 0.1M fluoride standard is reported to be stable at least two months. All other dilutions should be prepared fresh weekly.

7.9.1 0.1M Fluoride (1900 μg F/mℓ) – Dissolve 4.200 g of sodium fluoride in water and dilute to 1000 mℓ. This solution can also be purchased from Orion Research, Inc., Cambridge, Massachusetts.

7.9.2 10^{-2} M Fluoride (190 μg F/mℓ)– Dilute 10 mℓ of 7.9.1 to 100 mℓ with water.

7.9.3 10^{-3} M Fluoride (19 μg F/mℓ) – Dilute 10 mℓ of 7.9.2 to 100 mℓ with water.

7.9.4 10^{-4} M Fluoride (1.9 μg F/mℓ) – Dilute 10 mℓ of 7.9.3 to 100 mℓ with water.

7.9.5 10^{-5} M Fluoride (0.19 μg F/mℓ) – Dilute 10 mℓ of 7.9.4 to 100 mℓ with water.

8. **Procedure**

8.1 **Cleaning of Equipment.** All plastic and glassware must be rendered free of any fluoride before use.

8.2 **Collection and Shipping of Samples**

8.2.1 Urine samples representing pre-exposure (early morning) and after-exposure (end of working day) conditions should be taken. To minimize bacterial contamination, one should collect the mid-stream urine sample of a single voiding.

8.2.2 The urine samples are collected in chemically clean bottles containing 0.2 g of EDTA (ethylenedinitrilotetracetic acid).

8.2.3 Upon receipt at the laboratory, the volume of the urine sample should be determined. If the volume is greater than 100 mℓ, an amount of EDTA equivalent to 0.2 g per 100 mℓ in excess of the first 100 mℓ should be added.

8.2.4 Samples that cannot be analyzed within 48 hours should be refrigerated.

8.3 **Analysis of Urine Samples**

8.3.1 Transfer 10 mℓ of well mixed urine sample and 10 mℓ of TISAB buffer solution into a 50 mℓ plastic buffer, and add a magnetic stirring bar.

8.3.2 Immerse the electrodes in the solution. Adjust stirring speed and stirrer position to avoid splashing and hitting the electrodes.

8.3.3 After the EMF (voltage) reading stabilizes (that is, EMF drift less than 0.5 millivolt per minute) record the EMF to the nearest millivolt.

For most samples, the EMF will stabilize in a minute or two if the fluoride concentration is 2 μg/mℓ or more. Stabilization may take slightly longer at lower concentrations.

8.3.4 Rinse the electrodes with double distilled water and wipe dry with clean tissue before proceeding with next step.

9. **Calibration and Standards**

9.1 Use the standard fluoride solutions prepared in 7.9 and treat them in the same way as the samples, Section 8.2 start with the most dilute solution (7.9.5) and proceed to 7.9.2 (10^{-2} M fluoride).

9.2 Prepare a calibration curve by plotting the concentration – EMF data on semi-log paper with the fluoride concentration on the log axis.

9.3 Calibrations should be repeated at least twice daily.

10. **Calculations**

10.1 The concentration of fluoride is obtained by converting the millivolt readings into concentrations using the calibration curve.

10.2 The concentration is reported as mgF per liter (numerically equivalent to μg per mℓ) of urine.

11. References

11.1 *Instruction Manual for Fluoride Electrode Model 94-09*, Orion Research Incorporated, Cambridge, Massachusetts.

11.2 Sun, Mu-Wan, "Fluoride Ion Activity Electrode for Determination of Urinary Fluoride," *Amer. Inc. Hyg. Assoc. J.*, 30, 133 (1969).

CHLORIDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Chloride	Method No.: P&CAM 115
Matrix: Air	Range: 0.1 to 1750 mg/m^3 for a 200-liter air sample
Procedure: Collection with an impinger, specific ion electrode	Precision (CV): 0.02 (analytical) over the above range
Date Issued: 9/15/72	Classification: D (Operational)
Date Revised: 1/29/76	

1. Principle of the Method

Chloride compounds in air are collected in midget impingers containing acetate buffer (pH = 5.0). The resulting chloride solutions are analyzed with a calibrated specific ion electrode.

2. Range and Sensitivity

2.1 The sensitivity of the overall method has not been established at this time. However, an estimate of the detection limit can be made from the lower limit of detection of the analytical method alone, which is 1.8 μg/mℓ (5 x 10^{-5} M) . This corresponds to a concentration of 0.09 mg/m^3 for a 200-liter air sample drawn through 10 mℓ of acetate buffer.

2.2 The range of chloride concentrations easily detected with the electrode extends to 35,000 μg/mℓ (1M). However, the upper limit of the concentration range for the overall method has not been established. It is expected to be primarily a function of the collection capacity of the midget impinger. If the efficient collection of chloride is assumed, an estimate of the upper limit for a 200-liter air sample drawn through 10 mℓ of acetate buffer is 1750 mg/mℓ.

3. **Interferences**

3.1 To avoid interference, the sulfide ion concentration in the sample must not be greater than $10^{-7}M$.

3.2 Also, for interference-free operation, the chloride ion concentration must be at least 300 times the bromide ion concentration, 100 times the thiosulfate concentration, and 8 times the ammonium concentration. Traces of iodide and cyanide can be tolerated. (See Reference 11.2)

3.3 Nitrate, sulfate, and carbonate do not interfere.

4. **Precision and Accuracy**

4.1 The precision and accuracy of this method have not been established at this time. No collaborative tests have been performed on this method.

4.2 The precision of the analytical procedure alone is a function of electrode temperature, drift, and noise. (See Reference 11.1.) With frequent calibration, the reproducibility of measurements over the concentration range cited above yields a coefficient of variation of about 0.02. Very low concentrations of chloride ion can be measured accurately. Since the electrode response is proportional to the logarithm of the chloride ion concentration, the measurement of small differences between large values of concentration is not accurate.

5. **Advantages and Disadvantages of the Method**

5.1 Advantages include the simplicity, specificity, and speed of the method.

5.2 The interferences cited in Section 3 will inhibit the usefulness of this method in atmospheres where the specified ions are present in amounts comparable to the amount of chloride ion.

6. **Apparatus**

6.1 An Orion Chloride Ion Electrode, Model 94-17 or equivalent.

6.2 An Orion Double Junction Reference Electrode, Model 90-02 or equivalent.

6.3 A pH meter with expanded millivolt scale.

6.4 Midget impingers with graduation marks.

6.5 A properly calibrated personal sampling pump capable of sampling at a flow rate of 2.5 ℓ/min for time intervals of up to 80 min. The pump should be calibrated with the midget impinger containing 10 mℓ of the acetate buffer in the sampling train. A dry or wet test meter or a glass rotameter that will measure the appropriate flow rate within ± 5% may be used for the calibration. The meter should include a prefilter to prevent liquid droplets from reaching it.

6.6 Assorted laboratory glassware including pipettes, volumetric flasks, and graduated cylinders of appropriate sizes.

7. Reagents

All reagents must be analytical reagent grade. Doubly distilled or distilled deionized water should be used in the preparation of reagents and standards.

7.1 **Acetate Buffer (pH 5).** Dissolve 41 g of sodium acetate in water and dilute to 1 liter with water. Adjust the pH to 5 with acetic acid.

7.2 **Sodium Chloride Standard Solution** (0.1*M*, 3500 μg/mℓ). Dissolve 5.84 g of sodium chloride in water and dilute to 1 liter with water in a volumetric flask. Prepare more dilute standards by serial dilution of the 0.1 *M* standard with acetate buffer.

8. Procedure

8.1 **Cleaning of Equipment.** All glassware is washed in detergent solution and rinsed in tap water, followed by a rinse with doubly distilled water.

8.2 **Collection and Shipping of Samples**

8.2.1 Assemble a train composed of a midget impinger, a flow meter, and a pump. Insure that all fittings are vacuum tight.

8.2.2 Pipette exactly 10 mℓ of the acetate buffer into the midget impinger and sample at a flow rate of 2.5 ℓ/min. Sample about 200 liters of air. Note the exact volume of air sampled.

8.2.3 After sampling, seal the openings of the impingers with masking tape and return the impingers to the laboratory for analysis.

8.3 **Analysis of Samples**

8.3.1 Transfer each sample solution to a 50-mℓ beaker. Adjust the pH to 5, if necessary, with acetic acid or acetate buffer. Measure and record the final volume of the solution.

8.3.2 Place the chloride ion electrode and the double junction reference electrode in each solution. Let the meter stabilize before taking a reading. Record the resulting millivolt meter reading.

9. Calibration and Standards

9.1 Prepare appropriate standards in acetate buffer from the 0.1 *M* standard.

9.2 Obtain millivolt readings as in Section 8.3.2.

9.3 Construct a calibration curve by plotting the logarithms of chloride ion concentrations (in $\mu g/m\ell$) *versus* corresponding millivolt readings on "semilog" graph paper.

10. Calculations

10.1 From the calibration curve, note the chloride ion concentration (in $\mu g/m\ell$) of each sample solution.

10.2 Multiply the concentration of each sample by its volume to obtain the amount (in μg) of chloride ion in the sample.

10.3 The concentration of chloride ion in an air sample may be expressed as mg/m^3:

$$mg/m^3 = \mu g/V_s\ (\ell)$$

where: V_s = volume of air sampled.

11. References

11.1 "Orion Ionalyzer Instruction Manual, Chloride Electrode, Model 94-17," Orion Research Incorporated.

11.2 "Analytical Methods Guide," Orion Research Incorporated, 1973.

CYANIDE IN AIR

Physical and Chemical Analysis Branch
Analytical Method

Analyte:	Cyanide	**Method No.**	P&CAM 116
Matrix:	Air	**Range:**	0.013 -13 mg/m^3
Procedure:	Collection via Impinger/ Ion Specific Electrode		
Date Issued:	9/8/72	**Precision:**	Unknown
Date Revised:	12/1/73	**Classification:**	D (Operational)

1. **Principle of the Method**

   1.1 Atmospheric samples are taken using midget impingers that contain 10 mℓ of 0.1M NaOH.

   1.2 Samples are analyzed using the cyanide ion specific electrode.

2. **Range and Sensitivity**

   The range and sensitivity of the method have not been established at this time. The recommended range of the method is 0.013-13 mg/m^3 in air.

3. **Interferences**

   3.1 Sulfide ion irreversibly poisons the cyanide ion specific electrode and must be removed if found to be present in the sample. Check for the presence of sulfide ion by touching a drop of sample to a piece of lead acetate paper. The presence of sulfide is indicated by discoloration of the paper.

   3.2 Sulfide is removed by the addition of a small amount (spatula tip) of powdered cadmium carbonate to the pH 11-13 sample. Swirl to disperse the solid, and recheck the liquid by again touching a drop to a piece of lead acetate paper. If sulfide ion has not been removed completely, add more cadmium carbonate. Avoid a large excess of cadmium carbonate and long contact time with the solution.

3.3 When a drop of liquid no longer discolors a strip of lead acetate paper, remove the solid by filtering the sample through a small plug of glass wool contained in an eye dropper and proceed with the analysis.

4. **Precision and Accuracy**

The precision and accuracy of this method have not been completely determined at this time. No collaborative tests have been performed on this method.

5. **Advantages and Disadvantages of the Method**

Advantages are the simplicity, specificity, speed and accuracy of the method.

6. **Apparatus**

6.1 **Sampling Equipment.** The sampling unit for the impinger collection method consists of the following components:

6.1.1 A prefilter unit (if needed) which consists of the filter media and cassette filter holder.

6.1.2 A midget impinger containing the absorbing solution or reagent.

6.1.3 A pump suitable for delivering desired flow rates. The sampling pump is protected from splashover or water condensation by an adsorption tube loosely packed with a plug of glass wool and inserted between the exit arm of the impinger and the pump.

6.1.4 An integrating volume meter such as a dry gas or wet test meter.

6.1.5 Thermometer.

6.1.6 Manometer.

6.1.7 Stopwatch.

6.2 Orion 94-06 Cyanide ion specific electrode or equivalent.
6.3 Orion 90-01 Single junction reference electrode or equivalent.
6.4 Expanded scale millivolt – pH meter.
6.5 Associated Laboratory Glassware.
6.6 Plastic Bottles.
6.7 Magnetic Stirrer and Stirring Bars.

7. **Reagents**

The reagents described must be made up using ACS reagent grade or better grade of chemical.

7.1 Double distilled water.

7.2 Potassium Cyanide.

7.3 **Sodium Hydroxide 0.1M.** Dissolve 2.0 g NaOH in double distilled water and dilute to 500 mℓ.

7.4 **Potassium Cyanide Standards**

7.4.1 Dissolve 0.65 g KCN in 0.1M NaOH and dilute to 100 mℓ with additional 0.1M NaOH for 10^{-1} M[CN^-] (2600 μg/mℓ).

7.4.2 Dilute 10 mℓ of 10^{-1} M[CN^-] to 100 mℓ with 0.1M NaOH for 10^{-2} M[CN^-] (260 μg/mℓ).

7.4.3 Dilute 10 mℓ of 10^{-2} M[CN^-] to 100 mℓ with 0.1M NaOH for 10^{-3} M[CN^-] (26 μg/mℓ).

7.4.4 Dilute 10 mℓ of 10^{-3} M[CN^-] to 100 mℓ with 0.1M NaOH for 10^{-4} M[CN^-] (2.6 μg/mℓ).

7.4.5 Dilute 10 mℓ of 10^{-4} M[CN^-] to 100 mℓ with 0.1M NaOH for 10^{-5} M[CN^-] (0.26 μg/mℓ).

7.5 **Lead Acetate Paper.**

7.6 Cadmium Carbonate

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware is washed in detergent solution, rinsed in tap water and then rinsed with double distilled water.

8.2 **Collection and Shipping of Sample**

8.2.1 Pour 10 mℓ of the absorbing solution (Section 7) into the midget impinger, using a graduated cylinder to measure the volume.

8.2.2 Connect the impinger (via the adsorption tube) to the vacuum pump and the prefilter assembly (if needed) with a short piece of flexible tubing. The minimum amount of tubing necessary to make the joint between the prefilter and impinger should be used. The air being sampled should not be passed through any other tubing or other equipment before entering the impinger.

8.2.3 Turn on pump to begin sample collection. Care should be taken to measure the flow rate, time and/or volume as accurately as possible. The sample should be taken at a flow rate of 2.5 ℓ pm. A sample size of not more than 200 liters and no less than 10 liters should be collected. The minimum volume of air sampled will allow the measurement at least 1/10 times the TLV, 0.5 mg/m^3 (760 mm Hg, 25°C).

8.2.4 After sampling, the impinger stem can be removed and cleaned. Tap the stem gently against the inside wall of the impinger bottle to recover as much of the sampling solution as possible. Wash the stem with a small amount (1-2 mℓ) of unused absorbing solution and add the wash to the impinger. Then the impinger is sealed with a hard, non-reactive stopper (preferably Teflon). Do not seal with rubber. The stoppers on the impingers should be tightly sealed to prevent leakage during shipping. If it is preferred to ship the impingers with the stems in, the outlets of the stem should be sealed with Parafilm or other non-rubber covers, and the ground glass joints should be sealed (i.e., taped) to secure the top tightly.

8.2.5 Care should be taken to minimize spillage or loss by evaporation at all times. Refrigerate samples if analysis cannot be done within a day.

8.2.6 Whenever possible, hand delivery of the samples is recommended. Otherwise, special impinger shipping cases designed by NIOSH should be used to ship the samples.

8.2.7 A "blank" impinger should be handled as the other samples (fill, seal and transport) except that no air is sampled through this impinger.

8.2.8 Where a prefilter has been used, the filter cassettes are capped and placed in an appropriate cassette shipping container. One filter disc should be handled like the other samples (seal and transport) except that no air is sampled through, and this is labeled as a blank.

8.3 **Analysis of Samples**

8.3.1 The solution is quantitatively transferred from the impinger to a 50-cc beaker.

8.3.2 The cyanide ion electrode and the single junction reference electrode are placed in the solution and the resulting millivolt reading recorded. The reading should be taken after the meter has stabilized. Both the samples and standards should be stirred while the readings are being taken.

9. **Calibration and Standards**

9.1 Obtain the millivolt readings from each of the cyanide standards.

9.2 Plot the millivolt readings vs. the cyanide ion concentrations of the standards on semi-log paper. The cyanide ion concentration in $\mu g/m\ell$ is plotted on the log axis.

10. **Calculations**

10.1 The millivolt readings from the analysis of the sample are converted to $\mu gCN/m\ell$ of solution using the calibration curve.

10.2 The μg content of the sample is multipled by the sample volume to obtain the total μgCN in the sample.

10.3 Convert the volume of air sampled to standard conditions of 25°C and 760 mm Hg:

$$V_s = V \times \frac{P}{760} \times \frac{298}{T + 273}$$

where:

V_s = volume of air in liters at 25°C and 760 mm Hg
V = volume of air in liters as measured
P = Barometric Pressure in mm Hg
T = Temperature of air in degree centrigrade.

10.4 The concentration of CN in the air sampled can be expressed in μg CN per liter or mg CN per cubic meter.

$$mg/m^3 = \mu g/liter$$

$$mg/m^3 = \frac{\text{total } \mu g \text{ CN}}{V_s} \qquad \begin{matrix}\text{(Section 10.2)} \\ \text{(Section 10.3)}\end{matrix}$$

10.5 The concentration of CN can also be expressed in ppm, defined as $\mu\ell$ of component per liter of air.

$$\text{ppm} = \mu\ell\ CN/V_s = R/MW\ \mu g\ CN/V_s$$

$$= 0.94 \times \mu g\ CN/V_s$$

where:

R = 24.45 at 25°C, 760 mm Hg.
MW = 26

11. References

11.1 Cyanide Ion Specifications, Orion Research, Inc., Cambridge, MA.

11.2 Frant, M.S., "Detecting Pollutants with Chemical Sensing Electrodes," Environ. Sci. Tech., 8, 224 (1974).

FLUORIDE AND HYDROGEN FLUORIDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Total Fluoride	**Method No.:**	P&CAM 117
Matrix:	Air	**Range:**	0.05 to 475 mg/m^3 fluoride
Procedure:	Collection via impinger, ion specific electrode	**Precision:**	6.5% RSD for 100 μg HF
Date Issued:	9/7/72		
Date Revised:	11/15/76	**Classification:**	D (Operational)

1. **Principle of the Method**

   1.1 Atmospheric samples are taken using midget impingers containing 10 ml of 0.1M NaOH.

   1.2 Samples are diluted with Total Ionic Strength Activity Buffer (TISAB).

   1.3 The diluted samples are analyzed using the fluoride ion specific electrode.

2. **Range and Sensitivity**

   The range covered by the analytical method for a 40-liter sample is 0.05 to 475 mg/m^3. A larger volume sample is recommended for 0.05 to 0.5 mg/m^3 concentrations to minimize errors, particularly for sample loss.

3. **Interferences**

   Hydroxide ion is the most significant electrode interference but the use of TISAB eliminates this problem. Monofluorophosphate ion is an electrode interference that is not controlled in this method. The TISAB solution used in this method will control the fluoride complexing cations Si^{4+}, Fe^{3+} and Al^{3+} up to 80, 200, and 8 ppm respectively. The BF_4^- and PF_6^- ions dissociate to an unpredictable extent and their presence will confuse the measurement of fluoride.

4. **Precision and Accuracy**

   The precision for sampling and analysis of 16 samples of approximately 100 μg HF was 6.5% RSD. The analytical precision at 50 μg fluoride is 0.8% RSD. No collaborative tests or accuracy determinations have been performed.

5. **Advantages and Disadvantages of the Method**

   5.1 Advantages over previous methods include simplicity, accuracy, speed, specificity and elimination of distillation, diffusion and ashing of the samples.

   5.2 No significant disadvantages are known at present.

6. Apparatus

6.1 Sampling Equipment. The sampling unit for the impinger collection method consists of the following components:

6.1.1 A prefilter unit, which consists of a cassette filter holder with Teflon filter, may be used to remove particulates and allow sampling for gaseous fluorides only.

6.1.2 A midget impinger containing the absorbing solution or reagent.

6.1.3 A pump suitable for delivering desired flow rates. The sampling pump is protected from splashover or water condensation by an adsorption tube loosely packed with a plug of glass wool and inserted between the exit arm of the impinger and the pump.

6.1.4 An integrating volume meter such as a dry gas or wet test meter.

6.1.5 Thermometer.

6.1.6 Manometer.

6.1.7 Stopwatch.

6.2 Orion Model 94-09 Fluoride Specific Ion Electrode, or equivalent.

6.3 Reference Electrode, Orion 90-01 single junction, or equivalent calomel or silver/silver chloride electrode.

6.4 Expanded Scale Millivolt-pH Meter, capable of measuring to within 0.5 millivolt.

6.5 Polyethylene Beakers, 50-ml capacity.

6.6 Laboratory Glassware.

6.7 Magnetic Stirrer and Stirring bars for 50-ml Beakers.

7. Reagents

All chemicals must be ACS reagent grade or equivalent. Polyethylene beakers and bottles should be used for holding and storing all fluoride-containing solution.

7.1 Double Distilled Water.

7.2 Glacial Acetic Acid.

7.3 Absorbing Solution: 0.1M Sodium Hydroxide Solution. Dissolve 4 g sodium hydroxide pellets in 1 liter distilled water.

7.4 Sodium Hydroxide, 3.5M Solution. Dissolve 28 g sodium hydroxide pellets in sufficient distilled water to give 200 ml of solution.

7.5 Sodium Chloride.

7.6 Cyclohexane Diamine Tetraacetic acid monohydrate (CDTA).

7.7 Total Ionic Strength Activity Buffer (TISAB). Place 650 ml of double distilled water in a 1-liter beaker. Add 57 ml of glacial acetic acid, 58 g of sodium chloride and 30 g of CDTA. Stir the solution for 15 minutes. The CDTA may not go completely into solution until the sample volume approaches one liter during the following steps. Place the beaker in a water bath (for cooling) and slowly add 3.5M sodium hydroxide with stirring until the pH is 5.0 $\pm$ 0.1. Cool to room temperature and pour into a 1-liter volumetric flask and add double distilled water to the mark.

7.8 Sodium Fluoride, for preparation of standards.

7.9 Standard Fluoride Solution. The stock solution (0.1M [F^-]) is stable for two months. The dilution standards should be prepared every two weeks.

7.9.1 Dissolve 4.2 g of sodium fluoride in double distilled water and dilute to 1 liter. This solution contains 10^{-1} M [F^-] (1900 μg F^-/ml). The 0.1M fluoride solution may also be purchased from Orion Research, Inc., Cambridge, Mass.

7.9.2 Prepare 10^{-2} M [F^-] by diluting 10 ml of 10^{-1} M [F^-] to 100 ml with double distilled water (190 μg F^-/ml).

7.9.3 Prepare 10^{-3} M [F^-] by diluting 10 ml of 10^{-2} M [F^-] to 100 ml with double distilled water (19 μg F^-/ml).

7.9.4 Prepare 10^{-4} M [F^-] by diluting 10 ml of 10^{-3} M [F^-] to 100 ml with double distilled water (1.9 μg F^-/ml).

7.9.5 Prepare 10^{-5} M [F^-] by diluting 10 ml of 10^{-4} M [F^-] to 100 ml with double distilled water (0.19 μg F^-/ml).

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware and plastic ware are washed in detergent solution, rinsed in tap water, and then rinsed with double distilled water.

8.2 **Collection and Shipping of Samples**

8.2.1 Pour 10 ml of absorbing solution (Section 7) into the midget impinger, using a graduated cylinder to measure the volume.

8.2.2 Connect the impinger (via the adsorption tube) to the vacuum pump and the prefilter assembly (if needed) with a short piece of flexible tubing. The minimum amount of tubing necessary to make the joint between the prefilter and impinger should be used. The air being sampled should not be passed through any other tubing or other equipment before entering the impinger.

8.2.3 Turn on pump to begin sample collection. Care should be taken to measure the flow rate, time, and/or volume as accurately as possible. The sample should be taken at a flow rate of 2.5 lpm. A sample size of not more than 200 liters and no less than 40 liters should be collected. The minimum volume of air sampled will allow the measurement of at least 1/10 times the TLV, 0.2 mg/m^3 (760 mm Hg, 25°C).

8.2.4 Record the temperature and pressure of the atmosphere being sampled. If the pressure reading is not available, record the elevation. Also report the type of sampling pump used.

8.2.5 After sampling, the impinger stem can be removed and cleaned. Tap the stem gently against the inside wall of the impinger bottle to recover as much of the sampling solution as possible. Wash the stem with a small amount (1-2 ml) of unused absorbing solution and add the wash to the impinger. Then the impinger is sealed with a hard, non-reactive stopper (preferably Teflon). Do not seal with rubber. The stoppers on the impingers should be tightly sealed to prevent leakage during shipping. If it is preferred to ship the impingers with the stems in, the outlets of the stem should be sealed with Parafilm or other non-rubber covers, and the ground glass joints should be sealed (i.e., taped) to secure the top tightly.

8.2.6 Care should be taken to minimize spillage or loss by evaporation at all times. Refrigerate samples if analysis cannot be done within a day.

8.2.7 Whenever possible, hand delivery of the samples is recommended. Otherwise, special impinger shipping cases designed by NIOSH should be used to ship the samples.

95

8.2.8 A "blank" impinger should be handled as the other samples (fill, seal, and transport) except that no air is sampled through this impinger.

8.2.9 Where a prefilter has been used, the filter cassettes are capped and placed in an appropriate cassette shipping container. One filter disc should be handled as the other samples (seal and transport) except that no air is sampled through, and this is labeled as a blank.

8.3 **Analysis of Samples**

8.3.1 The sample is transferred from the impinger to a 25-ml graduated cylinder. Use 2 or 3 ml of double distilled water to rinse the impinger and add the rinse to the graduated cylinder. Add 10 ml of TISAB to the graduated cylinder and sufficient double distilled water to bring the volume to 25 ml. Stir or shake the solution to promote complete mixing. Pour the contents of the graduated cylinder into a 50-ml plastic beaker. Any drops remaining in the graduated cylinder can be disregarded since this will not affect the results.

8.3.2 Place the beaker on a stirrer and lower the fluoride selective electrode and the reference electrode into the stirred solution. After the electrodes have stabilized for 10 minutes, record the meter reading to the nearest 0.1 millivolt.

9. **Calibration and Standards**

Prepare a series of fluoride standard solutions by pipeting 10 ml of each fluoride standard (7.9) into clear polyethylene beakers. Then pipet 10 ml of TISAB and 5 ml of double distilled water into each of the beakers. Insert the fluoride ion electrode and the reference electrode into each of the stirred calibration solutions starting with the most dilute solution and record the resulting millivolt reading to the nearest 0.5 millivolt. Plot the millivolt reading vs. the fluoride ion concentration of the standard on semi-log paper. Use the concentration of the standards before dilution for plotting, not the diluted concentration. The fluoride ion concentration in μg/ml is plotted on the log axis. The calibration points should be repeated twice daily.

10. **Calculations**

10.1 The concentration (μg/ml) of fluoride in the sample solution is obtained from the calibration curve.

10.2 Total μg F^- in the sample = sample concentration (μg/ml) $\times$ 10.0 ml.

10.3 Convert the volume of air sampled to standard conditions of 25°C and 760 mm Hg.

$$V_s = V \times \frac{P}{760} \times \frac{298}{T + 273}$$

10.4 The total μg F^- is divided by the volume, in liters, of air sampled to obtain concentration in μg F^-/liter or mg F^-/m^3.

$$\text{mg } F^-/m^3 = \mu\text{g } F^-/\text{liter}$$

$$\text{mg } F^-/m^3 = \frac{\text{total } \mu\text{g } F^-}{V_s} \qquad \text{(Section 10.3)}$$

where:

V_s = volume of air in liters at 25°C and 760 mm Hg

V = volume of air in liters as measured

P = barometric pressure in mm Hg

T = temperature of air in degrees centigrade

96

10.5 The concentration can also be expressed in ppm, defined as μl of component per liter of air.

$$\text{ppm } F^- = \mu l\ F^-/V_s = \frac{24.45}{MW} \times \mu g\ F^-/V_s$$

$$= 1.29\ \mu g\ F^-/V_s$$

where:

24.45 = molar volume at 25°C and 760 mm Hg

MW = 19, weight of fluoride ion,
(i.e., 19 μg F^- = 24.45 μl at 25°C, 760 mm Hg)

10.6 To calculate the concentration of hydrogen fluoride as mg HF/m^3, multiply concentration of F^- (from 10.4) by 1.05. The concentration of hydrogen fluoride as μl/l or ppm is identical to the F^- concentration as μl F^-/l (from Sec. 10.5).

11. References

11.1 Elfers, L. A. and C. E. Decker, "Determination of Fluoride in Air and Stack Gas Samples by Use of an Ion Specific Electrode", Anal Chem 40(11):1658 (1968).

11.2 Harwood, J. E., "The Use of an Ion Selective Electrode for Routine Fluoride Analyses on Water Samples", in Water Research, Vol. 3, pp. 273-280, Pergamon Press Journal, 1969.

ACROLEIN IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Acrolein	**Method No.:**	P&CAM 118
Matrix:	Air	**Range:**	1 – 30 μg/10 mℓ
Procedure:	Complexation, Colorimetric	**Precision:**	Unknown
Date Issued:	4/6/72		
Date Revised:	1/10/74	**Classification:**	C (Tentative)

1. **Principle of Method**

Acrolein reacts with 4-hexylresorcinol in an ethyl alcohol-trichloroacetic acid solvent medium in the presence of mercuric chloride and forms a blue colored product. The strong absorption maximum at 605 nm is used as a quantitative measure of acrolein.

2. **Range and Sensitivity**

2.1 The linear range of the absorbance at 605 nm is at least 1-30 μg of acrolein in the 10 mℓ portions of mixed reagent.

2.2 A concentration of 0.01 ppm of acrolein can be determined in a 50 liter air sample based on a difference of 0.05 absorbance unit from the blank using a 1 cm cell. Greater sensitivity could be obtained by use of a longer path length cell.

3. **Interferences**

There is no interference from ordinary quantities of sulfur dioxide, nitrogen dioxide, ozone and most organic air pollutants. A slight interference occurs from dienes: 1.5 percent for 1,3-butadiene and 2 percent for 1,3-pentadiene. The red color produced by some other aldehydes and undetermined materials does not interfere in the spectrophotometric measurement.

4. **Precision and Accuracy**

The available information is insufficient to determine the precision and accuracy of this method.

5. Advantages and Disadvantages of the Method

5.1 The major advantage of this method is the sensitivity. A concentration of 0.01 ppm of acrolein can be determined in a 50 liter air sample.

5.2 Both the solid trichloroacetic acid and the solution are corrosive to the skin. Mercuric chloride is highly toxic. This reagent and the former should be handled carefully. This method is not attractive as a field technique since the absorbing solution tends to evaporate upon collection of large volumes of air and the colored complex begins to fade after 2 hours upon completion of sampling.

6. Apparatus

6.1 **Sampling Equipment.** The sampling unit for the impinger collection method consists of the following components:

6.1.1 A train of two glass standard midget impingers with fritted glass inlets containing the absorbing solution or reagent (Section 7.5). The fritted end should have a porosity approximately equal to that of Corning EC (170-220 μ maximum pore diameter).

6.1.2 A pump suitable for delivering desired flow rates; at least 2 ℓ per minute for 60 minutes. The sampling pump is protected from splashover or water condensation by an adsorption tube loosely packed with a plug of glass wool and inserted between the exit arm of the impinger and the pump.

6.1.3 An integrating volume meter such as a dry gas or wet test meter.

6.1.4 Thermometer.

6.1.5 Manometer.

6.1.6 Stopwatch.

6.2 **Water Bath.** Any bath capable of maintaining a temperature of 58-60°C is acceptable.

6.3 **Spectrophotometer.** This instrument should be capable of measuring the developed color at 605 nm. The absorption band is rather narrow and thus a lower absorptivity may be expected in a broad-band instrument.

6.4 Matched glass cells or cuvettes, 1 cm path length.

6.5 Assorted laboratory glassware – pipettes, volumetric flasks, graduated cylinders of appropriate capacities.

7. Reagents

All reagents must be analytical reagent grade. An analytical grade of distilled water must be used.

7.1 Ethanol (96%).

7.2 **Trichloroacetic Acid Solution, Saturated.** Dissolve 100 g of the acid (reagent grade) in 10 mℓ of water by heating on a water bath. The resulting solution has a volume of approximately 70 mℓ. Even reagent grade trichloroacetic acid has an impurity which affects product intensities. Every new batch of solution should be standardized with acrolein. It is convenient to prepare a large quantity of solution from a single batch of trichloroacetic acid to maintain a uniformity of response.

7.3 **Mercuric Chloride Solution (3%).** Dissolve 3 g of mercuric chloride in 100 mℓ of ethanol.

7.4 **4-Hexylresorcinol Solution.** Dissolve 5 g of 4-hexylresorcinol (MP 68°-70°C) in 5.5 mℓ of ethanol. This makes about 10 mℓ of solution.

7.5 **Mixed Absorbing Reagent.** Mix, in the specified order, the reagents in the following proportions: 5 mℓ ethanol, 0.1 mℓ 4-hexylresorcinol solution, 0.2 mℓ mercuric chloride solution, and 5 mℓ saturated trichloroacetic acid solution. The mixed reagent may be stored for a day at room temperature. Prepare the needed quantity by selecting an appropriate multiple of these amounts. Protect from direct sunlight.

7.6 **Acrolein, Purified.** Freshly prepare a small quantity (less than 1 mℓ is sufficient) by distilling 10 mℓ of the purest grade of acrolein commercially available. Reject the first 2 mℓ of distillate. (The acrolein should be stored in a refrigerator to retard polymerization.) The distillation should be done in a hood because the vapors are irritating to the eyes.

7.7 **Acrolein, Standard Solution "A" (1 mg/mℓ).** Weigh 0.1000 g (approximately 0.12 mℓ) of freshly prepared, purified acrolein into a 100 mℓ volumetric flask and dilute to volume with ethanol. This solution may be kept for as long as a month if properly refrigerated.

7.8 **Acrolein, Standard Solution "B" (10 μg/mℓ).** Dilute 1 mℓ of standard solution "A" to 100 mℓ with ethanol. This solution may be kept for as long as a month if properly refrigerated.

8. Procedure

8.1 **Cleaning of Equipment.** No specialized cleaning of glassware is required, however, since known interferences occur with dienes, cleaning techniques should insure the absence of all organic materials.

8.2 **Collection and Shipping of Samples**

8.2.1 Draw through the assembled sampling unit measured volumes of the vapor laden air at a rate of either 1 ℓ/minute for no more than 60 minutes or 2 ℓ/minute for no more than 30 minutes through 2 bubblers in series, each containing 10 mℓ of mixed absorbing reagent. An extra bubbler containing water may be added as a trap to protect the pump. A maximum of 60 ℓ of air can be sampled before possible reagent decomposition may occur. Care should be taken to measure flow rate, time and/or volume as accurately as possible. Note also the atmospheric pressure and temperature.

8.2.2 This sampling system collects 70-80 percent of the acrolein in the first bubbler and 95 percent of the acrolein in the first 2 bubblers, using absorbers with EC fritted glass inlets. The absorption efficiency might be increased by use of a C porosity frit (60μ maximum pore diameter).

8.2.3 Because of the two hour time limit for color development, it is probably best to analyze the samples soon after completion of sampling. For this reason shipping of the sample involving time periods of as much as two hours should not be practiced with this method.

8.3 **Analysis of Sample**

8.3.1 If evaporation has occurred during sampling the absorbing solution is diluted to its original 10 mℓ volume with ethanol.

8.3.2 Transfer the samples from each bubbler and backup bubbler to separate glass stoppered test tubes.

8.3.3 Immerse the tubes in a 60°C water bath for 15 minutes to develop the colors. A test tube containing only 10 mℓ of mixed absorbing reagent must be run similarly and simultaneously. This serves as the reagent blank.

8.3.4 Cool the test tubes in running water immediately upon removal from the water bath.

8.3.5 After 15 minutes read the absorbances at 605 nm in a suitable spectrophotometer using 1 cm cells. There is no appreciable loss in accuracy if the samples are allowed to stand up to 2 hours before reading the absorbances. For very low acrolein concentrations it may be convenient to use a longer path length cell.

9. Calibration and Standards

9.1 Preparation of standard curve.

9.1.1 Pipette 0, 0.5, 1.0, 2.0, and 3.0 mℓ of standard solution "B" into glass stoppered test tubes.

9.1.2 Dilute each standard to exactly 5 mℓ with ethanol.

9.1.3 Add in order, to each tube, exactly 0.1 mℓ of 4-hexylresorcinol solution, 0.2 mℓ of mercuric chloride solution, and 5 mℓ of trichloroacetic acid solution.

9.1.4 Mix, develop and read the colors as described in the analytical procedure (Sections 8.3.3 to 8.3.5).

9.2 Construct a calibration curve by plotting absorbance against micrograms of acrolein in the color developed solution.

10. Calculations

10.1 Subtract blank values, if any, from each sample.

10.2 Determine from the calibration curve the concentration of the acrolein present in each of the two bubblers, and add the values to get the total μg of acrolein in the air sampled.

$$\mu g \text{ acrolein} = \mu g_1 + \mu g_2$$

where:

μg_1 = microgram concentration of acrolein in the first bubbler.

μg_2 = microgram concentration of acrolein in the back up bubbler.

10.3 The concentration of acrolein in the sampled atmosphere can be calculated in ppm, defined as $\mu\ell$ acrolein per liter of air.

$$\text{ppm} = \frac{\mu\text{g acrolein}}{V_s} \times \frac{24.45}{MW}$$

where:

μg acrolein	=	total μg concentration as determined in 10.1. (Probably 95% of the actual concentration as stipulated in Section 8.2.2. A correction for efficiency may be used accordingly if deemed necessary.)
V_s	=	volume of air sampled in ℓ at 25°C and 760 mm Hg.
24.45	=	molar volume of an ideal gas at 25°C and 760 mm Hg.
MW	=	molecular weight of acrolein, 56.06.

11. References

11.1 Cohen, I.R., and Altshuller, A.P., "A New Spectrophotometric Method for the Determination of Acrolein in Combustion Gases and the Atmosphere." Anal. Chem. 33, 726 (1961).

11.2 Altshuller, A.P., and McPherson, S.P. "Spectrophotometric Analysis of Aldehydes in the Los Angeles Atmosphere," J. Air Poll. Control Assoc. 13, 109 (1963).

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BERYLLIUM IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Beryllium	**Method No.:**	P&CAM 121
Matrix:	Air, Settled Dust, Ore & Swipe Samples	**Range:**	0.03-8.0 μg/mℓ
Procedure:	Atomic Absorption	**Precision:**	Unknown
Date Issued:	4/7/72		
Date Revised:	January 10, 1974	**Classification:**	D (Operational)

1. **Principle of the Method**

   1.1 The samples are ashed with nitric acid to destroy the organic matrix. The ash is treated with 1:1 hydrochloric acid and dissolved in a weak hydrochloric acid solution maintaining a pH between 1 and 2.

   1.2 The liquid sample is aspirated into the nitrous oxide-acetylene flame of the atomic absorption spectrophotometer and the absorbance of the 2348.6 Å line of beryllium is measured.

2. **Range and Sensitivity**

   2.1 For aqueous solutions, the working range for beryllium is linear up to concentrations of approximately 4 μg/mℓ, but is useful up to concentrations of 8 μg/mℓ.

   2.2 The sensitivity under the standard operating conditions is about 0.03 μg beryllium/mℓ. This sensitivity can be increased with chemical concentration by solvent extraction.

3. **Interferences**

   High concentrations of aluminum (>500 μg/mℓ), silicon and magnesium depress the sensitivity of the beryllium determination. The interference can be controlled by adding oxine (8-hydroxyquinoline) to the sample and standards.

4. **Precision and Accuracy**

The precision and accuracy of this method have not been completely determined at this time. However, most standard atomic absorption procedures will provide a coefficient of variation of about 0.5 to 2% depending on the instrument used and the absorbance of the samples.

5. **Advantages and Disadvantages of the Method**

5.1 This method is rapid and does not require a high degree of technical skill.

5.2 The method is virtually free from spectral interferences, and has very good precision and accuracy. It is almost as sensitive as the emission spectrographic method, and far more precise.

5.3 The method is limited in that samples must be in enough volume of solution for a suitable aspiration time. This is rarely a problem when analyzing air samples, since the samples are usually collected for long periods of time.

6. **Apparatus**

6.1 Atomic Absorption Spectrophotometer. The instrument must be equipped with a nitrous oxide burner head.

6.2 Be Hollow Cathode Lamp of high spectral purity and adequate sensitivity.

6.3 Acetylene Gas and Regulator.

6.4 Nitrous Oxide and Regulator. Heating tape, with the temperature controlled by a rheostat, can be wound around the second stage regulator and connecting hose to prevent freeze-up of the line during operation.

6.5 125 mℓ Phillips borosilicate glass beakers with watchglasses.

6.6 15 mℓ graduated borosilicate glass centrifuge tubes.

7. **Reagents**

All reagents must be analytical reagent grade or better. All solutions are prepared with deionized water.

7.1 Redistilled concentrated nitric acid (16N).

7.2 Redistilled hydrochloric acid (6N).

7.3 **Stock Beryllium Solution.** A solution containing 1000 μg of beryllium per millileter is prepared by dissolving 1.000 g of beryllium metal in a minimum volume of 1:1 HCl. Dilute to 1 liter with 1% (V/V) HCl. (This stock solution can also be purchased from reputable laboratory supply firms.)

7.4 **Beryllium Standard Solutions.** These solutions are prepared by making appropriate dilutions from the stock beryllium solution, and acidifying with 0.5 mℓ of 1:1 redistilled HCl for each 10 mℓ of solution. These standard solutions are stable for at least several months.

8. **Procedure**

8.1 **Cleaning of Equipment.** Borosilicate glassware is recommended for this method. Glassware is soaked in a mild detergent solution immediately after use to remove any residual grease or chemicals and thus prevent the formation of an adsorptive surface. Before use, each piece is cleaned with a saturated solution of sodium dichromate in concentrated sulfuric acid and then rinsed thoroughly with warm tap water, concentrated nitric acid, tap water and deionized water.

8.2 **Collection and Handling of Samples**

8.2.1 **Air Samples** can be collected by:

1. A 0.45 μ cellulose membrane filter.
2. An electrostatic precipitator.
3. A chemically clean impinger containing a 10% HNO_3 solution.

The Hi-Vol sampler, which samples at a rate of about 1.5 m^3/min when operated 24 hours, will provide an adequate sample for the determination of beryllium in ambient air in concentrations as low as 0.002$\mu g/m^3$. Filters should not be overloaded with the sample to the point where the sample is falling off the filter. Each filter sample should be sealed in its own container for shipment. Plastic petri dishes are suitable for small membrane filters and cardboard file folders and large envelopes are suitable for hi-vol filters.

8.2.2 **Settled Dust and Ore Samples** can be collected directly in chemically clean jars.

8.2.3 **Swipe Samples** should be collected on Whatman filter paper by thoroughly wiping a *measured* area and placing the filter in a chemically clean jar.

8.3 Analysis of Samples

8.3.1 Ashing of Samples

a. **Air Samples.** The entire filter or impinger sample is transferred to a 125-mℓ Phillips beaker and ashed with nitric acid. If a fiberglass filter was used for sampling, it is necessary to first destroy the filter with hydrofluoric acid in a platinum or teflon dish prior to ashing with nitric acid in the Phillips beaker. When ashing is completed, as indicated by a white residue, convert the residue to the chloride form by three *successive* evaporations, just short of dryness, with 1:1 HCl. The residue is then dissolved in 0.1 mℓ of 1:1 HCl and deionized water and transferred with triplicate washings to a graduated centrifuge tube. The sample is evaporated to a volume of 2.0 mℓ for analysis. If a dilution is needed the solution is diluted with 0.3 N HCl to the appropriate volume for analysis.

b. **Ores and Settled Dusts.** Ore and settled dust samples are ground in a clean mill to pass a U.S. Standard 200-mesh sieve. The ground materials are shaken thoroughly in a mixer mill and a 0.2000g representative sample is weighed out. Dust samples are ashed with nitric acid and if insoluble beryllium minerals are suspected, a potassium fluoride-sodium pyrosulfate fusion is also performed. Ore samples are fused without prior nitric acid ashing. The resulting hydrochloric acid solution of the fused sample is diluted or concentrated to the volume required to place the anticipated beryllium concentration in the working range of the method, maintaining a final pH between 1 and 2.

8.3.2 Determination of Beryllium

The HCl solution of the ashed or fused sample is aspirated directly into the nitrous oxide – acetylene flame. A special nitrous oxide-acetylene burner head must be used. The 2349 Å resonance line of beryllium is used. The operating parameters vary according to the make of instrument being employed. Consult the instrument manufacturer's instructions for the particular instrument settings and procedure for lighting the nitrous oxide-acetylene flame. A reducing, fuel rich flame, is needed for the desired sensitivity in the beryllium analysis.

If the beryllium concentration falls between 0.2 and 8.0 μg/mℓ, the percent of absorption values of the samples are recorded on the 1X scale, or the absorbance or concentration values are read directly if these features are available on the instrument being employed. If the beryllium concentration is between 0 and 0.2 μg/mℓ, scale expansion can be used to

improve the readability of small signals and thereby improve the detection limit. However, longer aspiration time is needed when using scale expansion since higher noise suppression is required.

9. Calibration and Standards

9.1 Beryllium standards are prepared by appropriate dilutions of the stock solution to cover the range from 0 – 8 μg Be/mℓ. Each standard solution contains 0.5 mℓ of 1:1 redistilled HCl per every 10 mℓ of solution. Each standard solution is aspirated into the nitrous oxide-acetylene flame and either the % absorption is recorded or the absorbance or concentration are read directly. If the instrument records in % absorption, the readings of the standards and samples must be converted to absorbance.

9.2 The absorbance values are plotted against concentration to get the standard working curve. For low concentrations of beryllium, standards and samples can be run using scale expansion to improve the detection limit of the method. When scale expansion is used, it is not necessary to convert to absorbance before plotting.

10. Calculations

The concentration of Be in ambient air can be expressed as μg Be per cubic meter.

$$\mu g\ Be/m^3 = \frac{\mu g\ Be/m\ell}{V_s} \times m\ell\ soln$$

where:

μg Be/mℓ = the concentration of Be in the sample solution analyzed derived from the calibration curve (Section 9.2).

mℓ soln = final volume of sample solution analyzed including any dilution factor.

V_s = volume of air sampled in m^3 at 25°C and 760 mm Hg, (1000 ℓ = 1 m^3).

11. References

11.1 *Analytical Methods for Atomic Absorption Spectrophotometry.* The Perkin-Elmer Corporation, Norwalk, Connecticut (1971).

11.2 Sill, C.W. "Decomposition of Refractory Silicates in Ultramicro Analysis." Anal. Chem. 33; 1684 (1961).

11.3 Keenan, R.G., and J.L. Holtz. "Spectrographic Determination of Beryllium in Air, Biological Materials, and Ores Using the Sustaining A.C. Arc." American Ind. Hyg. Assoc. J, 25; 254 (1964).

11.4 Elwell, W.T., and J.A.F. Gidley. *Atomic Absorption Spectrophotometry*, 2nd Edition Pergamon Press, New York, 1966.

SELENIUM IN URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Selenium	**Method No.:** P&CAM 124
Matrix: Urine	**Range:** 0.017 to 0.500 mg/ℓ in 300 mℓ urine
Procedure: Reduction, turbidimetry	
Date Issued: 6/20/72	**Precision (CV_T) :** 0.04 at 45 μg/300mℓ urine
Date Revised: 1/29/76	**Classification:** D (Operational)

1. Principle of the Method

The method is based on the measurement of the turbidity produced by a colloidial suspension of red elemental selenium. Selenium in urine is oxidized by hot sulfuric acid in the presence of mercuric oxide to form nonvolatile oxyacids of selenium. The addition of hydrobromic acid and bromine results in the formation of volatile bromides of selenium, which are distilled from the digestion mixture. Red elemental selenium is precipitated from the distillate with hydroxylamine hydrochloride.

2. Range and Sensitivity

2.1 The detection limit is about 5 μg of selenium per sample, or 0.017 mg/ℓ of selenium in 300 mℓ of urine.

2.2 The range extends from the detection limit to about 150 μg. For a sample size of 300 mℓ,the range corresponds to 0.017 to 0.500 mg/ℓ of selenium in urine.

3. Interferences

No interferences are reported for this method.

4. Precision and Accuracy

4.1 The precision is about 2μg in the range 2 to 150 μg of selenium. Therefore, for a 300-mℓ urine sample containing a typical value of 45 μg of selenium (0.15 μg/mℓ), the coefficient of variation of the overall method (CV_T) is about 0.04.

4.2 The accuracy is within the limits of the precision. The measured values fall within 2μg of the "true" values in the range 2 to 150 μg of selenium.

5. **Advantages and Disadvantages of the Method**

5.1 One advantage is that this method requires no complex laboratory equipment.

5.2 The steps in the procedure involving the digestion of the sample, distillation of the bromides of selenium, and precipitation of elemental selenium are time consuming.

5.3 Also, the large amount of glassware required for each sample makes it impractical to analyze more than a few samples simultaneously.

6. **Apparatus**

6.1 Polyethylene bottles (500 mℓ) with Teflon-lined caps for collection of samples.

6.2 Kjeldahl flasks (800 mℓ).

6.3 Spectrophotometer or colorimeter.

6.4 An all-glass still consisting of a 250- or 500-mℓ round-bottom flask connected with a ground-glass joint to a Liebig condenser.

6.5 Compressed sulfur dioxide.

6.6 Asbestos filter mat in a Gooch crucible for vacuum filtration.

6.7 Water bath capable of maintaining a temperature of approximately 80°C.

6.8 Urinometer (or hydrometer capable of measuring in the range 1.000 to 1.040 ± 0.001 units).

6.9 Assorted laboratory glassware -- pipettes, volumetric flasks, and graduated cylinders of appropriate capacities.

7. **Reagents**

All reagents must be analytical reagent grade. Doubly distilled water should be used in preparing solutions and in carrying out the analytical procedure.

7.1 Thymol.

7.2 Sulfuric acid, concentrated.

7.3 Mercuric oxide.

7.4 **Hydrobromic Acid-Bromine (HBr-Br_2) Solution.**
Hydrobromic acid, concentrated (48%) containing 1% of liquid bromine by volume.

7.5 Sodium bisulfite or a cylinder of compressed sulfur dioxide.

7.6 Hydroxylamine hydrochloride and hydroxylamine hydrochloride solution (10% w/v in water).

7.7 Gum arabic solution (5% w/v in water).

7.8 Selenium metal (C.P.).

7.9 **Selenium, Standard Solution "A" (100 μg/ml).**

7.9.1 Prepare dry selenium dioxide by dissolving selenium metal in concentrated nitric acid, evaporating the solution to dryness, and twice subliming the solid selenium dioxide. Dissolve 0.1405 g of the dry selenium dioxide in water containing 5 mℓ of 0.5 *N* sodium hydroxide and dilute to 1 liter in a volumetric flask. (See Reference 11.2.)

7.9.2 An alternate and less difficult procedure is that described in Reference 11.3. Cautiously dissolve 0.1000 g of selenium metal in a few drops of concentrated nitric acid. Boil gently to expel brown fumes and dilute to 1 liter with water in a volumetric flask.

7.10 **Selenium, Standard Solution "B" (20 μg/ml).** Dilute 20 mℓ of selenium standard solution "A" to 100 mℓ with water in a volumetric flask.

8. Procedure

8.1 **Cleaning of Equipment.** All glassware should be thoroughly cleaned with acid prior to its usage. The following procedure provides sufficient cleaning.

8.1.1 Wash in detergent and tap water and follow with tap water and distilled water rinses.

8.1.2 Soak in a chromic acid solution in concentrated sulfuric acid at least 30 min and rinse with tap water and distilled water.

8.1.3 Soak in concentrated or 1:1 nitric acid for 30 min. and rinse with doubly distilled water. This entire procedure is necessary only for initial cleaning. For subsequent reuse of the glassware, it is necessary only to clean with nitric acid and rinse with distilled water.

Note: Polyethylene equipment can be effectively cleaned by subjecting it to steps 8.1.1 and 8.1.3. Chromic acid solution in concentrated sulfuric acid (as in step 8.1.2) cannot be used on polyethylene.

8.2 Collection and Shipping of Samples. Collect urine samples in polyethylene bottles. At least 300 mℓ should be collected. To preserve a sample, add 5 mg of thymol per 100 mℓ of urine and cool the sample to 0°C during shipment and storage. Urine treated with thymol and refrigerated is stable for 1 week.

8.3 Analysis of Samples

8.3.1 Determine the specific gravities of the urine samples after they have been allowed to warm to room temperature. This may be done with a urinometer.

8.3.2 To 300 mℓ of urine in an 800-mℓ Kjeldahl flask add 40 mℓ of concentrated sulfuric acid, 1 g of mercuric oxide, and a few glass beads. Boil over a gas flame until colorless. This usually requires about 4 hr.

8.3.3 Transfer the sample and beads to the round-bottom distillation flask; rinse the Kjeldahl flask with 60 mℓ of water containing 25 mℓ of concentrated sulfuric acid and add the rinse to the sample.

8.3.4 Allow the solution to cool and add 50 mℓ of the $HBr\text{-}Br_2$ solution.

8.3.5 Attach the Liebig condenser with a groundglass joint to the distillation flask. Adapt the delivery end of the condenser to glass tubing immersed in 3 mℓ of concentrated hydrogen bromide in an Erlenmeyer flask. Distill the solution until the temperature of the vapor reaches 130°C. (See Reference 11.4, p. 421.)

8.3.6 Bubble sulfur dioxide through the distillate until the bromine color just disappears.

8.3.7 Add about 0.5 g of hydroxylamine hydrochloride to the distillate and heat the resulting solution to 80°C on a water bath. Maintain the distillate at this temperature for about 15 min. If selenium is present, it will cause a pink turbidity.

8.3.8 Allow the precipitate to settle overnight.

8.3.9 Filter off the precipitate by suction through an asbestos filter mat in a Gooch crucible and wash it with water.

8.3.10 Dissolve the collected selenium by passing 2 mℓ of $HBr\text{-}Br_2$ solution dropwise through the filter. Rinse the filter twice with 2-mℓ portions of distilled water.

8.3.11 Transfer the solution quantitatively to a 10-mℓ volumetric flask and add 0.5 mℓ of gum arabic solution.

8.3.12 Bubble sulfur dioxide through the solution to discharge the color, add 0.5mℓ of hydroxylamine hydrochloride solution, and mix; heat in an 80°C water bath for 15 min, cool, and dilute to 10 mℓ in the volumetric flask.

8.3.13 Transfer to a suitable cuvette and measure the absorbance at 530 nm with a spectrophotometer or colorimeter. A reagent blank should be carried through the entire procedure. The absorbance of this blank should be subtracted from the absorbances of the samples.

9. Calibration and Standards

Add appropriate amounts of standard selenium solution "B" (0.5, 1.0, 2.0, 3.0, and 4.0 mℓ) to 2 mℓ of the HBr-Br_2 solution in a 10-mℓ volumetric flask. Continue as above from steps 8.3.11 through 8.3.13. A blank consisting of 2 mℓ of the HBr-Br_2 solution only should also be carried through the same procedure. The absorbance of the blank is subtracted from the absorbances of the standard solutions. A calibration curve of absorbance *versus* amount (in μg) of selenium is then constructed.

10. Calculations

The concentration of selenium in the urine sample can be expressed as mg of selenium per liter of urine:

$$\text{mg Se}/\ell = \frac{\mu\text{g Se (from calibration curve)}}{\text{m}\ell \text{ of urine}}$$

10.1 The use of a specific gravity correction factor to normalize values to 1.024, the average specific gravity of urine, has been recommended. (See Reference 11.5.)

10.2 The preferred method of normalization is to make a straightforward gravity correction as follows:

$$\text{corrected mg Se}/\ell = \text{mg Se}/\ell \times \frac{0.024}{(\text{specific gravity} - 1.000)}$$

11. References

11.1 Elkins, H.B., *"The Chemistry of Industrial Toxicology,"* Wiley, New York, N.Y., 1950, p. 351.

11.2 Wernimont, G., and F.J. Hopkinson, Ind. Eng. Chem., Anal. Ed., 12:308 (1940).

11.3 The Intersociety Committee, *"Methods of Air Sampling and Analysis."* American Public Health Association, Washington, D.C., 1972, pp. 316-321.

11.4 Horowitz, W., Ed., *"Official Methods of Analysis of the Association of Official Analytical Chemists,"* A.O.A.C., Washington, D.C., 1970, pp. 46-47, 420-422.

11.5 Elkins, H.B., "Concentration Adjustments in Urinalysis," Amer. Ind. Hyg. Ass. J., 35, 559 (1974).

FORMALDEHYDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Formaldehyde	**Method No:**	P&CAM 125
Matrix:	Air	**Range:**	0.1 ppm – 2.0 ppm
Procedure:	Spectrophotometric	**Precision:**	±5%
Date Issued:	9/24/73	**Classification:**	C (Tentative)
Date Revised:	1/15/74		

1. **Principle of the Method**

   1.1 Formaldehyde reacts with chromotropic acid-sulfuric acid solution to form a purple monocationic chromogen. The absorbance of the colored solution is read in a spectrophotometer at 580 nm and is proportional to the quantity of formaldehyde in the solution (References 11.2, 11.6).

   1.2 The chemistry of this color reaction is not known with certainty (Reference 11.3).

2. **Range and Sensitivity**

   2.1 From 0.1 μg/mℓ to 2.0 μg/mℓ of formaldehyde can be measured in the color developed solution.

   2.2 A concentration of 0.1 ppm of formaldehyde can be determined in a 25 liter air sample based on an aliquot of 4 mℓ from 20 mℓ of absorbing solution and a difference of 0.05 absorbance unit from the blank.

3. **Interferences**

   3.1 The chromotropic acid procedure has very little interference from other aldehydes. Saturated aldehydes give less than 0.01 percent positive interference, and the unsaturated aldehyde acrolein results in a few percent positive interference. Ethanol and higher molecular weight alcohols and olefins in mixtures with formaldehyde are negative interferences. However, concentrations of alcohols in

air are usually much lower than formaldehyde concentrations and, therefore, are not a serious interference.

3.2 Phenols result in a 10 to 20 percent negative interference when present at an 8:1 excess over formaldehyde. They are, however, ordinarily present in the atmosphere at lesser concentrations than formaldehyde and, therefore, are not a serious interference.

3.3 Ethylene and propylene in a 10:1 excess over formaldehyde result in a 5 to 10 percent negative interference and 2-methyl-1,3-butadiene in a 15:1 excess over formaldehyde showed a 15 percent negative interference. Aromatic hydrocarbons also constitute a negative interference (Reference 11.6). It has recently been found that cyclohexanone causes a bleaching of the final color (Reference 11.4).

4. Precision and Accuracy

The method was checked for reproducibility by having three different analysts in three different laboratories analyze standard formaldehyde samples. The results listed in Table 1 agreed within ±5 percent.

TABLE 1

COMPARISON OF FORMALDEHYDE RESULTS FROM THREE LABORATORIES

Micrograms Formaldehyde	Absorbance		
	Lab. 1	Lab. 2	Lab. 3
1	0.057	0.063	0.061
3	0.183	0.175	0.189
5	0.269	0.279	0.262
7	0.398	0.381	0.392
10	0.566	0.547	0.537
20	1.02	0.980	1.07

5. Advantages and Disadvantages

5.1 Effect of Storage – Disadvantage

5.1.1 The absorbance of the reaction product increases slowly on standing. An increase of 3 percent in absorbance was noted after one day standing and an increase of 10 percent after eight days standing (11.6).

5.1.2 No information is available on the effect of storage on the collected air sample.

5.2 **Precision – Advantage.** Results checked for reproducibility agreed within ±5 percent (see Table 1).

6. **Apparatus**

6.1 **Sampling Equipment.** The sampling unit for the impinger collection method consists of the following components:

6.1.1 A graduated midget impinger containing the absorbing solution or reagent.

6.1.2 A pump suitable for delivering flow rates of 1 liter per minute for 24 hours. The sampling pump is protected from splashover or water condensation by an adsorption tube loosely packed with a plug of glass wool and inserted between the exit arm of the impinger and the pump.

6.1.3 An integrating volume meter such as a dry gas or wet test meter.

6.1.4 Thermometer.

6.1.5 Manometer.

6.1.6 Stopwatch.

6.2 **Spectrophotometer or Colorimeter.** An instrument capable of measuring the absorbance of the color developed solution at 580 nm.

6.3 Associated laboratory glassware.

7. **Reagents**

7.1 **Chromotropic Acid Reagent.** Dissolve 0.10 g of 4,5-dihydroxy-2,7-naphthalenedisulfonic acid disodium salt (Eastman Kodak Company, Rochester, New York, Cat. No. P230) in water and dilute to 10 mℓ. Filter if necessary and store in a brown bottle. Make up fresh weekly.

7.2 Concentrated sulfuric acid.

7.3 **Formaldehyde Standard Solution "A"** (1 mg/mℓ). Dilute 2.7 mℓ of 37 percent formalin solution to 1 liter with distilled water. This solution must be standardized as described in Section 9.1. The solution is stable for at least a 3-month period. Alternatively sodium formaldehyde bisulfite (Eastman Kodak Company, Cat. No. P6450) can be used as a primary standard (Reference 11.4). Dissolve 4.4703 g in distilled water and dilute to 1 liter.

7.4 **Formaldehyde Standard Solution "B"** (10 μg/mℓ). Dilute 1 mℓ of standard solution "A" to 100 mℓ with distilled water. Make up fresh daily.

7.5 **Iodine, 0.1 N** (approximate). Dissolve 25 g of potassium iodide in about 25 mℓ of water, add 12.7 g of iodine and dilute to 1 liter.

7.6 **Iodine, 0.01 N.** Dilute 100 mℓ of the 0.1 N iodine solution to 1 liter. Standardize against sodium thiosulfate.

7.7 **Starch Solution, 1 Percent.** Make a paste of 1 g of soluble starch and 2 mℓ of water and slowly add the paste to 100 mℓ of boiling water. Cool, add several mℓ of chloroform as a preservative, and store in a stoppered bottle. Discard when a mold growth is noticeable.

7.8 **Sodium Carbonate Buffer Solution.** Dissolve 80 g of anhydrous sodium carbonate in about 500 mℓ of water. Slowly add 20 mℓ of glacial acetic acid and dilute to 1 liter.

7.9 **Sodium Bisulfite, 1 Percent.** Dissolve 1 g of sodium bisulfite in 100 mℓ of water. It is best to prepare a fresh solution weekly.

8. Procedure

8.1 **Cleaning of Equipment.** Care must be exercised to ensure the absence of probable contaminants like organic materials that can be charred by concentrated sulfuric acid. Soaking glassware for one hour in a 1:1 mixture of nitric and sulfuric acids, followed by thorough rinsing with double-deionized water will remove all possible organic contaminants.

8.2 **Collection and Shipping of Samples**

8.2.1 Pour 20 mℓ of the absorbing solution (distilled water) into each graduated midget impinger.

8.2.2 Connect two impingers in series to the vacuum pump (via the absorption tube) and the prefilter assembly (if needed) with short pieces of flexible tubing. The minimum amount of tubing necessary to make the joint between the prefilter and impingers should be used. The air being sampled should not be passed through any other tubing or other equipment before entering the impingers.

8.2.3 It has been recommended that two impingers must be used in series because under conditions of sampling, the collection efficiency of only one impinger is approximately 80 percent. With two impingers in series the total collection efficiency is approximately 95 percent. The contents of each impinger should be analyzed separately.

8.2.4 Turn on pump to begin sample collection. Care should be taken to measure the flow rate, time and/or volume as accurately as possible. The sample should be taken at a flow rate of 1 ℓpm for one hour. (References 11.1, 11.5). These conditions give a total of 60 liters of air that is drawn through the system. However, a shorter sampling time can be used providing enough formaldehyde is collected to be above the lower limit of sensitivity of the method.

8.2.5 After sampling, the impinger stem can be removed and cleaned. Tap the stem gently against the inside wall of the impinger bottle to recover as much of the sampling solution as possible. Wash the stem with a small amount (1-2 mℓ) of unused absorbing solution and add the wash to the impinger. Then the impinger is sealed with a hard, non-reactive stopper (preferably Teflon). Do not seal with rubber. The stoppers on the impingers should be tightly sealed to prevent leakage during shipping. If it is preferred to ship the impingers with the stems in, the outlets of the stem should be sealed with Parafilm or other non-rubber covers, and the ground glass joints should be sealed (i.e., taped) to secure the top tightly.

8.2.6 Care should be taken to minimize spillage or loss by evaporation at all times. Refrigerate samples if analysis cannot be done within a day.

8.2.7 Whenever possible, hand delivery of the samples is recommended. Otherwise, special impinger shipping cases designed by NIOSH should be used to ship the samples.

8.2.8 A "blank" impinger should be handled as the other samples (fill, seal and transport) except that no air is sampled through this impinger.

8.3 **Analysis**

8.3.1 Transfer the sample from each impinger to either a 25 mℓ- or 50 mℓ-graduate. Note the volume of each solution.

8.3.2 Pipet a 4 mℓ aliquot from each of the sampling solutions into glass stoppered test tubes. A blank containing 4 mℓ of distilled water must also be run. If the formaldehyde content of the aliquot exceeds the limit of the method, a smaller aliquot diluted to 4 mℓ with distilled water is used.

8.3.3 Add 0.1 mℓ of 1 percent chromotropic acid reagent to the solution and mix.

8.3.4 To the solution pipette slowly and cautiously 6 mℓ of concentrated sulfuric acid. The solution becomes extremely hot during the addition of the sulfuric acid. If the acid is not added slowly, some loss of sample could occur due to spattering.

8.3.5 Allow to cool to room temperature. Read at 580 nm in a suitable spectrophotometer using a 1 cm cell. No change in absorbance was noted over a 3 hour period after color development. Determine the formaldehyde content of the sampling solution from a curve previously prepared from standard formaldehyde solutions.

8.3.6 During the analysis procedure, it is good practice to group together the two impingers from each sampling series and label them as "A" and "B." The formaldehyde content calculated in "A" is added to that calculated in "B" to give the total amount in the sampled atmosphere by the impingers in series.

9. Calibration and Standards

9.1 Standardization of Formaldehyde Solution

9.1.1 Pipette 1 mℓ of formaldehyde standard solution "A" into an iodine flask. Into another flask pipette 1 mℓ of distilled water. This solution serves as the blank.

9.1.2 Add 10 mℓ of 1 percent sodium bisulfite and 1 mℓ of 1 percent starch solution.

9.1.3 Titrate with 0.1 N iodine to a dark blue color.

9.1.4 Destroy the excess iodine with 0.05 N sodium thiosulfate.

9.1.5 Add 0.01 N iodine until a faint blue end point is reached.

9.1.6 The excess inorganic bisulfite is now completely oxidized to sulfate, and the solution is ready for the assay of the formaldehyde bisulfite addition product.

9.1.7 Chill the flask in an ice bath and add 25 mℓ of chilled sodium carbonate buffer. Titrate the liberated sulfite with 0.01 N iodine, using a microburette, to a faint blue end point. The amount of iodine added in this step must be accurately measured and recorded.

9.1.8 One mℓ of 0.0100 N iodine is equivalent to 0.15 mg of formaldehyde. Therefore, since 1 mℓ of formaldehyde standard solution was titrated, the mℓ of 0.01 N iodine used in the final titration multiplied by the factor, 0.15, gives the formaldehyde concentration of the standard solution in mg/mℓ.

9.1.9 The factor, 0.15, must be adjusted or determined accordingly on the basis of the exact normality of the iodine solution.

9.2 **Preparation of Standard Curve**

9.2.1 Pipet 0, 0.1, 0.3, 0.5, 0.7, 1.0, and 2.0 mℓ of standard solution "B" into glass stoppered test tubes.

9.2.2 Dilute each standard to 4 mℓ with distilled water.

9.2.3 Develop the color as described in the analysis procedure (Section 8.3).

9.2.4 Plot absorbance against micrograms of formaldehyde in the color developed solution. Note that the microgram concentration of the formaldehyde is determined based on the standardization value of solution A.

10. **Calculations**

10.1 Convert the volume of air sampled (V) to the volume of air at standard conditions (V_s) of 760 mm of mercury and 25°C, using the correction formula:

$$V_s = V \times \frac{P}{760} \times \frac{298}{(T + 273)}$$

where:

V_s = volume of air in liters at standard conditions
V = volume of air sampled in liters
P = barometric pressure in mm of mercury
T = temperature of sample air, °C

10.2 Determine the total concentration (C_t) of formaldehyde present in the two sample impingers in series, A and B.

$$C_t = C_A \times F_A + C_B \times F_B$$

where:

C_t = total μg of formaldehyde in the sample.

C_A and C_B = respective formaldehyde concentration in μg of the sample aliquots taken from impingers A and B as determined from the calibration curve

F_A and F_B = respective aliquot factor; $\frac{\text{sampling soln. vol. in m}\ell}{\text{m}\ell \text{ aliquot used}}$

10.3 The concentration of formaldehyde in the sampled atmosphere may be calculated by using the following equation, assuming standard conditions are taken as 760 mm of mercury and 25°C:

$$\text{ppm (volume)} = \frac{C_t \times 24.47}{V_s \times \text{M.W.}}$$

where:

V_s = liters of air sampled at standard conditions

M.W. = molecular weight of formaldehyde (30.03)

24.47 = $\mu\ell$ of formaldehyde gas in one micromole at 760 mm Hg and 25°C.

11. References

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11.4 Feldstein, M. (Bay Area Air Pollution Control District) Personal Communication, March 1968.

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HYDROGEN SULFIDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Hydrogen Sulfide	Method No.:	P&CAM 126
Matrix:	Air	Range:	0.008 ppm – 50 ppm
Procedure:	Absorption – Methylene Blue – Spectrophotometric	Precision:	Unknown
		Classification:	C (Tentative)
Date Issued:	6/9/72		
Date Revised:	1/15/74		

1. **Principle of the Method**

1.1 Hydrogen sulfide is collected by aspirating a measured volume of air through an alkaline suspension of cadmium hydroxide (Reference 11.1). The sulfide is precipitated as cadmium sulfide to prevent air oxidation of the sulfide which occurs rapidly in an aqueous alkaline solution. STRactan 10® is added to the cadmium hydroxide slurry to minimize photo-decomposition of the precipitated cadmium sulfide (Reference 11.2). The collected sulfide is subsequently determined by spectrophotometric measurement of the methylene blue produced by the reaction of the sulfide with a strongly acid solution of N, N-dimethyl-p-phenylenediamine and ferric chloride (References 11.3, 11.4, 11.5). The analysis should be completed within 24-26 hours following collection of the sample.

1.2 Hydrogen sulfide may be present in the open atmosphere at concentrations of a few ppb or less. The reported odor detection threshold is in the 0.7-8.4 $\mu g/m^3$ (0.5-6.0 ppb) range (References 11.6, 11.7). Concentrations in excess of 140 $\mu g/m^3$ (100 ppb) are seldom encountered in the atmosphere.

1.3 Collection efficiency is variable below 10 $\mu g/m^3$ and is affected by the type of scrubber, the size of the gas bubbles and the contact time with the absorbing solution and the concentration of H_2S (References 11.8, 11.9, 11.10).

2. **Range and Sensitivity**

2.1 This method is intended to provide a measure of hydrogen sulfide in the range of 1.1-100 mg/m^3. For concentrations above 70 mg/m^3 the sampling period can be reduced or the liquid volume increased either before or after aspirating. (This

method is also useful for the mg/m^3 range of source emissions. For example, 100 mℓ cadmium $(OH)_2$- STRactan 10® media in Greenberg-Smith impingers and 5 minute sampling periods have been used successfully for source sampling.) The minimum detectable amount of sulfide is 0.008 μg/mℓ, which is equivalent to 0.2 μg/m^3 in an air sample of 1 m^3 and using a final liquid volume of 25 mℓ. When sampling air at the maximum recommended rate of 1.5 ℓ/minute for 2 hours, the minimum detectable sulfide concentration is 1.1 μg/m^3 at 760 mm Hg and 25°C.

2.2 Excellent results have been obtained by using this method for air samples having a hydrogen sulfide content in the range 5-50 ppm.

3. Interferences

3.1 The methylene blue reaction is highly specific for sulfide at the low concentrations usually encountered in ambient air. Strong reducing agents (e.g., SO_2) inhibit color development. Even sulfide solutions containing several micrograms sulfide/mℓ show this effect and must be diluted to eliminate color inhibition. If sulfur dioxide is absorbed to give a sulfite concentration in excess of 10 μg/mℓ, color formation is retarded. Up to 40 μg/mℓ,of this interference, however, can be overcome by adding 2-6 drops (0.5 mℓ/drop) of ferric chloride instead of a single drop for color development, and extending the reaction time to 50 minutes.

3.2 Nitrogen dioxide gives a pale yellow color with the sulfide reagents at 0.5 μg/mℓ or more. No interference is encountered when 0.3 ppm NO_2 is aspirated through a midget impinger containing a slurry of cadmium hydroxide-cadmium sulfide-STRactan 10®. If H_2S and NO_2 are simultaneously aspirated through cadmium hydroxide-STRactan 10® slurry, lower H_2S results are obtained, probably because of gas phase oxidation of the H_2S prior to precipitation as CdS (Reference 11.10).

3.3 Ozone at 57 ppb reduced the recovery of sulfide previously precipitated as CdS by 15 per cent (Reference 11.10).

3.4 Sulfides in solution are oxidized by oxygen from the atmosphere unless inhibitors such as cadmium and STRactan 10® are present.

3.5 Substitution of other cation precipitants for the cadmium in the absorbent (i.e., zinc, mercury, etc.) will shift or eliminate the absorbance maximum of the solution upon addition of the acid-amine reagent.

3.6 Cadmium sulfide decomposes significantly when exposed to light unless protected by the addition of 1 per cent STRactan® to the absorbing solution prior to sampling (Reference 11.2).

3.7 The choice of impinger used to trap H_2S with the $Cd(OH)_2$ slurry is very important when measuring concentration in the range 5-50 ppm. Impingers or bubblers having fritted-end gas delivery tubes are a problem source if the sulfide in solution is oxidized by oxygen from the atmosphere to free sulfur. The sulfur collects on the fritted-glass membrane and may significantly change the flow rate of the air sample through the system. One way of avoiding this problem is to use a midget impinger with standard, glass-tapered tips.

4. Precision and Accuracy

4.1 A relative standard deviation of 3.5 per cent and a recovery of 80 per cent has been established with hydrogen sulfide permeation tubes (Reference 11.2).

5. Advantages and Disadvantages of the Method

5.1 Effect of Light and Storage – Disadvantage

5.1.1 Hydrogen sulfide is readily volatilized from aqueous solution when the pH is below 7.0. Alkaline, aqueous sulfide solutions are very unstable because sulfide ion is rapidly oxidized by exposure to the air.

5.1.2 Cadmium sulfide is not appreciably oxidized even when aspirated with pure oxygen in the dark. However, exposure of an impinger containing cadmium sulfide to laboratory or to more intense light sources produces an immediate and variable photo-decomposition. Losses of 50-90 per cent of added sulfide have been routinely reported by a number of laboratories. Even though the addition of STRactan 10® to the absorbing solution controls the photo-decomposition (Reference 11.2), it is necessary to protect the impinger from light at all times. This is achieved by the use of low actinic glass impingers, paint on the exterior of the impingers, or an aluminum foil wrapping.

6. Apparatus

6.1 **Sampling Equipment.** The sampling unit for the impinger collection method consists of the following components:

6.1.1 A graduated 25 mℓ midget impinger with a standard glass-tapered gas delivery tube containing the absorbing solution or reagent.

6.1.2 A pump suitable for delivering desired flow rates with a minimum capacity of 2 ℓpm through the impinger. The sampling pump is protected from splashover or water condensation by an adsorption tube loosely packed with a plug of glass wool and inserted between the exit arm of the impinger and the pump.

6.1.3 An integrating volume meter such as a dry gas or wet test meter. The air meter must be capable of measuring the air flow within ±2 per cent. A wet or dry gas meter, with contacts on the 1-feet^3 or 10-l dial to record air volume, or a specially calibrated rotameter, is satisfactory. Instead of these, calibrated hypodermic needles may be used as critical orifices if the pump is capable of maintaining greater than 0.7 atmospheric pressure differential across the needle (Reference 11.11).

6.1.4 Thermometer.

6.1.5 Manometer.

6.1.6 Stopwatch.

6.2 Associated laboratory glassware.

6.3 Colorimeter with red filter or spectrophotometer at 670 nm..

6.4 Matched cells, 1 cm path length.

7. Reagents

All reagents must be ACS analytical reagent quality. Distilled water should conform to the ASTM Standards for Referee Reagent Water.

All reagents should be refrigerated when not in use.

7.1 **Amine-sulfuric Acid Stock Solution.** Add 50 mℓ concentrated sulfuric acid to 30 mℓ water and cool. Dissolve 12 g of N, N-dimethyl-p-phenylenediamine dihydrochloride (para-aminodimethylaniline) (redistilled if necessary) in the acid. Do not dilute. The stock solution may be stored indefinitely under refrigeration.

7.2 **Amine Test Solution.** Dilute 25 mℓ of the Stock Solution to 1 liter with 1:1 sulfuric acid.

7.3 **Ferric Chloride Solution.** Dissolve 100 g of ferric chloride, $FeCl_3 \cdot 6H_2O$, in water and dilute to 100 mℓ.

7.4 **Ammonium Phosphate Solution.** Dissolve 400 g of diammonium phosphate, $(NH_4)_2HPO_4$, in water and dilute to 1 liter.

7.5 **STRactan 10®**, (Arabinogalactan) Available from Chicago Scientific, Inc., 716 W. Irving Park Road, Bensenville, IL 60106.

7.6 **Absorbing Solution.** Dissolve 4.3 g of $3CdSO_4 . 8 H_2O$, and 0.3 g sodium hydroxide in separate portions of water. Mix and add 10 g STRactan 10® and dilute to 1 liter. Shake the resultant suspension vigorously before removing each aliquot. The STRactan®-cadmium hydroxide mixture should be freshly prepared. The solution is only stable for 3 to 5 days.

7.7 **H_2S Permeation Tube.** Prepare or purchase* a triple-walled or thick walled Teflon® permeation tube (References 11.10, 11.12, 11.13, 11.14, 11.15) which delivers hydrogen sulfide at a maximum rate of approximately 0.1 μg/minute at 25°C. This loss rate will produce a standard atmosphere containing 50 $\mu g/m^3$ (36 ppb H_2S when the tube is swept with a 2 ℓ/minute air flow. Tubes having H_2S permeation rates in the range of 0.004-0.33 μg/minute will produce standard air concentrations in the realistic range of 1-90 $\mu g/m^3$ H_2S with an air flow of 1.5 ℓ/min.

7.7.1 **Concentrated, Standard Sulfide Solution**

Transfer freshly boiled and cooled 0.1M NaOH to a liter volumetric flask. Flush with purified nitrogen to remove oxygen and adjust to volume. (Commercially available, compressed nitrogen contains trace quantities of oxygen in sufficient concentration to oxidize the small concentrations of sulfide contained in the standard and dilute standard sulfide standards. Trace quantities of oxygen should be removed by passing the stream of tank nitrogen through a Pyrex or quartz tube containing copper turnings heated to 400-450°C.) Immediately stopper the flask with a serum cap. Inject 300 mℓ of H_2S gas through the septum. Shake the flask. Withdraw measured volumes of standard solution with a 10 mℓ hypodermic syringe and fill the resulting void with an equal volume of nitrogen. Standardize with standard iodine and thiosulfate solution in an iodine flask under a nitrogen atmosphere to minimize air oxidation. The approximate concentration of the sulfide solution will be 440 μg sulfide/mℓ of solution. The exact concentration must be determined by iodine-thiosulfate standardization immediately prior to dilution.

For the most accurate results in the iodometric determination of sulfide in aqueous solution, the following general procedure is recommended:

1. Replace the oxygen from the flask by flushing with an inert gas such as carbon dioxide or nitrogen.

*Available from Metronics, Inc., 3201 Porter Drive, Palo Alto, California 94304, or PolyScience Corp., 909 Pitner Avenue, Evanston, Illinois 60202.

2. Add an excess of standard iodine, acidify, and back titrate with standard thiosulfate and starch indicator (Reference 11.16).

7.7.2 **Diluted Standard Sulfide Solution**

Dilute 10 mℓ of the concentrated sulfide solution to 1 liter with freshly boiled, distilled water. Protect the boiled water under a nitrogen atmosphere while cooling. Transfer the deoxygenated water to a flask previously purged with nitrogen and immediately stopper the flask. This sulfide solution is unstable. Therefore, prepare this solution immediately prior to use. This test solution will contain approximately 4 μg sulfide/mℓ.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware should be thoroughly cleaned; the following procedure is recommended:

8.1.1 Wash with a detergent and tap water solution followed by tap water and distilled water rinses.

8.1.2 Soak in 1:1 or concentrated nitric acid for 30 minutes and then follow with tap, distilled, and double distilled water rinses.

8.2 **Collection and Shipping of Samples**

8.2.1 Pipet 10 mℓ of the absorbing solution (Section 7.6) into the midget impinger. The addition of 5 mℓ of 95 per cent ethanol to the absorbing solution just prior to aspiration controls foaming for 2 hours (induced by the presence of STRactan 10®. In addition, 1 or 2 Teflon demister discs may be slipped up over the impinger air inlet tube to a height approximately 1-2" from the top of the tube.

8.2.2 Connect the impinger (via the absorption tube) to the vacuum pump with a short piece of flexible tubing. The air being sampled should not be passed through any tubing or other equipment before entering the impinger.

8.2.3 Turn on pump to begin sample collection. Care should be taken to measure the flow rate, time and/or volume as accurately as possible. The sample should be taken at a flow rate of 1.5 ℓpm.

8.2.4 After sampling, the impinger stem can be removed and cleaned. Tap the stem gently against the inside wall of the impinger bottle to recover as much of the sampling solution as possible. Wash the stem with a small amount (1-2 mℓ) of unused absorbing solution and add the wash to the impinger. Then the impinger is sealed with a hard, non-reactive stopper (preferably Teflon). Do not seal with rubber. The stoppers on the impingers should be tightly sealed to prevent leakage during shipping. If it is preferred to ship the impingers with the stems in, the outlets of the stem should be sealed with Parafilm or other non-rubber covers, and the ground glass joints should be sealed (i.e., taped) to secure the top tightly.

8.2.5 Care should be taken to minimize spillage or loss by evaporation at all times. Refrigerate samples if analysis cannot be done within a day.

8.2.6 Whenever possible, hand delivery of the samples is recommended. Otherwise, special impinger shipping cases designed by NIOSH should be used to ship the samples.

8.2.7 A "blank" impinger should be handled as the other samples (fill, seal and transport) except that no air is sampled through this impinger.

8.3 Analysis

8.3.1 Add 1.5 mℓ of the amine-test solution to the midget impinger through the air inlet tube and mix.

8.3.2 Add 1 drop of ferric chloride solution and mix. (*Note:* See Section 3.1 if SO_2 exceeds 10 μg/mℓ in the absorbing media.)

8.3.3 Transfer the solution to a 25 mℓ volumetric flask. Discharge the color due to the ferric ion by adding 1 drop ammonium phosphate solution. If the yellow color is not destroyed by 1 drop ammonium phosphate solution, continue dropwise addition until solution is decolorized. Make up to volume with distilled water and allow to stand for 30 minutes.

8.3.4 Prepare a zero reference solution in the same manner using a 10 mℓ volume of absorbing solution, through which no air has been aspirated.

8.3.5 Measure the absorbance of the color at 670 nm in a spectrophotometer or colorimeter set at 100 per cent transmission against the zero reference.

9. Calibration and Standards

9.1 Aqueous Sulfide

9.1.1 Place 10 mℓ of the absorbing solution in each of a series of 25 mℓ volumetric flasks and add the diluted standard sulfide solution, equivalent to 1, 2, 3, 4, and 5 μg of hydrogen sulfide to the different flasks.

9.1.2 Add 1.5 mℓ of amine-acid test solution to each flask and mix.

9.1.3 Add 1 drop of ferric chloride solution to each flask. Mix, make up to volume and allow to stand for 30 minutes.

9.1.4 Determine the absorbance in a spectrophotometer at 670 nm, against the sulfide-free reference solution.

9.1.5 Prepare a standard curve of absorbance vs. μg H_2S/mℓ.

9.2 **Gaseous Sulfide.** Commercially available permeation tubes containing liquefied hydrogen sulfide may be used to prepare calibration curves for use at the upper range of atmospheric concentration. Triple-walled tubes, drilled rod and micro bottles which deliver hydrogen sulfide within a minimum range of 0.1-1.2 μg/minute at 25°C have been prepared by Thomas (Reference 11.10); O'Keeffe (References 11.12, 11.13); Scaringelli (References 11.14, 11.15). Preferably the tubes should deliver hydrogen sulfide within a loss rate range of 0.003-0.28 μg/minute to provide realistic concentrations of H_2S (1.5-140 μg/m^3, 1.1-100 ppb) without having to resort to a dilution system to prepare the concentration range required for determining the collection efficiency of midget impingers. Analyses of these known concentrations give calibration curves which simulate all of the operational conditions performed during the sampling and chemical procedure. This calibration curve includes the important correction for collection efficiency at various concentrations of hydrogen sulfide.

9.2.1 Prepare or obtain a Teflon® permeation tube that emits hydrogen sulfide at a rate of 0.1-0.2 μg/minute (0.07-0.14 μℓ/minute at standard conditions of 25°C and 1 atmosphere). A permeation tube with an effective length of 2-3 cm and a wall thickness of 0.318 cm will yield the desired permeation rate if held at a constant temperature of 25°C ± 0.1°C. Permeation tubes containing hydrogen sulfide are calibrated under a stream of dry nitrogen to prevent the precipitation of sulfur in the walls of the tube.

9.2.2 To prepare standard concentrations of hydrogen sulfide, assemble the apparatus consisting of a water-cooled condenser, constant temperature bath maintained at 25°C ±0.1°C cylinders containing pure dry nitrogen

and pure dry air with appropriate pressure regulators, needle valves and flow meters for the nitrogen and dry air, diluent-streams. The diluent gases are brought to temperature by passage through a 2-meter-long copper coil immersed in the water bath. Insert a calibrated permeation tube into the central tube of the condenser, maintained at the selected constant temperature by circulating water from the constant-temperature bath, and pass a stream of nitrogen over the tube at a fixed rate of approximately 50 mℓ/minute. Dilute this gas stream to obtain the desired concentration by varying the flow rate of the clean, dry air. This flow rate can normally be varied from 0.2-15 ℓ/minute. The flow rate of the sampling system determines the lower limit for the flow rate of the diluent gases. The flow rate of the nitrogen and the diluent air must be measured to an accuracy of 1-2 per cent. With a tube permeating hydrogen sulfide at a rate of 0.1 μℓ/minute, the range of concentration of hydrogen sulfide will be between 6-400 $\mu g/m^3$ (4-290 ppb), a generally satisfactory range for ambient air conditions. When higher concentrations are desired, calibrate and use longer permeation tubes.

9.2.3 **Procedure for Preparing Simulated Calibration Curves**

Obviously one can prepare a multitude of curves by selecting different combinations of sampling rate and sampling time. The following description represents a typical procedure for ambient air sampling of short duration, with a brief mention of a modification for 24 hour sampling.

1. The system is designed to provide an accurate measure of hydrogen sulfide in the 1.4-84 $\mu g/m^3$ (1-60 ppb) range. It can be easily modified to meet special needs.

2. The dynamic range of the colorimetric procedure fixes the total volume of the sample at 186 ℓ; then, to obtain linearity between the absorbance of the solution and the concentration of hydrogen sulfide in ppm, select a constant sampling time. This fixing of the sampling time is desirable also from a practical standpoint: In this case, select a sampling time of 120 minutes. Then to obtain a 186 ℓ sample of air requires a flow rate of 1.55 ℓ/minute. The concentration of standard H_2S in air is computed as follows:

$$C = \frac{P_r \times M}{(R + r)}$$

where:

C = Concentration of H_2S in ppm
P_r = Permeation rate in μg/minute
M = Reciprocal of vapor density, 0.719 $\mu\ell/\mu g$
R = Flow rate of diluent air, liter/minute
r = Flow rate of diluent nitrogen, liter/minute

3. The data for a typical calibration curve are listed in Table 1.

TABLE 1

TYPICAL CALIBRATION DATA

Concentrations H_2S, ppb	Amount of H_2S in μl/186 liters	Absorbance of Sample
1	.144	.010
5	.795	.056
10	1.44	.102
20	2.88	.205
30	4.32	.307
40	5.76	.410
50	7.95	.512
60	8.64	.615

4. A plot of the concentration of hydrogen sulfide in ppm (x – axis) against absorbance of the final solution (y – axis) will yield a straight line, the reciprocal of the slope of which is the factor for conversion of absorbance to ppm. This factor includes the correction for collection efficiency. Any deviation from the linearity at the lower concentration range indicates a change in collection efficiency of the sampling system. If the range of interest is below the dynamic range of the method the total volume of air collected should be increased to obtain sufficient color within the dynamic range of the colorimetric procedure. Also, once the calibration factor has been established under simulated conditions the conditions can be modified so that the concentration of H_2S is a simple multiple of the absorbance of the colored solution.

5. For long-term sampling of 24-hour duration, the conditions can be fixed to collect 1200 ℓ of sample in a larger volume of STRactan 10®-cadmium hydroxide. For example, for 24 hours at 0.83 ℓ/min, approximately 1200 ℓ of air are scrubbed. An aliquot representing 0.1 of the entire amount of sample is taken for the analysis.

6. The remainder of the analytical procedure is the same as described in the previous paragraph.

9.2.4 The permeation tubes must be stored in a wide-mouth glass bottle containing silica gel and solid sodium hydroxide to remove moisture and hydrogen sulfide. The storage bottle is immersed to two-thirds its depth in a constant temperature water bath in which the water is controlled at 25°C ±0.1°C.

Periodically, (every 2 weeks or less) the permeation tubes are removed and rapidly weighed on a semimicro balance (sensitivity ±0.01 mg) and then returned to the storage bottle. The weight loss is recorded. The tubes are ready for use when the rate of weight loss becomes constant (within ±2 per cent).

10. Calculations

10.1 Gaseous Sulfide

10.1.1 Determine the sample volume in liters from the gas meter or flow meter readings and time of sampling. Adjust volume to 760 mm mercury and 25°C (V_S).

10.1.2 Determine the concentration of H_2S in $\mu g/m^3$.

$$\mu g\ H_2S/m^3 = \mu g\ H_2S/V_S \times 10^{-3}$$

where:

$\mu g\ H_2S$ = micrograms hydrogen sulfide determined
10^{-3} = conversion factor, m^3/ℓ

10.2 Gaseous Sulfide from Aqueous Sulfide

10.2.1 Determine the sample volume (V) in liters from the gas meter or flow meter readings and time of sampling. Adjust volume to 760 mm mercury and 25°C (V_S), using the correction formula:

$$V_S = V \times \frac{P}{760} \times \frac{298}{(T + 273)}$$

where:

V_S = Volume of air in liters at standard conditions
V = Volume of air sampled in liters
P = Barometric pressure in mm Hg
T = Temperature of sample air in °C

10.2.2 Using the Beers-Law Standard curve of absorbance vs. $\mu g\ S^=$ ion, determine $\mu g\ S^=$ ion in the sampling impinger corresponding to its absorbance reading at 670 nm.

10.2.3 Calculate the concentration of H_2S in the aspirated volume of air using the formula:

$$\text{ppm, } H_2S = \frac{\mu g\ S^= \times 24.45}{V_S \times MW}$$

where:

$\mu g\ S^=$ = micrograms sulfide ion (Section 10.2.2)
24.45 = molar volume of an ideal gas at 25°C and 760 mm Hg
MW = mass of sulfide ion, 32.06

11. References

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ORGANIC SOLVENTS IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Organic Solvents (See Table 1)	**Method No.:**	P&CAM 127
Matrix:	Air	**Range:**	For the specific compound, refer to Table 1
Procedure:	Adsorption on charcoal desorption with carbon disulfide, GC		
Date Issued:	9/15/72	**Precision:**	10.5% RSD
Date Revised:	2/15/77	**Classification:**	See Table 1

1. **Principle of the Method**

   1.1 A known volume of air is drawn through a charcoal tube to trap the organic vapors present.

   1.2 The charcoal in the tube is transferred to a small, graduated test tube and desorbed with carbon disulfide.

   1.3 An aliquot of the desorbed sample is injected into a gas chromatograph.

   1.4 The area of the resulting peak is determined and compared with areas obtained from the injection of standards.

2. **Range and Sensitivity**

   The lower limit in mg/sample for the specific compound at 16 × 1 attenuation on a gas chromatograph fitted with a 10:1 splitter is shown in Table 1. This value can be lowered by reducing the attenuation or by eliminating the 10:1 splitter.

3. **Interferences**

   3.1 When the amount of water in the air is so great that condensation actually occurs in the tube, organic vapors will not be trapped. Preliminary experiments indicate that high humidity severely decreases the breakthrough volume.

   3.2 When two or more solvents are known or suspected to be present in the air, such information (including their suspected identities), should be transmitted with the sample, since with differences in polarity, one may displace another from the charcoal.

   3.3 It must be emphasized that any compound which has the same retention time as the specific compound under study at the operating conditions described in this method is an interference. Hence, retention time data on a single column, or even on a number of columns, cannot be considered as proof of chemical identity. For this reason it is important that a sample of the bulk solvent(s) be submitted at the same time so that identity(ies) can be established by other means.

141

3.4 If the possibility of interference exists, separation conditions (column packing, temperatures, etc.) must be changed to circumvent the problem.

4. Precision and Accuracy

4.1 The mean relative standard deviation of the analytical method is 8% (11.4).

4.2 The mean relative standard deviation of the analytical method plus field sampling using an approved personal sampling pump is 10% (11.4). Part of the error associated with the method is related to uncertainties in the sample volume collected. If a more powerful vacuum pump with associated gas-volume integrating equipment is used, sampling precision can be improved.

4.3 The accuracy of the overall sampling and analytical method is 10% (NIOSH-unpublished data) when the personal sampling pump is calibrated with a charcoal tube in the line.

5. Advantages and Disadvantages of the Method

5.1 The sampling device is small, portable, and involves no liquids. Interferences are minimal, and most of those which do occur can be eliminated by altering chromatographic conditions. The tubes are analyzed by means of a quick, instrumental method. The method can also be used for the simultaneous analysis of two or more solvents suspected to be present in the same sample by simply changing gas chromatographic conditions from isothermal to a temperature-programmed mode of operation.

5.2 One disadvantage of the method is that the amount of sample which can be taken is limited by the number of milligrams that the tube will hold before overloading. When the sample value obtained for the backup section of the charcoal tube exceeds 25% of that found on the front section, the possibility of sample loss exists. During sample storage, the more volatile compounds will migrate throughout the tube until equilibrium is reached (33% of the sample on the backup section).

5.3 Furthermore, the precision of the method is limited by the reproducibility of the pressure drop across the tubes. This drop will affect the flow rate and cause the volume to be imprecise, because the pump is usually calibrated for one tube only.

6. Apparatus

6.1 An approved and calibrated personal sampling pump for personal samples. For an area sample, any vacuum pump whose flow can be determined accurately at 1 liter per minute or less.

6.2 Charcoal tubes: glass tube with both ends flame sealed, 7 cm long with a 6-mm O.D. and a 4-mm I.D., containing 2 sections of 20/40 mesh activated charcoal separated by a 2-mm portion of urethane foam. The activated charcoal is prepared from coconut shells and is fired at 600°C prior to packing. The absorbing section contains 100 mg of charcoal, the backup section 50 mg. A 3-mm portion of urethane foam is placed between the outlet end of the tube and the backup section. A plug of silylated glass wool is placed in front of the absorbing section. The pressure drop across the tube must be less than one inch of mercury at a flow rate of 1 lpm.

6.3 Gas chromatograph equipped with a flame ionization detector.

6.4 Column (20 ft × ⅛ in) with 10% FFAP stationary phase on 80/100 mesh, acid-washed DMCS Chromosorb W solid support. Other columns capable of performing the required separations may be used.

6.5 A mechanical or electronic integrator or a recorder and some method for determining peak area.

6.6 Microcentrifuge tubes, 2.5 ml, graduated.

6.7 Hamilton syringes: 10 μl, and convenient sizes for making standards.

6.8 Pipets: 0.5-ml delivery pipets or 1.0-ml type graduated in 0.1-ml increments.

6.9 Volumetric flasks: 10 ml or convenient sizes for making standard solutions.

7. **Reagents**

7.1 Spectroquality carbon disulfide (Matheson Coleman and Bell).

7.2 Sample of the specific compound under study, preferably chromatoquality grade.

7.3 Bureau of Mines Grade A helium.

7.4 Prepurified hydrogen.

7.5 Filtered compressed air.

8. **Procedure**

8.1 **Cleaning of Equipment:** All glassware used for the laboratory analysis should be detergent washed and thoroughly rinsed with tap water and distilled water.

8.2 **Calibration of Personal Pumps.** Each personal pump must be calibrated with a representative charcoal tube in the line. This will minimize errors associated with uncertainties in the sample volume collected.

8.3 **Collection and Shipping of Samples**

8.3.1 Immediately before sampling, the ends of the tube should be broken to provide an opening at least one-half the internal diameter of the tube (2 mm).

8.3.2 The small section of charcoal is used as a back-up and should be positioned nearest the sampling pump.

8.3.3 The charcoal tube should be vertical during sampling to reduce channeling through the charcoal.

8.3.4 Air being sampled should not be passed through any hose or tubing before entering the charcoal tube.

8.3.5 The flow, time, and/or volume must be measured as accurately as possible. The sample should be taken at a flow rate of 1 lpm or less to attain the total sample volume required. The minimum and maximum sample volumes that should be collected for each solvent are shown in Table 1. The minimum volume quoted must be collected if the desired sensitivity is to be achieved.

8.3.6 The temperature and pressure of the atmosphere being sampled should be measured and recorded.

8.3.7 The charcoal tubes should be capped with the supplied plastic caps immediately after sampling. Under no circumstances should rubber caps be used.

8.3.8 One tube should be handled in the same manner as the sample tube (break, seal, and transport), except that no air is sampled through this tube. This tube should be labeled as a blank.

8.3.9 Capped tubes should be packed tightly before they are shipped to minimize tube breakage during shipping.

143

8.3.10 Samples of the suspected solvent(s) should be submitted to the laboratory for qualitative characterization. These liquid bulk samples should not be transported in the same container as the samples or blank tube. If possible, a bulk air sample (at least 50 l air drawn through tube) should be shipped for qualitative identification purposes.

8.4 **Analysis of Samples**

8.4.1 Preparation of Samples. In preparation for analysis, each charcoal tube is scored with a file in front of the first section of charcoal and broken open. The glass wool is removed and discarded. The charcoal in the first (larger) section is transferred to a small stoppered test tube. The separating section of foam is removed and discarded; the second section is transferred to another test tube. These two sections are analyzed separately.

8.4.2 Desorption of Samples. Prior to analysis, one-half ml of carbon disulfide is pipetted into each test tube. (All work with carbon disulfide should be performed in a hood because of its high toxicity.) Tests indicate that desorption is complete in 30 minutes if the sample is stirred occasionally during this period.

8.4.3 GC Conditions. The typical operating conditions for the gas chromatograph are:

1. 85 cc/min. (70 psig) helium carrier gas flow.
2. 65 cc/min. (24 psig) hydrogen gas flow to detector.
3. 500 cc/min. (50 psig) air flow to detector.
4. 200°C injector temperature.
5. 200°C manifold temperature (detector).
6. Isothermal oven or column temperature — refer to Table 1 for specific compounds.

8.4.4 Injection. The first step in the analysis is the injection of the sample into the gas chromatograph. To eliminate difficulties arising from blowback or distillation within the syringe needle, one should employ the solvent flush injection technique. The 10 μl syringe is first flushed with solvent several times to wet the barrel and plunger. Three microliters of solvent are drawn into the syringe to increase the accuracy and reproducibility of the injected sample volume. The needle is removed from the solvent, and the plunger is pulled back about 0.2 μl to separate the solvent flush from the sample with a pocket of air to be used as a marker. The needle is then immersed in the sample, and a 5-μl aliquot is withdrawn, taking into consideration the volume of the needle, since the sample in the needle will be completely injected. After the needle is removed from the sample and prior to injection, the plunger is pulled back a short distance to minimize evaporation of the sample from the tip of the needle. Duplicate injections of each sample and standard should be made. No more than a 3% difference in area is to be expected.

8.4.5 Measurement of area. The area of the sample peak is measured by an electronic integrator or some other suitable form of area measurement, and preliminary results are read from a standard curve prepared as discussed below.

8.5 **Determination of Desorption Efficiency**

8.5.1 Importance of determination. The desorption efficiency of a particular compound can vary from one laboratory to another and also from one batch of charcoal to another. Thus, it is necessary to determine at least once the percentage of the specific compound that is removed in the desorption process for a given compound, provided the same batch of charcoal is used. NIOSH has found that the desorption efficiencies for the compounds in Table 1 are between 81% and 100% and vary with each batch of charcoal.

8.5.2 Procedure for determining desorption efficiency. Activated charcoal equivalent to the amount in the first section of the sampling tube (100 mg) is measured into a 5-cm, 4-mm I.D. glass tube, flame-sealed at one end (similar to commercially available culture tubes). This charcoal must be from the same batch as that used in obtaining the samples and can be obtained from unused charcoal tubes. The open end is capped with Parafilm. A known amount of the compound is injected directly into the activated charcoal with a microliter syringe, and the tube is capped with more Parafilm. The amount injected is usually equivalent to that present in a 10-liter sample at a concentration equal to the federal standard.

At least five tubes are prepared in this manner and allowed to stand for at least overnight to assure complete absorption of the specific compound onto the charcoal. These five tubes are referred to as the samples. A parallel blank tube should be treated in the same manner except that no sample is added to it. The sample and blank tubes are desorbed and analyzed in exactly the same manner as the sampling tube described in Section 8.4.

Two or three standards are prepared by injecting the same volume of compound into 0.5 ml of CS_2 with the same syringe used in the preparation of the sample. These are analyzed with the samples.

The desorption efficiency equals the difference between the average peak area of the samples and the peak area of the blank divided by the average peak area of the standards, or

$$\text{desorption efficiency} = \frac{\text{Area sample} - \text{Area blank}}{\text{Area standard}}$$

9. Calibration and Standards

It is convenient to express concentration of standards in terms of mg/0.5 ml CS_2 because samples are desorbed in this amount of CS_2. To minimize error due to the volatility of carbon disulfide, one can inject 20 times the weight into 10 ml of CS_2. For example, to prepare a 0.3 mg/0.5 ml standard, one would inject 6.0 mg into exactly 10 ml of CS_2 in a glass-stoppered flask. The density of the specific compound is used to convert 6.0 mg into microliters for easy measurement with a microliter syringe. A series of standards, varying in concentration over the range of interest, is prepared and analyzed under the same GC conditions and during the same time period as the unknown samples. Curves are established by plotting concentration in mg/0.5 ml versus peak area.

NOTE: Since no internal standard is used in the method, standard solutions must be analyzed at the same time that the sample analysis is done. This will minimize the effect of known day-to-day variations and variations during the same day of the FID response.

10. Calculations

10.1 The weight, in mg, corresponding to each peak area is read from the standard curve for the particular compound. No volume corrections are needed, because the standard curve is based on mg/0.5 ml CS_2 and the volume of sample injected is identical to the volume of the standards injected.

10.2 Corrections for the blank must be made for each sample.

$$\text{Correct mg} = \text{mg}_s - \text{mg}_b$$

where:

mg_s = mg found in front section of sample tube

mg_b = mg found in front section of blank tube

A similar procedure is followed for the backup sections.

10.3 The corrected amounts present in the front and backup sections of the same sample tube are added to determine the total measured amount in the sample.

10.4 This total weight is divided by the determined desorption efficiency to obtain the corrected mg per sample.

10.5 The concentration of the analyte in the air sampled can be expressed in mg per m^3.

$$mg/m^3 = \frac{\text{Corrected mg (Section 10.4)} \times 1000 \text{ (liters/m}^3\text{)}}{\text{Air volume sampled (liters)}}$$

10.6 Another method of expressing concentration is ppm (corrected to standard conditions of 25°C and 760 mm Hg).

$$\text{ppm} = mg/m^3 \times \frac{24.45}{MW} \times \frac{760}{P} \times \frac{(T + 273)}{298}$$

where:

P = pressure (mm Hg) of air sampled
T = temperature (°C) of air sampled
24.45 = molar volume (liter/mole) at 25°C and 760 mm Hg
MW = molecular weight
760 = standard pressure (mm Hg)
298 = standard temperature (°K)

11. References

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TABLE 1

Parameters Associated With P&CAB Analytical Method No. 127

Organic Solvent	Method Classification	Detection limit (mg/sample)	Sample Volume (liters) Minimum[a]	Maximum[b]	GC Column Temp.(°C)	Molecular Weight
Acetone	D	–	0.5	7.7	60	58.1
Benzene	A	0.01	0.5	55	90	78.1
Carbon tetrachloride	A	0.20	10	60	60	154.0
Chloroform	A	0.10	0.5	13	80	119
Dichloromethane	D	0.05	0.5	3.8	85	84.9
p-Dioxane	A	0.05	1	18	100	88.1
Ethylene dichloride	D	0.05	1	12	90	99.0
Methyl ethyl ketone	B	0.01	0.5	13	80	72.1
Styrene	D	0.10	1.5	34	150	104
Tetrachloroethylene	B	0.06	1	25	130	166
1,1,2-trichloroethane	B	0.05	10	97	150	133
1,1,1-trichloroethane (methyl chloroform)	B	0.05	0.5	13	150	133
Trichloroethylene	A	0.05	1	17	90	131
Toluene	B	0.01	0.5	22	120	92.1
Xylene	A	0.02	0.5	31	100	106

(a) Minimum volume, in liters, required to measure 0.1 times the OSHA standard

(b) These are breakthrough volumes calculated with data derived from a potential plot (11.2) for activated coconut charcoal. Concentrations of vapor in air at 5 times the OSHA standard (11.3) or 500 ppm, whichever is lower, 25°C, and 760 torr were assumed. These values will be as much as 50% lower for atmospheres of high humidity. The effects of multiple contaminants have not been investigated, but it is suspected that less volatile compounds may displace more volatile compounds (See 3.1 and 3.2)

ARSENIC IN URINE AND AIR

Measurements Research Branch

Analytical Method

Analyte:	Arsenic	**Method No.:**	P&CAM 139
Matrix:	Urine and air	**Range:**	0.05 to 2.0 μg/sample
Procedure:	Filter/urine collection, arsine generation, AAS	**Precision:**	10.7% RSD (Analytical)
Date Issued:	3/24/72		
Date Revised:	12/20/76	**Classification:**	D (Operational)

1. **Principle of the Method**

   1.1 Samples are ashed with a mixture either of nitric, perchloric, and sulfuric acids or a mixture of nitric and sulfuric acids to destroy the organic matrix.

   1.2 Arsine is generated by the addition of $NaBH_4$ and is flushed through the burner of an atomic absorption spectrophotometer for the determination of arsenic content.

2. **Range and Sensitivity**

   2.1 For a 25-ml urine sample, the range extends from 0.001 mg/l to 0.040 mg/l.

   2.2 For a 30-liter air sample, the range extends from 0.002 mg/m³ to 0.060 mg/m³.

   2.3 The range can be extended by taking smaller (or larger) aliquots for analysis.

3. **Interferences**

   There are no known interferences for this method, providing that appropriate background correcting techniques are applied.

4. **Precision and Accuracy**

   The accuracy and precision of this method have not been completely determined at this time, although 31 determinations of 1 μg As analyzed on six different days gave an overall RSD of 10.7%.

5. **Advantages and Disadvantages of the Method**

   5.1 This method has the advantage of being free of antimony interference. It is somewhat faster, and several times as sensitive as the colorimetric measurement of arsenic.

   5.2 The disadvantages include the requirement of specialized equipment and the use of rather large volumes of expensive gases. The sampling method will not collect arsine gas.

6. **Apparatus**

   6.1 Sampling Equipment. The sampling unit for the collection of personal air samples has the following components:

   6.1.1 The filter unit, consisting of the filter media (Section 6.2) and appropriate cassette

filter holder, either a 2- or 3-piece filter cassette (Millipore Filter Corporation, Bedford, Massachusetts).

6.1.2 A vacuum pump such as a personal sampling pump. This pump must be properly calibrated so the volume of air sampled can be measured as accurately as possible. The pump must be calibrated with a representative filter unit in the line. Flow rate, times, and/or volume must be known.

6.1.3 Thermometer.

6.1.4 Manometer.

6.1.5 Stopwatch.

6.1.6 Various clips, tubing, spring connectors, and belt for connecting sampling apparatus to worker being sampled.

6.2 Type AA Millipore filter (or equivalent), 37-mm.

6.3 125-ml borosilicate Phillips beakers.

6.4 Atomic absorption spectrophotometer equipped with arsine generator and argon-hydrogen or nitrogen-hydrogen system. (Figure 1)

6.5 Hood facilities capable of handling perchloric acid fumes if perchloric acid digestion is used.

6.6 Specific gravity meter or hydrometer capable of measuring specific gravities in the range of 1.000 to 1.040 $\pm$ 0.001 for urine samples.

6.7 25-ml Pyrex volumetric flasks.

7. Reagents

All chemicals must be ACS reagent grade or better.

7.1 Nitric acid, distilled reagent grade.

7.2 Perchloric acid, 72%.

7.3 Sulfuric acid, 90%.

7.4 Hydrochloric acid, 36%.

7.5 Sodium borohydride, 11/32-in pellets. (Available from Alfa Inorganics.)

7.6 Arsenic Standards

7.6.1 Arsenic Standard Stock Solution, 1000 ppm. Dissolve 0.4165 g of $Na_2AsO_4 \cdot 7H_2O$ in a mixture of double distilled water, 5 ml of concentrated H_2SO_4 and 20 ml of concentrated HCl and bring volume to 100 ml with double distilled water. Commercially prepared stock standard solutions obtained from a reliable supplier may also be used.

7.6.2 Working Standards. Dilute stock solution with appropriate amounts of solution prepared by adding 100 ml concentrated HCl and 25 ml concentrated H_2SO_4 to 300 ml double distilled water and diluting to volume of 500 ml with double distilled water.

7.7 All water used is double distilled in Pyrex.

7.8 Gases

7.8.1 Hydrogen — electrolytic grade.

7.8.2 Argon or nitrogen — high purity.

8. Procedure

8.1 **Cleaning of Equipment.** All glassware must be cleaned with a detergent solution followed by both tap water and distilled water rinses. Then the glassware is cleaned with hot concentrated nitric acid and thoroughly rinsed with tap water followed by distilled water. Arsine generators must be rinsed twice with double deionized water between determinations, leaving no evidence of contamination from previous determination.

8.2 **Collection and Shipping of Samples**

8.2.1 Urine samples are collected in polyethylene bottles which are precleaned in nitric acid. About 0.1 g EDTA is added as a preservative. At least 75 ml should be collected. Care should be taken to prevent bottles from leaking in transit.

8.2.2 Air samples are collected on 37-mm AA Millipore filter (or equivalent) as follows:

1. Assemble the filter unit by mounting the filter disc in the filter cassette.
2. Connect the exit end of the filter unit to the pump with a short piece of flexible tubing.
3. Turn on pump to begin sample collection. The flow rate, times, and or volume must be measured as accurately as possible. Record atmospheric pressure and temperature. The sample should be taken at a flow rate of 1.7 liters per minute. A minimum sample of 30 liters should be collected. Larger sample volumes are encouraged, provided the filters do not become loaded with dust to the point that loose material might fall off or the filter become plugged.
4. The sample cassettes should be shipped in a suitable container designed by NIOSH to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample is dislodged from the filter, nor that the sample surface be disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the cassette on top of the sample filter.
5. With each batch of samples, one filter, labeled as a blank, must be submitted. No air should be drawn through this filter.

8.3 **Analysis of Samples**

8.3.1 Determine the specific gravity of the urine sample at room temperature. This may be done with the use of a specific gravity meter or a reliable hydrometer.

8.3.2 Transfer 25 ml of the urine sample or the filter paper sample into a 125-ml Phillips beaker.

8.3.3 Wet-ash the sample by treating with 5 ml of a mixture of 3 parts HNO_3, 1 part H_2SO_4, and 1 part $HClO_4$ and heating on a hot plate at 130°C-150°C. Keep on adding small amounts of redistilled HNO_3 until a colorless (liquid) ash is obtained. If the ashing is still incomplete, additional $HClO_4$ can be added drop by drop. If a perchloric acid fume hood is not available, the sample can be wet-ashed by treating with 5 ml of a mixture of 3 parts HNO_3 and 2 parts H_2SO_4 and heating on a hot plate at 130°C-150°C, adding small amounts of redistilled HNO_3 until a colorless (liquid) ash is obtained. Continue heating until fumes of SO_3 are produced.

8.3.4 Allow the mixture to cool, transfer to a 25-ml volumetric flask, and make up to volume with distilled water.

8.3.5 Pipet a 5-ml aliquot of the 25-ml sample into an arsine generation flask.

8.3.6 The arsine is then generated by reduction with sodium borohydride. Add 25 ml of distilled water and 3 ml concentrated HCl and mix well. Connect the sample flask to the generating system. Add to the sample solution, via the addition stopcock, a

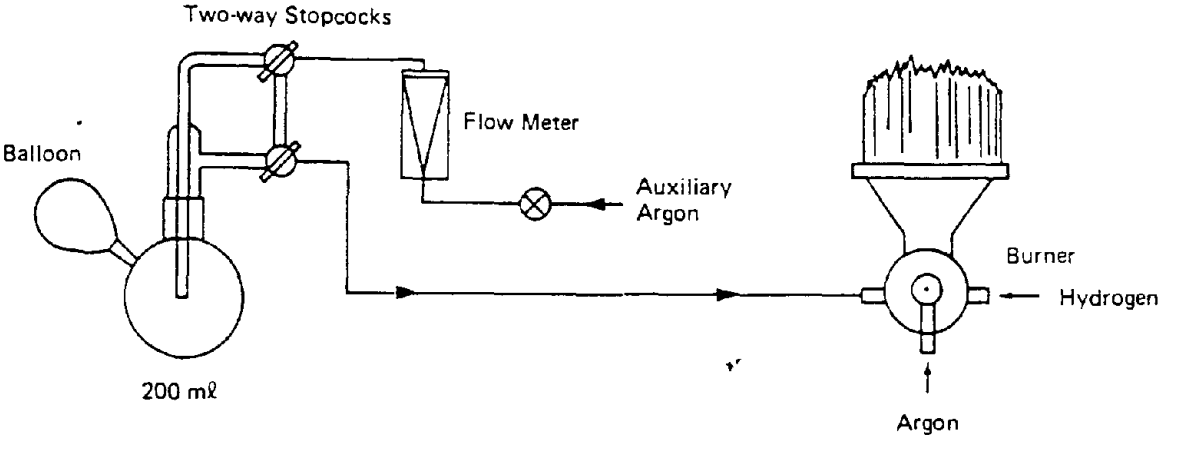


FIGURE 1. Schematic of Apparatus for Arsine Generation. Instrument conditions for arsine measurement by atomic absorption should be set following manufacturer's recommendation

single sodium borohydride pellet (11/32 in. diameter, 200 mg). Care should be exercised to maintain the same total volume of solution in the arsine generator for all samples and standards analyzed at one time.

8.3.7 The reaction is vigorous and the balloon fills with the evolved gases thus acting as a reservoir for the generating system.

8.3.8 After one minute, turn the lower stopcock allowing the pressure in the balloon to flush the gases into the flame of the atomic absorption instrument. Before the balloon is deflated, turn the upper stopcock allowing the argon to flush through the generator flask and into the flame. The absorption signal is recorded on a rapid response strip chart recorder. Larger (or smaller) aliquots of the sample solution may be taken if the signal is not in the proper range.

9. Calibration and Standards

9.1 Prepare working standards of 1.0, 3.0, 5.0, 7.5, and 10.0 μg of arsenic in 25 milliliters of solution by dilution of the standard stock solution. These standards should be prepared fresh each time.

9.2 Construct a calibration curve by pipetting 5 ml of each of the working standards into arsine generators and proceeding with the analysis from Section 8.3.6. A calibration curve of absorption versus micrograms of arsenic is then drawn and used for the determination of arsenic content of the samples.

10. Calculations

10.1 The concentration of arsenic in the urine sample can be expressed as mg As per liter of urine.

$$\text{mg As/l} = \frac{\mu\text{g As}}{\text{ml of urine}} \qquad \text{(from calibration curve, Section 9.2)}$$

10.1.1 The use of a specific gravity correction factor to normalize values to 1.024, the average specific gravity of urine, has been recommended (11.4).

10.1.2 The preferred method of normalization is to make a straightforward gravity correction as follows:

$$\text{corrected mg As/l} = \text{mg As/l} \times \frac{0.024}{(\text{specific gravity} - 1.000)}$$

10.2 The concentration of arsenic in air can be expressed as mg As per cubic meter of air, which is numerically equal to micrograms As per liter of air.

$$\text{mg As/m}^3 = \mu\text{g As/V}_s$$

where:

μg As = micrograms As from calibration curve (Section 9.2)

V_s = volume of air sampled in liters at 25°C and 760 mm Hg.

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ARSENIC IN URINE AND AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Arsenic	Method No.:	P&CAM 140
Matrix:	Urine and Air	Range:	1.0 to 25 μg/sample
Procedure:	Arsine Generation – Colorimetric	Precision:	4-9% Average deviation
Date Issued:	3/24/72		
Date Revised:	1/10/74	Classification:	A (Recommended)

1. Principle of the Method

   1.1 Samples are ashed with a mixture of nitric, perchloric, and sulfuric acids to destroy the organic matrix.

   1.2 The sample ash is treated with ammonium oxalate and taken to fumes of SO_3 to remove all traces of nitric acid.

   1.3 The sample is transferred to an arsine generator where the arsenic is converted to the trivalent form by the addition of KI, HCl, and $SnCl_2$.

   1.4 Arsine is generated by the addition of metallic zinc and is absorbed by a solution of pyridine – silver diethyldithiocarbamate. The absorbance of this solution is measured spectrophotometrically at 560 mμ.

2. Range and Sensitivity

   2.1 For a 25 mℓ urine sample, the range extends from 0.01 mg/ℓ to 1.0 mg/ℓ.

   2.2 For a 30 liter air sample, the range extends from 0.01 mg/m^3 to 0.8 mg/m^3.

   2.3 The range can be extended by taking smaller (or larger) aliquots for analysis.

3. Interferences

   Antimony will interfere when present in relatively high concentrations, but such concentrations are not likely to be found.

4. **Precision and Accuracy**

This method deviates only slightly from the A.C.G.I.H. recommended method for the determination of arsenic in air. For that method eight cooperating laboratories analyzed four arsenic samples (0.05-1.5 $\mu g/m\ell$) with a percent average deviation from the known of 4.0 to 9.1.

5. Advantages and Disadvantages of the Method

5.1 This method has the advantage of being a well established, reliable method for the analysis of arsenic. In addition, very little specialized equipment is required.

5.2 The disadvantages are that the method is tedious and that the use of pyridine as a solvent renders it somewhat objectionable. The sampling method will not collect arsine gas.

6. Apparatus

6.1 **Sampling Equipment.** The sampling unit for the collection of personal air samples has the following components:

6.1.1 The filter unit, consisting of the filter media (Section 6.2) and appropriate cassette filter holder, either a 2- or 3-piece filter cassette. (Millipore Filter Corporation, Bedford, Massachusetts.)

6.1.2 Battery operated diaphragm pump with belt clip – this should operate for 4-5 hours continuously at 1.7 ℓpm and 20 cm water resistance. (Monitaire Sample – Mine Safety Appliances Co., Pittsburgh, Pennsylvania.)

6.1.3 Thermometer.

6.1.4 Manometer.

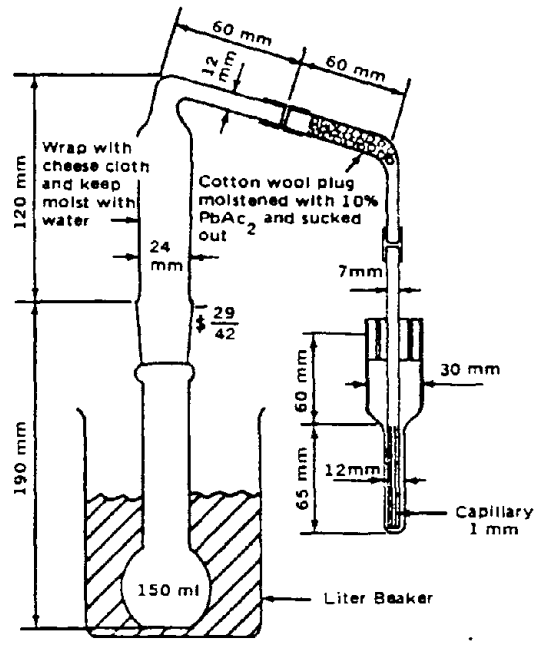
6.1.5 Stopwatch.

6.1.6 Various clips, tubing, spring connectors, and belt for connecting sampling apparatus to worker being sampled.

6.2 Type HA millipore filter (or equivalent), 37 mm.

6.3 125 $m\ell$ borosilicate Phillips beakers.

6.4 Arsine generation equipment (Reference 11.3, and attached illustration).



APPARATUS FOR ARSINE GENERATION

6.5 Hood facilities capable of handling perchloric acid fumes.

6.6 Spectrophotometer capable of measurements at 560 mμ.

6.7 1 cm spectrophotometer cells.

6.8 Specific gravity meter or hydrometer capable of measuring in the range 1.000 to 1.040 ± 0.001 units.

6.9 150 mℓ polyethylene bottles for collection of urine samples.

7. Reagents

All reagents must be ACS reagent grade or better.

7.1 Silver Diethyldithiocarbamate.

7.2 Pyridine.

7.3 **Arsine Absorbing Solution.** Dissolve 1.0 g of silver diethyldithiocarbamate in 200 mℓ of pyridine, shake well and filter.

7.4 Hydrochloric acid, 36%.

7.5 Nitric Acid, 68%, distilled reagent grade.

7.6 Perchloric Acid, 72%.

7.7 Sulfuric Acid, 98%.

7.8 **Ammonium Oxalate.** Prepare a saturated solution in double distilled water.

7.9 **Potassium Iodide Solution.** Dissolve 15 g of potassium iodide in 100 mℓ double distilled water.

7.10 **Stannous Chloride Solution.** Dissolve 40 g of stannous chloride dihydrate in 100 mℓ hydrochloric acid.

7.11 **Zinc,** 20 mesh granular, low arsenic.

7.12 **Lead Acetate.** Dissolve 10 g of lead acetate in 100 mℓ double distilled water. The glass wool in the scrubber is soaked in this solution, drained and dried.

7.13 **Arsenic Standard Stock Solution.** Dissolve 1.320 g of arsenic trioxide in 10 mℓ of 40% sodium hydroxide and dilute to 1 liter with distilled water. Working standards are made by diluting this solution.

7.14 The water used is double distilled in pyrex.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware must be cleaned with a detergent solution followed by both tap water and distilled water rinses. Then the glassware is treated with hot concentrated nitric acid and thoroughly rinsed with tap water followed by distilled water. (Arsine generators are rinsed with concentrated HCl following the nitric acid wash.)

8.2 **Collection and Shipping of Samples**

8.2.1 **Urine Samples** are collected in polyethylene bottles which are precleaned in nitric acid. About 0.1 g EDTA is added as a preservative. At least 75 mℓ should be collected. Care should be taken to prevent leaking of bottles in transit.

8.2.2 **Air Samples** are collected on 37 mm HA millipore filter (or equivalent) as follows:

1. Assemble the filter unit by mounting the filter disc in the filter cassette.

2. Connect the exit end of the filter unit to the pump with a short piece of flexible tubing.

3. Turn on pump to begin sample collection. The flow rate, times, and/or volume must be measured as accurately as possible. Record atmospheric pressure and temperature. The sample should be taken at a flow rate of 1.7 liters per minute. A minimum sample of 30 liters should be collected. Larger sample volumes are encouraged, provided the filters do not become loaded with dust to the point that loose material might fall off or the filter become plugged.

4. The sample cassettes should be shipped in a suitable container designed by NIOSH to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample is dislodged from the filter, nor that the sample surface may be disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the cassette on top of the sample filter.

5. With each batch of samples, one filter, labeled as a blank, must be submitted. No air should be drawn through this filter.

8.3 **Analysis of Samples**

8.3.1 Determine the specific gravity of the urine sample at room temperature. This may be done with the use of a specific gravity meter or a reliable hydrometer.

8.3.2 Transfer 25 mℓ of the urine sample or the filter paper sample into a 125 mℓ Phillips beaker.

8.3.3 Ash the sample by treating with 5 mℓ of a mixture of 3 parts HNO_3, 1 part H_2SO_4, and 1 part $HClO_4$ and heating on a hot plate at 130°-150°C. Keep on adding small amounts of redistilled HNO_3 until a colorless (liquid) ash is obtained. If the ashing is still incomplete, additional $HClO_4$ can be added dropwise.

8.3.4 Continue heating to fumes of SO_3. After cooling the colorless or nearly colorless liquid, add 10 mℓ of distilled water and 5 mℓ of a saturated

solution of ammonium oxalate. The mixture is again taken to fumes of SO_3 to free the ashed solution of all traces of nitric acid.

8.3.5 Allow the mixture to cool and then transfer to the flask of an arsine generator with 20 mℓ of distilled water. Rinse the Phillips beaker with 5 mℓ of concentrated HCl and add these washings to the arsine generator. (If high As concentrations are expected, the solution may be made to a known volume, and only an aliquot is added to the arsine generator.)

8.3.6 Add to the arsine receiver 3 mℓ of the pyridine – silver diethyldithiocarbamate absorbing solution.

8.3.7 To the sample solution in the arsine generator flask, add *in order* 2 mℓ of 15% KI solution and 0.5 mℓ of 40% $SnCl_2$. Swirl the flask and allow to stand 15 minutes to insure conversion of all the pentavalent arsenic to the trivalent form.

8.3.8 Add to the sample flask 7.0 g of zinc (granular 20 mesh), and close the flask *immediately* (sealing ground glass joints by wetting with distilled water).

8.3.9 Heat the flask in a beaker of hot water and allow thirty minutes for the evolution of arsine.

8.3.10 Transfer the arsine absorbing solution to a 1 cm cell and read the absorbance of this solution on a spectrophotometer at 560 mμ.

9. Calibration and Standards

9.1 Prepare working standards of 1.0, 5.0, 10.0, 15.0, and 20.0 μg of arsenic per milliliter of solution by dilution of the standard stock solution. These standards should be prepared fresh weekly.

9.2 Construct a calibration curve by pipetting 1 mℓ of each of the working standards into arsine generators, adding 20 mℓ double distilled water, 5 mℓ concentrated hydrochloric acid, and proceeding from Step 8.3.6 of the procedure. A calibration curve of absorbance versus micrograms of arsenic is then drawn and used for determination of arsenic content of the samples.

10. Calculations

10.1 The concentration of arsenic in the urine sample can be expressed as mg As per liter of urine:

$$\text{mg As}/\ell = \frac{\mu\text{g As}}{\text{m}\ell \text{ of urine}} \text{ (from calibration curve, Section 9.2)}$$

10.1.1 The use of a specific gravity correction factor to normalize values to 1.024, the average specific gravity of urine, has been proposed. There are two ways of using this correction.

10.1.2 One way is to use a straightforward gravity correction, in which case the magnitude of the factor is small.

$$\text{corrected mg As/}\ell = \text{mg As/}\ell \times \frac{1.024}{\text{specific gravity}}$$

10.1.3 The other method corrects for differences in total solids or salt content of various urine specimens, whereby the specific gravity of water is initially subtracted from the urine specific gravities. Because of the wide range of reported urine specific gravities, 1.002 to 1.040, the use of such a correction factor can lead to unusually or erroneously high results.

$$\text{corrected mg As/}\ell = \text{mg As/}\ell \times \frac{(1.024 - 1.000)}{(\text{specific gravity} - 1.000)}$$

10.2 The concentration of arsenic in air can be expressed as mg As per cubic meter of air, which is numerically equal to micrograms As per liter of air.

$$\text{mg As/m}^3 = \mu\text{g As/V}_s$$

where:

μg As = micrograms As from calibration curve (Section 9.2)

V_s = volume of air sampled in ℓ at 25°C and 760 mm Hg.

11. References

11.1 "Determination of Arsenic in Air," *Manual of Analytical Methods*, A.C.G.I.H. (1958).

11.2 Thomas and Collier, "The Concentration of Arsenic in Tobacco Smoke Determined by a Rapid Titrimetric Method," Journal of Industrial Hygiene and Toxicology, 27, 201 (1945).

11.3 *Methods of Analysis*, Association of official Agricultural Chemists, p. 632, 10th Edition, 1965.

11.4 Fernandez, Frank, J., "Atomic Absorption Determination of Gaseous Hydrides Utilizing Sodium Borohydride Reduction," Atomic Absorption Newsletter, 12, pp. 93-97 (1973).

2,4 TOLUENEDIISOCYANATE (TDI) IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	TDI	**Method No.:**	P&CAM 141
Matrix:	Air	**Range:**	0.007 ppm – 0.140 ppm
Procedure:	Colorimetric		
Date Issued:	11/17/72	**Precision:**	Unknown
Date Revised:	1/10/74	**Classification:**	D (Operational)

1. Principle of the Method

   1.1 TDI is hydrolyzed by the absorbing solution to the corresponding toluenediamine derivative.

   1.2 The diamine is diazotized by the sodium nitrite – sodium bromide solution.

   1.3 The diazo compound is coupled with N-(1-Naphthyl)-ethylenediamine to form a colored complex.

   1.4 The amount of colored complex formed is in direct proportion to the amount of TDI present. The amount of colored complex is determined by reading the absorbance of the solution at 550 nm.

   1.5 Toluenediamine is formed via hydrolysis of TDI on a mole to mole basis. This amine is used in place of the TDI for standards. This accomplishes two things. First, the amine is not as toxic as the TDI. Second, TDI liquifies to a semi-solid at room temperature. Weighing the semi-solid is more difficult than weighing the dry amine. Both compounds have been tested by this method and the results compare favorably.

   1.6 TDI kits based on the Marcali method are commercially available but have not been thoroughly tested to date.

2. Range and Sensitivity

   2.1 The range of the standards used is equivalent to 1.0 – 20.0 μg TDI. In a 20 liter air sample, this range converts to 0.007 ppm – 0.140 ppm.

2.2 For samples of high concentration whereby absorbance is greater than the limits of the standard curve (1.0 – 20.0 μg TDI) sample dilution with absorbing solution and rereading the absorbance extends the upper limit of the range.

2.3 It is not known how much TDI would saturate 15 mℓ of the absorbing solution. It may be possible that, in extremely high concentrations, some of the TDI would not be absorbed by the absorbing solution. Therefore, if a sample is diluted and reread, it could give an erroneously low value.

2.4 A single bubbler absorbs 95% of the diisocyanate if the air concentration is below 2 ppm. Above 2 ppm, about 90% of the diisocyanate is recovered. At high levels, it is suggested that two impingers in series be used.

3. Interferences

3.1 Any free aromatic amine may give a coupling color and thus may be a positive interference.

3.2 Methylene-di-(4-phenylisocyanate) (MDI) will form a colored complex in this reaction. However, its color development time is about 1 – 2 hours compared with 5 minutes for TDI. Therefore, MDI is not a serious problem.

4. Precision and Accuracy

4.1 This method is a modification of the Marcali method. Its precision and accuracy are unknown.

4.2 There has been no collaborative testing.

5. Advantages and Disadvantages of the Method

5.1 This method is based on the well tested Marcali method.

5.2 Any free organic amine will interfere. The method cannot be considered specific for TDI.

6. Apparatus

6.1 **Sampling Equipment.** The sampling unit for personal samples by the impinger collection method consists of the following components:

6.1.1 An all glass midget impinger containing the absorbing solution or reagent.

6.1.2 Battery operated personal sampling pump – MSA Model G or equivalent. The sampling pump is protected from splashover or water condensation by an adsorption tube loosely packed with a plug of glass wool and inserted between the exit arm of the impinger and the pump.

6.1.3 An integrating volume meter such as a dry gas or wet test meter.

6.1.4 Thermometer.

6.1.5 Manometer.

6.1.6 Stopwatch.

6.1.7 Various clips, tubing, spring connectors, and belt for connecting sampling apparatus to worker being sampled.

6.2 Beckman Model B spectrophotometer or equivalent.

6.3 Cells, 1-cm and 5-cm matched quartz cells.

6.4 Volumetric flasks (several of each), glass stoppered: 50, 100, 1000 mℓ.

6.5 Balance capable of weighing to at least three decimal places, preferably 4 decimal places.

6.6 Pipets: 0.5, 1, 15 mℓ.

6.7 Graduated cylinders: 25, 50 mℓ.

7. **Reagents**

All reagents must be made using ACS reagent grade or a better grade.

7.1 Double distilled water.

7.2 2,4-toluenediamine.

7.3 Hydrochloric acid, concentrated, 11.7 N.

7.4 Glacial acetic acid, concentrated, 17.6 N.

7.5 Sodium nitrite.

7.6 Sodium bromide.

7.7 **Sodium nitrite solution:** Dissolve 3.0 g sodium nitrite and 5.0 g sodium bromide in about 80 mℓ double distilled water. Adjust volume to 100 mℓ with double distilled water. This solution is stable for one week if refrigerated.

7.8 Sulfamic acid.

7.9 **Sulfamic acid solution, 10% w./v.:** Dissolve 10 g sulfamic acid in 100 mℓ double distilled water.

7.10 N-(1-Naphthyl)-ethylenediamine dihydrochloride.

7.11 **N-(1-Naphthyl)-ethylenediamine solution:** Dissolve 50 mg in about 25 mℓ double distilled water. Add 1 mℓ concentrated hydrochloric acid and dilute to 50 mℓ with double distilled water. Solution should be clear and colorless; coloring is due to contamination by free amines, and solution should not be used. The solution is stable for four days.

7.12 **Absorbing solution:** Add 35 mℓ concentrated hydrochloric acid and 22 mℓ glacial acetic acid to approximately 600 mℓ double distilled water. Dilute the solution to 1-liter with double distilled water. 15 mℓ is used in each impinger.

7.13 **Standard solution A:** Weigh out 140 mg of 2,4 – toluenediamine (equivalent to 200 mg of 2,4 – toluenediisocyanate). Dissolve in 660 mℓ of glacial acetic acid, transfer to a 1-liter glass stoppered volumetric flask, and make up to volume with distilled water.

7.14 **Standard solution B:** Transfer 10 mℓ of standard solution A to a glass-stoppered 1-liter volumetric flask. Add 27.8 mℓ of glacial acetic acid so that when solution B is diluted to 1-liter with distilled water, it will be 0.6N with respect to acetic acid. This solution contains an equivalent of 2 μg TDI/mℓ.

8. **Procedure**

8.1 **Cleaning of Equipment**

8.1.1 Wash all glassware in a hot detergent solution, such as Alconox to remove any oil.

8.1.2 Rinse well with hot tap water.

8.1.3 Rinse well with double distilled water. Repeat this rinse several times.

8.2 Collection and Shipping of Samples

8.2.1 Pipet 15 mℓ of the absorbing solution (Section 7) into the midget impinger.

8.2.2 Connect the impinger (via the absorption tube) to the vacuum pump and the prefilter assembly (if needed) with a short piece of flexible tubing. The minimum amount of tubing necessary should be used between the breathing zone and impinger. The air being sampled should not be passed through any other tubing or other equipment before entering the impinger.

8.2.3 Turn on pump to begin sample collection. Care should be taken to measure the flow rate, time and/or volume as accurately as possible. Record atmospheric pressure and temperature. The sample should be taken at a flow rate of 1 ℓpm. Sample for 20 minutes, making the final air volume 20 liters.

8.2.4 After sampling, the impinger stem can be removed and cleaned. Tap the stem gently against the inside wall of the impinger bottle to recover as much of the sampling solution as possible. Wash the stem with a small amount (1-2 mℓ) of unused absorbing solution and add the wash to the impinger. Then the impinger is sealed with a hard, non-reactive stopper (preferably Teflon or glass). Do not seal with rubber. The stoppers on the impingers should be tightly sealed to prevent leakage during shipping. If it is preferred to ship the impingers with the stems in, the outlets of the stem should be sealed with Parafilm or other non-rubber covers, and the ground glass joints should be sealed (i.e., taped) to secure the top tightly.

8.2.5 Care should be taken to minimize spillage or loss by evaporation at all times. Refrigerate samples if analysis cannot be done within a day.

8.2.6 Whenever possible, hand delivery of the samples is recommended. Otherwise, special impinger shipping cases designed by NIOSH should be used to ship the samples.

8.2.7 A "blank" impinger should be handled as the other samples (fill, seal and transport) except that no air is sampled through this impinger.

8.3 Analysis of Samples

8.3.1 Remove bubbler tube, if it is still attached, taking care not to remove any absorber solution.

8.3.2 Start reagent blank at this point by adding 15 mℓ fresh absorbing solution to a clean bubbler cylinder.

8.3.3 To each bubbler, add 0.5 mℓ of 3% sodium nitrite solution, gently agitate, and allow solution to stand for 2 minutes.

8.3.4 Add 1 mℓ of 10% sulfamic acid solution, agitate for 30 seconds and allow solution to stand 2 minutes to destroy all the excess nitrous acid present.

8.3.5 Add 1 mℓ of 0.1% N-(1-Naphthyl)-ethylenediamine solution. Agitate and allow color to develop. Color will be developed in 5 minutes. A reddish blue color indicates the presence of TDI.

8.3.6 Add double distilled water to adjust the final volume to 20 mℓ in the bubbler cylinder. Mix.

8.3.7 Transfer each solution to a 1-cm or 5-cm quartz cell.

8.3.8 Using the blank, adjust the spectrophotometer to 0 absorbance at 550 nm.

8.3.9 Determine the absorbance of each sample at 550 nm.

9. **Calibration and Standards**

9.1 To each of a series of eight graduated cylinders add 5 mℓ of 1.2N hydrochloric acid.

9.2 To these cylinders add the following amounts of 0.6N acetic acid: 10.0, 9.5, 9.0, 8.0, 7.0, 6.0, 5.0 and 0.0 mℓ, respectively.

9.3 To the cylinder add standard solution B in the same order as the acetic acid was added: 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 10 mℓ, so that the final volume is 15 mℓ (i.e., 0.0 mℓ of the standard is added to the 10 mℓ acetic acid; 0.5 mℓ of the standard is added to the 9.5 mℓ acid; et cetera). The cylinders now contain the equivalent of 0.0, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 20.0 μg TDI, respectively. The standard containing 0.0 mℓ standard solution is a blank.

9.4 Add 0.5 mℓ of the 3.0% sodium nitrite reagent to each cylinder. Mix. Allow to stand 2 minutes.

9.5 Add 1 mℓ of the 10% sulfamic acid solution. Mix. Allow to stand for 2 minutes.

9.6 Add 1 mℓ of the N-(1-Naphthyl)-ethylenediamine solution. Mix. Let stand for 5 minutes.

9.7 Make up to 20 mℓ with double distilled water.

9.8 Transfer each solution to a 1-cm or 5-cm quartz cell.

9.9 Using the blank, adjust the spectrophotometer to 0 absorbance at 550 nm.

9.10 Determine the absorbance of each standard at 550 nm.

9.11 A standard curve is constructed by plotting the absorbance against micrograms TDI.

10. **Calculations**

10.1 Subtract the blank absorbance, (Section 8.2.7), if any, from the sample absorbance.

10.2 From the calibration curve (Section 9.11), read the micrograms TDI corresponding to the absorbance of the sample.

10.3 Calculate the concentration of TDI in air in ppm, defined as $\mu\ell$ TDI per liter of air.

$$ppm = \frac{\mu g}{V_s} \times \frac{24.45}{MW}$$

where:

ppm = parts per million TDI
μg = micrograms TDI (Section 10.2).
V_s = corrected volume of air in ℓ (Section 10.4).
24.45 = molar volume of an ideal gas at 25°C and 760 mm Hg
MW = molecular weight of TDI, 174.15.

10.4 Convert the volume of air sampled to standard conditions of 25°C and 760 mm Hg:

$$V_s = V \times \frac{P}{760} \times \frac{298}{T + 273}$$

where:

V_s = volume of air in liters at 25°C and 760 mm Hg
V = volume of air in liters as measured
P = Barometric Pressure in mm Hg
T = Temperature of air in degree centigrade.

11. References

11.1 Marcali, K., "Microdetermination of Toluenediisocyanates in Atmosphere," Analytical Chemistry, 4, 552 (1957).

11.2 Larkin, R.L. and R.E. Kupel, "Microdetermination of Toluenediisocyanate Using Toluenediamine as the Primary Standard," American Industrial Hygiene Association Journal, 30, 640 (1969).

TIO

p,p- DIPHENYLMETHANE DIISOCYANATE (MDI) IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	MDI	Method No.:	P&CAM 142
Matrix:	Air	Range:	0.007-0.073 ppm
Procedure:	Colorimetric	Precision:	Unknown
Date Issued:	9/13/72		
Date Revised:	1/10/74	Classification:	D (Operational)

1. Principle of the Method

   1.1 MDI is hydrolyzed by the absorbing solution to methylene dianiline.

   1.2 The methylene dianiline is diazotized by sodium nitrite – sodium bromide.

   1.3 The diazo compound is coupled with N-(1-Naphthyl)-ethylenediamine to form the colored complex.

   1.4 The amount of colored complex formed is in direct proportion to the amount of MDI present. The amount of colored complex is determined by reading the absorbance of the solution at 555 nm.

2. Range and Sensitivity

   2.1 The range of the standards used is 1.5-15 μg MDI. In a 20 liter air sample, this range converts to 0.007 ppm-0.073 ppm.

   2.2 For samples of high concentration whereby absorbance is greater than the limits of the standard curve, sample dilution with absorber solution and rereading absorbance can extend the upper limit of the range.

   2.3 It is not known how much MDI would saturate 15 mℓ of the absorber solution. Therefore, if a sample is diluted and reread, it could give an erroneously low value. It may be possible that, in extremely high concentrations, some of the MDI would not be absorbed by the absorber solution. In such cases two impingers should be used in series, and the appropriate corrections are made for efficiency.

3. Interferences

3.1 Any free aromatic amine may be diazotized and coupled forming a positive interference.

3.2 Toluenediisocyanate (TDI) also interferes.

4. Precision and Accuracy

4.1 The precision and accuracy are unknown.

4.2 There has been no collaborative testing.

5. Advantages and Disadvantages of the Method

5.1 This method is a modification of the Marcali method. This method has altered the concentration of acidity during the coupling reaction, reducing the final reaction time to fifteen minutes compared to two hours for the Marcali method.

5.2 Since any free aromatic amine may interfere and TDI definitely interferes the method is not specific for MDI.

6. Apparatus

6.1 **Sampling Equipment.** The sampling unit for personal samples by the impinger collection method consists of the following components:

6.1.1 A midget impinger containing the absorbing solution or reagent.

6.1.2 Battery operated personal sampling pump – MSA Model G or equivalent. The sampling pump is protected from splashover or water condensation by an adsorption tube loosely packed with a plug of glass wool and inserted between the exit arm of the impinger and the pump.

6.1.3 An integrating volume meter such as a dry gas or wet test meter.

6.1.4 Thermometer.

6.1.5 Manometer.

6.1.6 Stopwatch.

6.1.7 Various clips, tubing, spring connectors, and belt for connecting sampling apparatus to worker being sampled.

6.2 Beckman Model B spectrophotometer or equivalent.

6.3 Cells, 5-cm matched quartz cells.

6.4 Volumetric flasks (several of each): 100 mℓ and 1-liter.

6.5 Balance capable of weighing to at least three places, preferably four places.

6.6 Pipets, delivery: 0.5, 1, 2, 5, 10, 15 mℓ.
graduated: 2 mℓ

6.7 Graduated cylinders: 50, 100 mℓ.

7. **Reagents**

All reagents must be made using ACS reagent grade or a better grade.

7.1 Double distilled water.

7.2 Sodium nitrite.

7.3 Sodium bromide.

7.4 Sulfamic acid.

7.5 Concentrated hydrochloric acid, 11.7N.

7.6 Glacial acetic acid, 17.6N.

7.7 N-(1-Naphthyl)-ethylenediamine dihydrochloride.

7.8 Sodium carbonate.

7.9 Methylene Dianiline (MDA).

7.10 **Sodium Nitrite – Sodium Bromide Solution:** Dissolve 3.0 g sodium nitrite and 5.0 g sodium bromide in double distilled water and dilute to 100 mℓ. The solution maybe stored in the refrigerator for one week.

7.11 **Sulfamic Acid Solution.** Dissolve 10.0 g sulfamic acid in 100 mℓ double distilled water.

7.12 **Absorbing Solution.** Add 35 mℓ concentrated hydrochloric acid and 22 mℓ glacial acetic acid to about 600 mℓ double distilled water and dilute to 1-liter with double distilled water.

7.13 **Coupling Solution.** Dissolve 0.1g N-(1-Naphthyl)-ethylenediamine dihydrochloride in 50 mℓ water, add 2 mℓ concentrated hydrochloric acid and dilute to 100 mℓ with double distilled water. This solution is stable for about ten days.

7.14 **Sodium Carbonate.** Dissolve 16.0 g sodium carbonate in double distilled water and dilute to 100 mℓ.

7.15 **Solution A.** Dissolve 0.238 g MDA in 700 mℓ glacial acetic acid. Dilute to 1-liter with double distilled water. This is equivalent to 300 mg MDI/1.

7.16 **Solution B.** Immediately after making solution A, pipet 10 mℓ solution A into a 1-liter volumetric flask. Add 35 mℓ concentrated hydrochloric acid, 15 mℓ glacial acetic acid, dilute to volume with double distilled water. This solution contains the equivalent of 3µg MDI/ml.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 Wash all glassware in hot detergent solution, such as alconox, to remove any oil.

8.1.2 Rinse well with hot tap water.

8.1.3 Rinse well with double distilled water. Repeat this rinse several times.

8.2 Collection and Shipping of Samples

8.2.1 Pipet 15 mℓ of the absorbing solution (Section 7) into the midget impinger.

8.2.2 Connect the impinger (via the absorption tube) to the personal sampling pump with a short piece of flexible tubing. The minimum amount of tubing should be used between the sampling zone and impinger. The air being sampled should not be passed through any other tubing or other equipment before entering the impinger.

8.2.3 Turn on pump to begin sample collection. Care should be taken to measure the flow rate, time and/or volume as accurately as possible. Record atmospheric pressure and temperature. The sample should be taken at a flow rate of 1 ℓpm. Sample for 20 minutes making the final volume 20 liters.

8.2.4 After sampling, the impinger stem can be removed and cleaned. Tap the stem gently against the inside wall of the impinger bottle to recover as much of the sampling solution as possible. Wash the stem with a small amount (1-2 mℓ) of unused absorbing solution and add the wash to the impinger. Then the impinger is sealed with a hard, non-reactive stopper (preferably Teflon or glass). Do not seal with rubber. The stoppers on the impingers should be tightly sealed to prevent leakage during shipping. If it is preferred to ship the impingers with the stems in, the outlets of the stem should be sealed with Parafilm or other non-rubber covers, and the ground glass joints should be sealed (i.e., taped) to secure the top tightly.

8.2.5 Care should be taken to minimize spillage or loss by evaporation at all times. Refrigerate samples if analysis cannot be done within a day.

8.2.6 Whenever possible, hand delivery of the samples is recommended. Otherwise, special impinger shipping cases designed by NIOSH should be used to ship the samples.

8.2.7 A "blank" impinger should be handled as the other samples (fill, seal and transport) except that no air is sampled through this impinger.

8.3 **Analysis of Samples**

8.3.1 Remove the bubbler tube, if it is still attached, taking care not to remove any absorbing solution.

8.3.2 Start reagent blank at this point by adding 15 mℓ fresh absorbing solution to a clean bubbler cylinder.

8.3.3 To each cylinder, add 0.5 mℓ sodium nitrite solution, stir well, and allow to stand for 2 minutes.

8.3.4 Add 1 mℓ 10% sulfamic acid solution, stir for 30 seconds, and allow to stand for 2 minutes.

8.3.5 Add 1.5 mℓ sodium carbonate solution and stir.

8.3.6 Add 1 mℓ coupling solution and stir. Allow color to develop for 15 to 30 minutes.

8.3.7 Transfer each solution to a 5-cm quartz cell.

8.3.8 Using the blank, adjust the spectrophotometer to 0 absorbance at 555 nm.

8.3.9 Determine the absorbance of each sample at 555 nm.

9. Calibration and Standards

9.1 To a series of five impinger cylinders, add the following amounts of absorbing solution: 15.0, 14.5, 14.0, 13.0, 10.0 mℓ respectively.

9.2 To the cylinder add standard solution B in the same order as the absorbing solution was added: 0.0, 0.5, 1.0, 2.0, 5.0 mℓ, so that the final volume is 15 mℓ (i.e., 0.0 mℓ of standard is added to the 15 mℓ absorbing solution; 0.5 mℓ of standard is added to the 14.5 absorber solution, etc.). The cylinders now contain an amount of MDA equivalent to 0.0, 1.5, 3.0, 6.0, 15 µg MDI, respectively. The standard containing 0.0 ml standard solution is a blank.

9.3 To each cylinder, add 0.5 mℓ sodium nitrite solution, stir well and allow to stand for 2 minutes.

9.4 Add 1 mℓ of 10% sulfamic acid solution, stir for 30 seconds, and allow to stand for 2 minutes.

9.5 Add 1.5 mℓ sodium carbonate solution and stir.

9.6 Add 1 mℓ coupling solution and stir. Allow color to develop for 15 to 30 minutes.

9.7 Transfer each solution to a 5-cm quartz cell.

9.8 Using the blank, adjust the spectrophotometer to 0 absorbance at 555 nm.

9.9 Determine the absorbance of each standard at 555 nm.

9.10 A standard curve is constructed by plotting the absorbance against micrograms MDI.

10. Calculations

10.1 Blank values (Section 8.2.7), if any, should first be subtracted from each sample.

10.2 From the calibration curve (Section 9.10), read the micrograms MDI corresponding to the absorbance of the sample.

10.3 Calculate the concentration of MDI in the air sampled in ppm, defined as µℓ MDI per liter of air.

$$ppm = \frac{\mu g}{V_s} \times \frac{24.45}{MW}$$

where:

ppm = parts per million MDI

μg = micrograms MDI (Section 10.2)

V_s = corrected volume of air (Section 10.4)

24.45 = molar volume of an ideal gas at 25°C and 760 mm Hg

MW = molecular weight of MDI, 250.27.

10.4 Connect the volume of air sampled to standard conditions of 25°C and 760 mm Hg:

$$V_s = V \times \frac{P}{760} \times \frac{298}{T + 273}$$

where:

V_s = volume of air in liters at 25°C and 760 mm Hg

V = volume of air in liters as measured

P = Barometric Pressure in mm Hg

T = Temperature of air in degree centigrade.

11. References

11.1 Grim, K.E., and A.L. Linch, "Recent Isocyanate-in-Air Analysis Studies," American Industrial Hygiene Association Journal, 25, 285 (1964).

MERCURY IN URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Mercury	Method No.: P&CAM 145
Matrix: Urine	Range: 0.04 to 0.15 mg/ℓ in 100 mℓ of urine
Procedure: Colorimetry	Precision: Unknown
Date Issued: 9/11/72	Classification: D (Operational)
Date Revised: 1/29/76	

1. Principle of the Method

The urine sample is digested with concentrated nitric acid and potassium permanganate. After destruction of the excess permanganate with hydroxylamine hydrochloride, the divalent mercury is extracted from the acid solution into chloroform with dithizone. The mercury is then selectively stripped from the organic layer with an acid solution of potassium bromide. The pH of this aqueous solution is adjusted to 6 and the isolated mercury is extracted again with dithizone into chloroform. The absorbance of the chelate at 485 nm is measured with a spectrophotometer.

2. Range and Sensitivity

2.1 The detection limit is about 4 μg of mercury in a 100-mℓ urine sample. The sensitivity may be improved by digesting a larger volume of urine.

2.2 The maximum amount of mercury that can be determined with the procedure described in Section 8 is 15 μg. In 100 mℓ of urine, this corresponds to 0.15 μg/mℓ. Larger amounts may be measured if the amount of dithizone solution used for extraction is increased.

3. Interferences

Many metals that form stable complexes with dithizone, such as copper, lead, silver, gold, palladium, and divalent platinum, are prevented from interfering by control of pH and by the selective complexation of mercury with bromide ion.

4. Precision and Accuracy

The precision and accuracy of measurements of mercury in urine by this method have not been determined.

5. Advantages and Disadvantages of the Method

5.1 The method is selective for mercury.

5.2 The analytical procedure is time-consuming and limits the number of samples that can be analyzed in 1 day.

6. Apparatus

6.1 Polyethylene or borosilicate bottles (125 mℓ).

6.2 A urinometer (or hydrometer capable of measuring specific gravities in the range of 1.000 to 1.040 with an uncertainty of only ±0.001 units).

6.3 Kjeldahl flasks (500 mℓ).

6.4 Separatory funnels (1 liter, 250 mℓ, and 125 mℓ) with a support rack.

6.5 Spectrophotometer or colorimeter.

6.6 Assorted laboratory glassware including graduated cylinders, volumetric flasks, and volumetric pipettes of appropriate sizes.

7. Reagents

All reagents must be analytical reagent grade. Doubly distilled or distilled deionized water should be used in preparing reagents and in the experimental procedure.

7.1 EDTA (ethylenediaminetetraacetic acid) or potassium persulfate.

7.2 Nitric acid, concentrated.

7.3 Potassium permanganate.

7.4 Hydroxylamine hydrochloride, solution "A" (20% w/v in water).

7.5 **Sodium Hydroxide Solution (0.05 *N*.)** Dissolve 2.0 g in water and dilute to 1 liter with water.

7.6 ***m*-Cresol Purple Indicator Solution (0.05% w/v).** Dissolve 0.05 g of the powder in 6 mℓ of 0.05 *N* NaOH and dilute with water to 100 mℓ in a volumetric flask.

7.7 **Phenol Red Indicator Solution (0.04% w/v).** Dissolve 0.1 g of the indicator in 6 mℓ of 0.05 *N* NaOH and dilute with water to 250 mℓ in a volumetric flask.

7.8 Ammonium hydroxide solution (1% v/v in water).

7.9 **Hydroxylamine Hydrochloride, Solution "B" (0.5% w/v).** Dissolve 5 g of hydroxylamine hydrochloride in water and dilute to 1 liter. Neutralize to pH 7.5 with 1% ammonium hydroxide, using phenol red indicator solution. The phenol red will change in color from yellow to red near pH 7.5.

7.10 **Treated Chloroform.** Extract 1 liter of reagent-grade chloroform with 100 mℓ of hydroxylamine hydrochloride solution "B". (See Reference 11.3.)

7.11 **Dithizone, Stock Solution (0.5 mg/mℓ).** Dissolve 0.5 g of dithizone (diphenylthiocarbazone) in treated chloroform and dilute with treated chloroform to 1 liter in a volumetric flask. Prepare more dilute solutions as needed by serial dilution with treated chloroform. Allow the dithizone solutions to stabilize overnight before use.

7.12 **Hydrochloric Acid (0.1*N*).** Dilute 8.6 mℓ of concentrated hydrochloric acid to 1 liter with water.

7.13 Potassium bromide solution (40% w/v in water).

7.14 **Buffer Solution.** Dissolve 300 g of anhydrous sodium hydrogen phosphate and 75 g of potassium carbonate in water and dilute to 2 liters with water (MacIlvaine's Buffer Solutions).

7.15 **Mercury Standard Solution.** Dissolve 0.1354 g of mercuric chloride, C.P., special reagent grade, in 1 *N* HCl and dilute to 100 mℓ with the acid. This solution contains 1 mg/mℓ of mercury in an acid solution and is quite stable. If any cloud or sediment develops, the solution should be discarded. More dilute standards may be made by serial dilutions with water.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware must be rendered free of mercury before use. This can be accomplished by using the following three-step procedure:

8.1.1 Wash in a detergent-tap water solution and follow with tap-water and distilled-water rinses.

8.1.2 Soak in a chromic acid-sulfuric acid solution and follow with tap and distilled-water rinses.

8.1.3 Soak in a concentrated nitric acid or concentrated nitric acid diluted 1:1 with distilled water for 30 min and follow with distilled and doubly distilled-water rinses.

This entire procedure must be used when initially preparing glassware for use in the mercury determination. For each successive use of equipment that has previously been subjected to the entire cleaning procedure, it is necessary only to use the nitric acid cleaning procedure described in step 8.1.3.

8.2 Collection and Shipping of Samples

8.2.1 Urine samples may be collected in mercury-free borosilicate or polyethylene bottles. However, a recent publication suggests that loss of mercury in urine may be attributed to storage in polyethylene bottles. (See Reference 11.4.) At least 125 mℓ should be collected. Preserve the sample by addition of 100 to 200 mg of EDTA to each sample of urine. It has recently been found that the addition of 100 mg of potassium persulfate per 100 mℓ of urine preserves urine satisfactorily. (See Reference 11.4.)

8.2.2 The samples should be shipped to the laboratory as quickly as possible and kept as cool as possible without freezing during storage and shipment.

8.3 Analysis of Samples

8.3.1 Measure the specific gravity of the urine with a urinometer (or hydrometer).

8.3.2 Digest 100 mℓ of the urine with 15 mℓ of concentrated nitric acid and 7.5 g of potassium permanganate by refluxing for 1 hr in a 500-mℓ Kjeldahl flask.

8.3.3 After cooling the sample to room temperature, reduce the permanganate by slow addition of hydroxylamine hydrochloride, solution "A". Then add 5 mℓ in excess.

8.3.4 After additional cooling, adjust the pH of the solution to approximately 2 with a dilute sodium hydroxide solution. This is conveniently done by adding the alkaline *m*-cresol purple indicator solution described in Section 7.6 until the color change (red to yellow) indicates a pH of approximately 2. Cool the solution. If the mercury content of the sample is completely unknown, it is advisable to take a small aliquot and make a preliminary measurement of mercury before proceeding with this method.

8.3.5 Transfer the digestion mixture or a proper aliquot to a 250-mℓ separatory funnel.

8.3.6 Extract with successive 5-mℓ portions of a 20-mg/ℓ dithizone solution. Withdraw the chloroform layers into another 250-mℓ separatory funnel.

8.3.7 Continue the extractions until the dithizone in the chloroform layer does not change color.

8.3.8 Wash the aqueous phase with two 3- to 5-mℓ portions of treated chloroform and add these to the extracts. Discard the aqueous phase.

8.3.9 To the chloroform extracts, add 50 mℓ of 0.1 *N* HCl and 10 mℓ of the 40% KBr solution.

8.3.10 Shake for 2 min. The mercury goes into the aqueous phase as H_2HgBr_4 while copper and bismuth remain in the dithizone, which is discarded. Wash the aqueous phase with a few milliliters of treated chloroform.

8.3.11 Let the phases separate well and discard completely all chloroform droplets. An aliquot of the stripping solution may be taken if necessary so that the amount of mercury will fall on the standard curve. If an aliquot is taken, dilute it to 50 mℓ in a volumetric flask with 0.1 *N* HCl and transfer the resulting solution to a clean 250-mℓ separatory funnel.

8.3.12 Add 10 mℓ of buffer solution to the appropriate separatory funnel to bring the pH to 6. Also add 10 mℓ of 10-mg/ℓ dithizone solution.

8.3.13 Shake the funnel well for 2 min and allow the phases to separate well. Avoid any exposure to direct sunlight or exceedingly bright artificial light.

8.3.14 Drain the aqueous layer from the separatory funnel and discard it. Allow the chloroform layer to fill the bore of the stopcock. Remove any traces of moisture from the stem of the funnel with a cotton swab on an applicator stick.

8.3.15 Rinse a cuvette twice with 1- to 2-mℓ portions of the chloroform layer and draw off the remainder into the cuvette.

8.3.16 Measure the absorbance at 485 nm against distilled chloroform in a spectrophotometer or colorimeter. A reagent blank should be carried through the entire procedure. The absorbance of the blank should be subtracted from the absorbances of the samples.

9. Calibration and Standards

9.1 **Preparation of Standards.** Three or four standards are sufficient to establish the calibration curve. The preparation of each standard is carried out in the following manner:

9.1.1 Place in a 125-mℓ separatory funnel 5 mℓ of the 50% KBr solution, 10 mℓ of the buffer solution, and aliquots of the appropriate mercury standard solution containing 0 to 15 μg of mercury. Add enough 0.1 *N* HCl to make the final volume 65 mℓ.

9.1.2 Add 10 mℓ of a 10-mg/ℓ dithizone solution, shake the funnel for 2 min, and allow the phases to separate well. Follow the procedural steps in 8.3.14, 8.3.15, and 8.3.16. The 10-mg/ℓ dithizone solution is of sufficient strength to cover the range from 0 to 15 μg of mercury. By using 20 mℓ instead of the standard 10 mℓ of this reagent, the concentration range covered can be doubled. It is not recommended to add more than 20 mℓ of 10-mg/ℓ dithizone to any sample.

9.2 Construction of the Calibration Curve

9.2.1 Plot the absorbance (corrected for a reagent blank) of each standard *versus* the amount (in μg) of mercury in it.

9.2.2 The amount of mercury (in μg) in a sample or sample aliquot (see Section 8.3.5) can be read directly from the curve. For an occasional mercury determination, it is better to bracket the sample with standards than to prepare the complete calibration curve.

10. Calculations

The concentration of mercury in the urine sample can be expressed as mg of mercury per liter of urine:

$$\text{mg Hg/ℓ} = \frac{\text{μg Hg (from calibration curve)}}{\text{mℓ of urine}}$$

10.1 The use of a specific gravity correction factor to normalize values to 1.024, the average specific gravity of urine, has been recommended. (See Reference 11.5.)

10.2 The preferred method of normalization is to make a straightforward gravity correction as follows:

$$\text{corrected mg Hg/ℓ} = \text{mg Hg/ℓ} \times \frac{0.024}{(\text{specific gravity} - 1.000)}$$

11. References

11.1 *"Manual of Analytical Methods,"* American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio, 1958.

11.2 Kinser, R.E., and J.L. Holtz in *"The Industrial Environment – Its Evaluation and Control, Syllabus,"* Powell, C.H., and A.D. Hosey, Eds., Public Health Service Publication No. 614, U.S. Department of Health, Education, and Welfare, Washington, D.C. 1965, p. B-19-8.

11.3 Hubbard, D.M., "Determination of Lead," Ind. Eng. Chem., Anal. Ed., 9, 493 (1937).

11.4 Trujillo, P., P. Stein, and E. Campbell, "The Preservation and Storage of Urine Samples for the Determination of Mercury," Amer. Ind. Hyg. Ass. J., 35, 257 (1974).

11.5 Elkins, H.B., "Concentration Adjustments in Urinalysis," Amer. Ind. Hyg. Ass. J., 35, 559 (1974).

SULFUR DIOXIDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Sulfur Dioxide	**Method No:**	P&CAM 146
Matrix:	Air	**Range**	0.01-10 ppm
Procedure:	Collection via Impinger Peroxide Absorption/Titration	**Precision:**	± 10% below the 0.1 ppm level
Date Issued:	9/14/72		
Date Revised:	1/15/74	**Classification:**	C (Tentative)

1. **Principle of the Method**

   1.1 Sulfur dioxide in air is absorbed via midget impingers, in 0.3N hydrogen peroxide (H_2O_2) reagent, adjusted to about pH 5.

   1.2 The stable and nonvolatile sulfuric acid formed in this process is then titrated with standardized sodium hydroxide.

2. **Range and Sensitivity**

   The method is applicable to the determination of SO_2 in air in the concentration range from about 0.01 to 10.0 ppm. For SO_2 concentrations greater than 0.8 ppm a second impinger connected in series can be used to maintain a 98% recovery efficiency.

3. **Interferences**

   3.1 Strong acidic gases trapped in the sample solution give erroneously high results, whereas the presence of alkaline gases or reactive solids such as NH_3 and CaO gives erroneously low results.

   3.2 Sulfuric acid does not interfere since it is not appreciably separated from the air stream owing to its small particle size. One possible exception when the relative humidity is greater than 85%, which could result in particle sizes greater than 1 micron (Reference 11.3).

   3.3 Sulfur trioxide gas, if present, would be a positive interference.

3.4 Sulfates do not interfere. Carbon dioxide does not interfere since it is not absorbed in the acid-absorbing reagent.

3.5 If large amounts of solid materials are present, a filter may be employed upstream advantageously. However, SO_2 may be lost, the extent of loss depending upon the composition of the particulate matter and the nature and retentive capacity of the filter used.

4. Precision and Accuracy

The error for the combined sampling and analytical technique is ± 10% at concentrations below 0.1 ppm with increasing accuracy at concentrations of 0.1 to 1 ppm. Measurements should be reported to the nearest 0.01 ppm.

5. Advantages and Disadvantages

5.1 Aside from the advantages and disadvantages indicated by the discussion of interferences, this method has other distinct advantages. It requires only simple equipment and can be performed by analysts having lesser skills.

5.2 It is preferable to the West-Gaeke method if SO_2 is the principal gaseous pollutant and if the samples must be stored for long periods prior to analysis.

6. Apparatus

6.1 **Sampling Equipment.** The sampling unit for the impinger collection method consists of the following components:

6.1.1 A prefilter unit (if needed) which consists of the filter media and cassette filter holder.

6.1.2 A midget impinger containing the absorbing solution or reagent.

6.1.3 A pump suitable for delivering desired flow rates. The sampling pump is protected from splashover or water condensation by an adsorption tube loosely packed with a plug of glass wool and inserted between the exit arm of the impinger and the pump.

6.1.4 An integrating volume meter such as a dry gas or wet test meter.

6.1.5 Thermometer.

6.1.6 Manometer.

6.1.7 Stopwatch.

6.2 **Burette.** A 25 or 50 mℓ burette graduated in 0.1 mℓ subdivisions, preferably with Teflon plug, should be capable of measuring volume with an accuracy of 0.05 mℓ. Use a 10-ml burette for the final titration in 8.3.2.

6.3 Assorted Glassware

7. **Reagents**

7.1 **Absorbing Solution.** Hydrogen Peroxide, 0.3N, pH 5. Dilute 17 mℓ of 30% H_2O_2 solution to 1.0 liter with distilled water. Determine the alkalinity of the solution by taking a measured aliquot, adding 3 drops of mixed indicator, and titrating with 0.002N HCl or HNO_3 until a pink color persists (pH 5). Calculate the amount of acid necessary to adjust the acidity of the bulk absorbing solution and add the required amount. The zero blank obtained by titrating an aliquot of this adjusted reagent with 0.002N NaOH to the equivalence point (green) should be no more than a few drops. The reagent is stable for at least one month at room temperature.

7.2 **Mixed Indicator, 0.1%.** Dissolve 0.06 bromocresol green and 0.04 methyl red in 100 mℓ of methanol. When stored in an amber bottle at room temperature the reagent is stable for at least six months.

7.3 **Sulfuric Acid Solution, 1N.** Prepare by slowly adding 28 mℓ of concentrated sulfuric acid (36N) to about 700 mℓ of distilled water in a 1-liter volumetric flask. Cool mixture and dilute to volume.

7.4 **Standard Sulfuric Acid Solution, 0.002N.** Prepare by diluting 4.0 mℓ of the 1N sulfuric acid solution to volume with distilled water in a 2-liter volumetric flask. Determine the normality of this solution either by a gravimetric barium sulfate method or with a primary standard such as $Na_2B_4O_7 \cdot 10H_2O$ or sodium carbonate. This standard sulfuric acid solution may be stored indefinitely without change in strength.

7.5 **Sodium Hydroxide Solution, 1N.** Dissolve 40 g of sodium hydroxide in 1 liter of water.

7.6 **Standard Sodium Hydroxide Solution, 0.002N.** Prepare 2 liters of this solution by diluting 4.0 mℓ of the 1N solution with freshly boiled CO_2 free distilled water. This solution is standardized as described in Section 9. Store the reagent in a polyethylene or other alkali-resistant bottle and restandardize bimonthly.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware used in this method must be free of acidity and alkalinity. The following procedure is recommended:

8.1.1 Wash in detergent – tap water and follow with tap water and distilled water rinses.

8.1.2 Soak in chromic acid for at least 30 minutes and follow with tap and distilled water rinses.

8.1.3 Soak in concentrated nitric acid for at least 30 minutes and follow with several tap, distilled, and double distilled water rinses.

This entire procedure need only be used when initially preparing glassware. For each following successive use of equipment previously subjected to the entire cleaning procedure, it is necessary only to wash the glassware in detergent and water, making sure to rinse it thoroughly with distilled and double distilled water.

8.2 **Collection and Shipping of Samples**

8.2.1 Pour 15 mℓ of the absorbing solution (Section 7) into the midget impinger, using a graduated cylinder to measure the volume.

8.2.2 Connect the impinger (via the adsorption tube) to the vacuum pump and the prefilter assembly (if needed) with a short piece of flexible tubing. The minimum amount of tubing necessary to make the joint between the prefilter and impinger should be used. The air being sampled should not be passed through any other tubing or other equipment before entering the impinger.

8.2.3 Turn on pump to begin sample collection. Care should be taken to measure the flow rate, time and/or volume as accurately as possible. The sample should be taken at a flow rate of 1-2 ℓpm. A sample size of not more than 100 liters and no less than 10 liters should be collected. The minimum volume of air sampled will allow the measurement at least 1/10 times the TLV, 1.3 mg/m^3 (760 mm Hg, 25°C).

8.2.4 After sampling, the impinger stem can be removed and cleaned. Tap the stem gently against the inside wall of the impinger bottle to recover as much of the sampling solution as possible. Wash the stem with a small amount (1-2 mℓ) of unused absorbing solution and add the wash to the impinger. Then the impinger is sealed with a hard, nonreactive stopper (preferably Teflon). Do not seal with rubber. The stoppers on the impingers should be tightly sealed to prevent leakage during shipping. If it is preferred to ship the impingers with the stems in, the outlets of the stem should be sealed with Parafilm or other nonrubber covers, and the ground glass joints should be sealed (i.e., taped) to secure the top tightly.

8.2.5 Care should be taken to minimize spillage or loss by evaporation at all times. Refrigerate samples if analysis cannot be done within a day.

8.2.6 Whenever possible, hand delivery of the samples is recommended. Otherwise, special impinger shipping cases designed by NIOSH should be used to ship the samples.

8.2.7 A "blank" impinger should be handled as the other samples (fill, seal and transport) except that no air is sampled through this impinger.

8.2.8 Where a prefilter has been used, the filter cassettes are capped and placed in an appropriate cassette shipping container. One filter disc should be handled like the other samples (seal and transport) except that no air is sampled through, and this is labeled as a blank.

8.3 Analysis of Samples

8.3.1 Transfer the collected sample from the impingers to a convenient titration-vessel. Rinse each impinger with two to three 1-mℓ portions of distilled water. Add the rinsings to the titration vessel.

8.3.2 Add three drops of mixed indicator solution and titrate the solution with standard 0.002N sodium hydroxide until the color changes from red to green.

8.3.3 A reagent blank should be titrated at the same time and in the same manner. The result, which should be less than 0.1 mℓ, should be subtracted from the sample titer.

9. Calibration and Standards

The measured concentration of absorbed SO_2 in the samples is based on the proper standardization of the sodium hydroxide solution. The solution is standardized by pipetting 25 mℓ of the standard sulfuric acid solution into an Erlenmeyer flask, adding 3 drops of mixed indicator and titrating with the NaOH solution to the green equivalence point. This determination must be done at least in duplicate and the solution must be restandardized periodically.

10. Calculations

10.1 Determine the normality of the standard sulfuric acid solution.

10.2 Determine the normality of the standard sodium hydroxide solution:

$$m\ell_{NaOH} \times N_{NaOH} = m\ell_{H_2SO_4} \times N_{H_2SO_4}$$

10.3 The analytical results are computed on the basis of the following reactions:

$$SO_2 + H_2O_2 \longrightarrow H_2SO_4$$

$$H_2SO_4 + 2\,NaOH \longrightarrow Na_2SO_4 + 2\,H_2O$$

Therefore,

$$\mu g\ SO_2 = m\ell_{NaOH} \times N_{NaOH} \times \frac{64}{2000} \times 10^6$$

Where:

$m\ell_{NaOH}$ = volume of NaOH standard needed to titrate the sample minus the blank value

N_{NaOH} = normality of NaOH standard

$\frac{64}{2000}$ = gram-milliequivalent weight of SO_2

10^6 = equivalent μg per gram

10.4 Convert the volume of air sampled to standard conditions of 25°C and 760 mm Hg.

$$V_s = V \times \frac{P}{760} \times \frac{298}{(T + 273)}$$

where:

V_s = volume of air in liters at 25°C and 760 mm Hg
V = volume of air in liters as measured
P = Barometric pressure in mm Hg
T = Temperature of air in degrees centigrade

10.5 The concentration of the SO_2 in the sample can be expressed in $\mu g/\ell$ which is numerically equal to mg/m^3.

$$mg/m^3 = \mu g/\ell = \frac{\mu g\ SO_2}{V_s}$$ (as calculated in 10.3) (as calculated in 10.4)

10.6 The SO_2 concentration in the sample can also be expressed in ppm, defined as $\mu\ell$ of SO_2 per liter of air.

$$ppm = \frac{\mu\ell SO_2}{V_s}$$

$$= \frac{24.45}{MW} \times \frac{\mu g\ SO_2}{V_s}$$

$$= \frac{0.382 \times \mu g\ SO_2}{V_s}$$

where:

24.45 = molar volume at 25°C and 760 mm Hg

MW = 64, molecular weight of SO_2

11. References

11.1 Jacobs, M.B., Greenburg, L., "Sulfur Dioxide in New York City Atmosphere," *Ind.*, 48:1517, 1956.

11.2 Jacobs, M.B., "The Chemical Analysis of Air Pollutants," *Interscience*, New York, 1960.

11.3 Hochheiser, Seymour, "Methods of Measuring and Monitoring Atmospheric Sulfur Dioxide," PHS Publication No. 999-AP-6.

11.4 Croxford, S.R., Slimming, D.W., Wilkins, E.T., "The Measurement of Atmospheric Pollution: The Accuracy of the Instruments and the Significance of the Results," Proceeding of the Harrogate Conference, London, England, 1960.

TOTAL PARTICULATE CHROMIUM IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Total Particulate Chromium	**Method No:**	P&CAM 152
Matrix:	Air	**Range:**	0.01 to < 0.4 mg/m^3 Air
Procedure:	Atomic Absorption		
Date Issued:	11/20/72	**Precision:**	Less than 5% relative standard deviation
Date Revised:	12/1/73	**Classification:**	D (Operational)

1. Principle of the Method

   1.1 Atmospheric samples are obtained by drawing a measured volume of air through a 0.8-μ filter.

   1.2 The filter is wet ashed and analyzed for chromium by atomic absorption spectroscopy using a nitrous oxide-acetylene flame.

2. Range and Sensitivity

   2.1 The sensitivity for chromium in solution by atomic absorption using a nitrous oxide-acetylene flame is about 0.1 $\mu g/m\ell$ and the linear range extends to about $4\mu g/m\ell$.

   2.2 The range in air, therefore, is from 0.01 mg/m^3 to greater than 0.4 mg/m^3 using the recommended volumes of 100 liters of air and 10 $m\ell$ of solution.

3. Interferences

   3.1 A number of metallic elements may interfere with the atomic absorption analysis of chromium, but the interferences that are possible are only serious at high levels of interfering ion concentration. These levels are not likely in the present analysis. Most notable among the interfering metals are iron and nickel. The interference is usually a suppression effect.

   3.2 These spectral interferences in the chromium determination are minimized by the proper choice of flame conditions. A nitrous oxide-acetylene flame is recommended in this method. If greater sensitivity is needed, an air-acetylene flame can

be used and sensitivity is aided by making the flame luminous (fuel rich), while interferences are minimized in a blue (lean, oxidizing) flame.

4. **Precision and Accuracy**

The relative standard deviation for determination of 10 spiked filters near the threshold limit value was found to be less than 5%. The overall accuracy is not known but good recoveries are indicated.

5. **Advantages and Disadvantages**

5.1 The method has the advantage of using filter paper for convenience in sampling and shipping. The method is straightforward and requires few chemicals.

5.2 This method does not differentiate between hexavalent chromium and other forms. Moreover, the ashing procedure is long and somewhat tedious.

5.3 The possible degradation of the filters when H_2SO_4 mist is present has not been evaluated.

6. **Apparatus**

6.1 **Sampling Equipment.** The sampling unit for the filter-collection method has the following components:

6.1.1 The filter unit, consisting of the filter media and appropriate cassette filter holder, either a 2- or 3-piece filter cassette.

6.1.2 A vacuum pump such as a personal sampling pump. This pump must be properly calibrated so the volume of air sampled can be measured as accurately as possible. The pump must be calibrated with a representative filter unit in the line. Flow rate, times, and/or volume must be known.

6.1.3 Thermometer.

6.1.4 Manometer.

6.1.5 Stopwatch.

6.2 Millipore 37 mm HA type filter.

6.3 Atomic absorption spectrophotometer with a nitrous oxide-acetylene burner head, and requisite gas equipment.

6.4 Laboratory glassware for standards and sample handling.

7. **Reagents**

7.1 Double distilled water.

7.2 Redistilled HNO_3.

7.3 Chromium standard stock solution: Weigh 3.735 g of dry K_2CrO_4 into a clean 1000-mℓ volumetric flask, dissolve in double distilled water, add 50 mℓ of redistilled HNO_3, and dilute to mark with double distilled water. This solution contains 1000 μg Cr per mℓ.

8. **Procedure**

8.1 **Cleaning of Glassware**

8.1.1 All glassware should be detergent washed and tapwater rinsed.

8.2 **Collection and Shipping of Samples**

8.2.1 Weigh the filter disc, if needed. Assemble the filter unit by mounting the filter disc in the filter cassette.

8.2.2 Connect the exit end of the filter unit to the pump with a short piece of flexible tubing.

8.2.3 Turn on pump to begin sample collection. The flow rate, time, and/or volume must be measured as accurately as possible. The sample should be taken at a flow rate of 2 liters per minute. A minimum sample of 100 liters should be collected. Larger sample volumes are encouraged, provided the filters do not become loaded with dust to the point that loose material might fall off or the filter become plugged.

8.2.4 The sample cassettes should be shipped in a suitable container designed by NIOSH to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample is dislodged from the filter nor that the sample surface be disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the cassette on top of the sample filter.

8.2.5 With each batch of samples, one filter, labelled as a blank, must be submitted. No air should be drawn through this filter.

8.3 Analysis

8.3.1 To prepare the sample, place the filter carefully in a 125 mℓ Phillips beaker and add 3 mℓ of redistilled HNO_3. Cover the beaker with a watch glass and place on a hotplate. Heat the beaker to dryness. Continue adding HNO_3 one mℓ at a time and taking to dryness until no organic matter remains. Then add 0.5 mℓ of HNO_3 and several mℓ of water, swirl and place on the hotplate but do not take to dryness. Transfer the solution to a clean graduated centrifuge tube with several washings until the volume is 10 mℓ. Mix the contents well.

8.3.2 Analyze the samples for chromium by atomic absorption spectrophotometry using aspiration into a nitrous oxide-acetylene flame, a 357.9 nanometer wavelength setting and a 0.3-nanometer spectral band width unless sensitivity and signal-to-noise ratio are favorable to a narrower slit width. Record the concentration of chromium in the sample.

8.3.3 If necessary the samples can be diluted to bring the concentration down to the working range.

8.3.4 The blanks are treated and analyzed in the same manner as the sample.

9. Calibration

Working standards covering the analytical range of 0.1 μg/mℓ to 4 μg/mℓ should be made by diluting the standard stock solution with double distilled water and redistilled HNO_3 in sufficient quantity to maintain the 5% acid level. These should be used to make a calibration curve of μg Cr/mℓ solution versus absorbance.

10. Calculations

10.1 The concentration of Cr for the samples is read from the calibration curve and multiplied by the solution volume of 10 mℓ to give total Cr content.

10.2 The total chromium content can be expressed as CrO_3 by the following equation.

$$\frac{\mu g\ Cr}{0.52} = \mu g\ CrO_3$$

10.3 Convert the volume of air sampled to standard conditions of 25°C and 760 mm Hg.

$$V_s = V \times \frac{P}{760} \times \frac{298}{T + 273}$$

V_s = volume of air in liters at 25°C and 760 mm Hg

V = volume of air in liters as measured

P = barometric pressure in mm Hg

T = temperature of air in degree centigrade.

10.4 The concentration of CrO_3 in the air sample can be expressed in mg CrO_3 per cubic meter or μg CrO_3 per liter of air.

$$\text{mg } CrO_3/m^3 = \mu\text{g } CrO_3/V_s$$

11. References

11.1 *Industrial Toxicology*, Lawrence T. Fairhall, 2nd Edition, The Williams and Wilkins Company, pp. 37-38, 1957.

11.2 "Atomic Absorption Newsletter," F.L. Vogliotti, Vol. 9, No. 6, pp. 125-126, 1970.

11.3 "Analytical Methods for Atomic Absorption Spectrophotometry," Perkin-Elmer Corp., Cr, Standard Conditions, p. 2, 1971.

11.4 *Atomic Absorption Spectroscopy*, Christian, G.D., and Feldman, F.J., Wiley Interscience, pp. 285-292, 1970.

199

OZONE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Ozone	**Method No.:**	P&CAM 153
Matrix:	Air	**Range:**	0.01 to 10 ppm
Procedure:	KI Absorption – Colorimetric	**Precision:**	± 5% Deviation from the mean (Ref. 11.1)
Date Issued:	9/8/72	**Classification:**	B (Accepted)
Date Revised:	1/15/74		

1. **Principle of the Method**

   1.1 Air containing ozone is drawn through a midget impinger containing 10 mℓ of 1% potassium iodide in a neutral (pH 6.8) buffer composed of 0.1M disodium hydrogen phosphate and 0.1M potassium dihydrogen phosphate.

   1.2 The iodine liberated in the absorbing reagent is determined spectrophotometrically by measuring the absorption of the tri-iodide ion at 352 nm.

   1.3 The stoichiometry (Reference 11.1) is approximated by the following reaction:

$$O_3 + 3\,KI + H_2O \rightarrow KI_3 + 2KOH + O_2$$

   1.4 The analysis must be completed within 30 minutes to 1 hour after sampling.

2. **Range and Sensitivity**

   2.1 The range extends from 0.01 ppm to about 10 ppm.

   2.2 The sensitivity of the method is dependent on the volume of air sampled.

3. Interferences

3.1 The negative interference of reducing gases such as sulfur dioxide and hydrogen sulfide are very serious (probably on a mole-to-mole equivalency).

3.2 Interference from high concentrations of sulfur dioxide can be eliminated by using a prefilter consisting of a U-tube filled with strips of glass fiber paper impregnated with chromium trioxide.

3.3 The procedure is very sensitive to reducing dusts which may be present in the air or on the glassware. Losses of iodine occur even on clear glass surfaces, and thus the manipulations should minimize the exposure.

3.4 Halogens, hydrogen peroxide, organic peroxides, organic nitrites and various other oxidants will liberate iodine as well as ozone by this method.

3.5 Peroxyacetyl nitrate gives a response approximately equivalent to 50% of that of an equimolar concentration of ozone (Reference 11.1). Concentrations in the atmosphere may range up to 0.1 ppm.

4. Precision and Accuracy

The accuracy and precision of this method have not been completely determined at this time. No collaborative tests have been performed on this method.

5. Advantages and Disadvantages

5.1 The method is simple.

5.2 The analysis must be completed during the period of 30 to 60 minutes after sampling due to the instability of the colors.

6. Apparatus

6.1 Sampling Equipment. The sampling unit for the impinger collection method consists of the following components:

6.1.1 A graduated midget impinger containing the absorbing solution or reagent.

6.1.2 A pump suitable for delivering desired flow rates. The sampling pump is protected from splashover or water condensation by an absorption tube loosely packed with a plug of glass wool and inserted between the exit arm of the impinger and the pump.

6.1.3 An integrating volume meter such as a dry gas or wet test meter.

6.1.4 Thermometer.

6.1.5 Manometer.

6.1.6 Stopwatch.

6.2 Spectrophotometer capable of measuring the yellow color at 352 nm.

6.3 Matched cells or cuvettes of 2 cm path length.

6.4 Associated laboratory glassware.

7. **Reagents**

The reagents described must be made up using ACS reagent grade or better grade of chemical.

7.1 **Double distilled water.** Double distilled water should be used for all reagents. The double distilled water can be prepared in an all-glass still by adding potassium permanganate to produce a faint pink color and barium hydroxide to alkalize the distilled water before the second distillation.

7.2 **Absorbing reagent.** (1% KI in 0.1 M phosphate buffer). Dissolve 13.61g of potassium dihydrogen phosphate, 14.20g of anhydrous disodium hydrogen phosphate (or 35.82g of dodecahydrate salt) and 10.00g of potassium iodide successively and dilute the mixture to exactly 1 liter with double distilled water. Age at room temperature for at least 1 day before using. This solution may be stored for several weeks in a glass stoppered brown bottle in the refrigerator or for shorter periods of time at room temperature. Do *not* expose to sunlight.

7.3 **Standard iodine solution.** 0.025 M I_2 (0.05N). Dissolve successively 16.0g of potassium iodide and 3.1730g of iodine with double distilled water and make up to a volume of exactly 500 mℓ. Age for at least 1 day before using. Standardize shortly before use against 0.025 M $Na_2S_2O_3$. The sodium thiosulfate is standardized against primary standard bi-iodate, $KH(IO_3)_2$, or potassium dichromate, $K_2Cr_2O_7$.

8. Procedure

8.1 Cleaning of equipment. All glassware should be cleaned with *dichromate* cleaning solution followed by thorough rinsing with tap water and distilled water.

8.2 Collection and Shipping of Samples

8.2.1 Pipet 10 mℓ of the absorbing solution (Section 7) into the midget impinger.

8.2.2 Connect the impinger (via the absorption tube) to the vacuum pump and the prefilter assembly (if needed) with a short piece of flexible tubing (not rubber). The minimum amount of tubing necessary to make the joint between the prefilter and impinger should be used. The air being sampled should not be passed through any other tubing or other equipment before entering the impinger.

8.2.3 Turn on pump to begin sample collection. Care should be taken to measure the flow rate, time and/or volume as accurately as possible. The sample should be taken at a flow rate of 1-2 ℓpm. If the sample air temperature and pressure deviate greatly from 25°C and 760mmHg, measure and record these values.

8.2.4 Sufficient air should be sampled so that the equivalent of 0.5 to 10 μl of ozone is absorbed. Sampling periods of longer than 30 minutes should be avoided. For a flow rate of 2 liters per minute, a 30 minute sample should yield a sensitivity of 0.01 ppm. Do *not* expose the absorbing reagent to direct sunlight.

8.2.5 After sampling, the impinger stem can be removed and cleaned. Tap the stem gently against the inside wall of the impinger bottle to recover as much of the sampling solution as possible. Wash the stem with a small amount (<1 mℓ) of unused absorbing solution and add the wash to the impinger. Then the impinger is sealed with a hard, non-reactive stopper (preferably Teflon). Do not seal with rubber.

8.2.6 The sample solutions must be analyzed within 60 minutes after sampling.

8.2.7 A "blank" impinger should be handled as the other samples (fill and seal) except that no air is sampled through this impinger.

8.3 Analysis

8.3.1 Record the total volume of sample solution present in the graduated impinger. This is usually 10 mℓ.

8.3.2 Transfer the exposed absorbing reagent (avoiding dilution with rinse water) to a clean cell or cuvette.

8.3.3 Determine the absorbance at 352 nm using a matched cell or cuvette freshly filled with distilled water as the reference. This measurement must be done within 30 to 60 minutes after sampling.

8.3.4 Determine daily the blank correction (to be deducted from sample absorbance) by reading the absorbance of the "blank" sample using distilled water as the reference.

8.3.5 Samples having a color too dark to read may be quantitatively diluted with additional absorbing reagent and the absorbance of the diluted solution read. The dilution factor must then be introduced into the calculations.

9. Calibration and Standards

9.1 Freshly prepare 0.00125 M (0.0025 N) iodine standard by pipetting exactly 5 mℓ of the 0.025 M (0.05 N) standard stock solution into a 100 mℓ volumetric flask and diluting to the mark with the absorbing reagent.

9.2 Pipet 0.2, 0.4, 0.6, and 0.9 mℓ portions of the diluted standard iodine in separate 25 mℓ volumetric flasks and dilute to the mark with absorbing reagent. Mix thoroughly. The concentrations of these solutions (must be based on exact molar concentration as determined from the molarity of the standard iodine solution) are respectively – 0.25, 0.50, 0.75, and 1.125 μmoles I_2 per 25 mℓ; or more conveniently expressed as 0.10, 0.20, 0.30, and 0.45 μmoles of I_2 per 10 mℓ of absorbing reagent.

9.3 Immediately after the preparation of this known series, read the absorbance of each at 352 nm in the usual manner.

9.4 Plot the corrected absorbances (absorbance minus absorbance of reagent blank) of the known standards against the exact calculated molar concentrations (in μmoles per 10 mℓ). Draw the straight line that gives the best fit. Beer's Law is followed.

10. Calculations

10.1 The absorbance of the "blank" sample (Section 8.3.7) must first be subtracted from the sample absorbances.

10.2 As indicated by the stoichiometric equation in Section 1.3, it has been empirically determined that 1 mole of ozone (O_3) liberates 1 mole of iodine (I_2) by this procedure. Thus, one can read directly from the calibration curve (Section 9.4) the equivalent μmoles of O_3 per 10 mℓ of absorbing reagent. If the total volume of the sample solution is not 10 mℓ, or if the sample solution has been diluted prior to analysis, the dilution factor must be included in the calculation.

10.3 The concentration of ozone in the air sampled can be expressed as ppm of ozone, defined as $\mu\ell$ ozone per liter of air.

$$\text{ppm, } O_3 = \frac{\mu\text{moles } O_3 \times 24.45}{V_s}$$

where:

μmoles O_3 = micromoles ozone determined from calibration curve (Section 9.4) including the dilution factor, if any.

24.45 = molar volume of ozone ($\mu\ell/\mu$mole) at 25°C and 760 mm Hg.

V_s = volume of air sampled in ℓ at 25°C and 760 mm Hg.

11. References

11.1 "Tentative Method for the Manual Analysis of Oxidizing Substances in the Atmosphere," 44101-02-70T. American Public Health Association, Washington, D.C., 1972.

11.2 "Selected Methods for the Measurement of Air Pollutants," USDHEW Public Health Service Publication Number 999-AP-11, 1965.

OZONE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Ozone	Method No.: P&CAM 154
Matrix: Air	Range: 0.1 to 0.4 mg/m³ in a 45 - liter sample
Procedure: Collection *via* impinger, colorimetric alkaline KI method	Precision (CV_T): 0.08 in the range 0.1 to 0.4 mg/m³ in 45 - liter samples
Date Issued: 9/1/72	Classification: C (Tentative)
Date Revised: 1/29/76	

1. **Principle of the Method**

   Air containing ozone is drawn through an alkaline solution of potassium iodide. Ozone reacts to form a stable product that can be stored in solution with little loss for several days. Addition of acid to the solution liberates iodine in proportion to the collected ozone. The absorption of iodine at 352 nm is then measured spectrophotometrically.

2. Range and Sensitivity

   2.1 This method has been validated over the range 0.1 to 0.4 mg/m³. This is the limit of the useful range for the 45-liter sample size. The method is capable of measuring smaller or larger concentrations if the sampling size is adjusted, but it has not been validated for other sample sizes.

   2.2 The detection limit for a 45-liter sample size is estimated to be 0.04 mg/m³.

3. Interferences

   3.1 Chlorine, hydrogen peroxide, organic peroxides, and various other oxidants will liberate iodine by this method. The presence of strong oxidants such as chlorine causes serious positive interferences.

   3.2 The positive response in proportion to the nitrogen dioxide present is limited to about 10% by the use of sulfamic acid in the procedure to destroy nitrite, thus minimizing error due to the collection of nitrogen dioxide.

3.3 The negative interferences from reducing gases such as sulfur dioxide and hydrogen sulfide are serious (probably on a mole-to-mole equivalency). It has been shown that the interference due to sulfur dioxide can be eliminated by incorporating into the sampling train upstream from the impinger a glass-fiber paper impregnated with chromium trioxide and sulfuric acid. All of the sulfur dioxide can be removed from a mixture of 15 ppm of sulfur dioxide and 0.15 ppm of ozone when the sample is passed through such an absorber. However, since the absorber is also capable of oxidizing nitric oxide to nitrogen dioxide, it may increase the interference cited in Section 3.2. (See Reference 11.2.)

3.4 The procedure is very sensitive to reducing dusts that may be present in the air or on the glassware. Losses of iodine also occur even on clean glass surfaces and thus the manipulations should minimize this exposure.

4. Precision and Accuracy

4.1 The coefficient of variation (CV_T) for the total analytical and sampling method in the range of 0.1 to 0.4 mg/m³ is 0.08. Statistical information and details of the validation and experimental test procedures can be found in Reference 11.3.

4.2 The average recovery of ozone from vapor samples in the range 0.1 to 0.4 mg/m³ was 98% for the total analytical and sampling method.

5. Advantages and Disadvantages

5.1 The method is simple, accurate, and precise in the absence of interferences.

5.2 Those compounds listed in 3.1 will liberate iodine by this method causing positive interferences. Reducing gases or dusts cause negative interferences.

5.3 A delay of several days is believed to be permissible between sampling and completion of analysis. However, the exact relationship between delay and analytical results has not been established.

6. Apparatus

6.1 Midget impinger with a 1-mm i.d. nozzle, graduation marks, and a Teflon-coated stopper to replace the impinger top during shipment and storage.

6.2 A properly calibrated personal sampling pump capable of sampling at a flow rate of 1 ℓ/min for time intervals of up to 45 min. The pump should be calibrated with the midget impinger containing 10 mℓ of the absorbing reagent in the sampling train. A dry or wet test meter or a glass rotameter that will measure the appropriate flow rate within ±5% may be used for the calibration. The meter should include a prefilter to prevent liquid droplets from reaching it.

6.3 Spectrophotometer capable of measuring the absorbance at 352 nm with stoppered tubes or cuvettes, preferably of quartz or fused silica.

6.4 Associated laboratory glassware.

7. Reagents

The reagents described must be analytical reagent grade. Doubly distilled water should be used in the preparation of solutions and in the analytical procedure.

7.1 **Absorbing Reagent.** Dissolve 40.0 g of sodium hydroxide in almost a liter of water, then dissolve 10.0 g of potassium iodide and dilute the mixture to 1 liter with water. Store in a clean glass bottle with a screw cap (with inert liner) or rubber stopper (previously boiled for 30 min in alkali and washed). Age for at least 1 day before using.

7.2 **Acidifying Reagent.** Dissolve 5.0 g of sulfamic acid in 100 mℓ of water. then add 84 mℓ of 85% phosphoric acid and dilute to 200 mℓ with water.

7.3 **Potassium Iodate, Standard Solution.** Dissolve 0.758 g of potassium iodate in water and dilute to 1 liter in a volumetric flask. One milliliter of this stock solution is eqivalent to 400 μℓ of ozone. Prepare a dilute standard solution just before it is required by pipetting exactly 5 mℓ of stock solution into a 50-mℓ volumetric flask and diluting to the mark with water.

8. Procedure

8.1 **Cleaning of Equipment.** All glassware should be cleaned with a chromic acid – sulfuric acid cleaning solution followed by three rinses with tap water and three with distilled water.

8.2 **Collection and Shipping of Samples**

8.2.1 Assemble a train composed of a midget impinger, a flow meter. and a pump. Use ground-glass connections upstream from the impinger. Insure that all fittings are vacuum tight.

8.2.2 Pipette exactly 10 mℓ of the absorbing solution into the midget impinger and sample at a flow rate of 1 liter per minute. Note the volume of air sampled. Record atmospheric temperature and pressure.

8.2.3 Cap the impinger with the Teflon-coated stopper supplied for shipment to the laboratory.

8.3 **Analysis of Samples**

8.3.1 If the liquid level is below the 10-mℓ mark in the impinger, add distilled water to bring the level up to the mark.

8.3.2 Add 2 mℓ of the acidifying reagent with a volumetric pipette. Swirl gently to expel the carbon dioxide released immediately. Then cap and shake vigorously until bubbling ceases. It is imperative that *all* of the carbon dioxide is expelled; otherwise more may be released while the absorbance measurement is being made.

8.3.3 Place the stoppered impinger in a water bath at room temperature for 5 to 10 min to dissipate the heat of neutralization.

8.3.4 Transfer a portion of the sample to a cuvette and measure the absorbance at 352 nm. A cuvette containing distilled water is used as the reference. Do not delay the reading since reducing impurities sometimes cause rapid fading of the color.

8.3.5 Prepare a reagent blank by adding 2 mℓ of the acidifying reagent to 10 mℓ of unexposed absorbing reagent. Cool and determine the blank absorbance. The blank absorbance should be determined each day and should be subtracted from the absorbance of the samples.

8.3.6 Aliquots of samples may be taken before or after acidification if very large concentrations of oxidant are expected. When taking aliquots before acidification, dilute the aliquot to 10 mℓ with unexposed absorbing reagent and proceed in the usual manner. When taking aliquots after acidification, dilute the aliquot to 10 mℓ with reagent blank mixture. Dilution after acidification is not as reliable as before acidification and should be used only to save a sample when unexpectedly large concentrations of oxidant are encountered. The calculations should include the dilution factor.

9. Calibration and Standards

9.1 Add the freshly prepared, dilute standard iodate solution in graduated amounts of 0.1 to 0.5 mℓ (measured accurately in a graduated pipette or small burette) to a series of 25-mℓ glass-stoppered volumetric flasks. Make at least four standards.

9.2 Add alkaline potassium iodide solution (measured accurately in a graduated pipette) to make the total volume of each exactly 10 mℓ.

9.3 Acidify and determine the absorbances of each standard as was done for the samples.

9.4 Plot the absorbance of each standard (after correction for the blank) against its equivalent ozone concentration in 10 mℓ of absorbing solution. One milliliter of the diluted standard iodate solution in a total of 10 mℓ of the absorbing solution (see Section 9.2) is equivalent to 78 μg (40 μℓ) of ozone.

10. Calculations

10.1 After measuring the absorbances of the sample solutions, use the standard absorbance curve to find the micrograms of ozone in each sample.

10.2 The concentration of ozone in air may be expressed in mg/m^3 :

$$mg/m^3 = \frac{\text{corrected weight (in } \mu g)}{\text{volume of air sampled (in } \ell)}$$

10.3 The reaction of ozone with alkaline KI is not quantitative and the yield of iodine is concentration dependent. The following correction equation has been developed (see Reference 11.3):

$$O_3 \ (mg/m^3) = 0.038 + 1.038A - 0.010505A^2$$

where A is the concentration of ozone (also in mg/m^3) as determined above.

10.4 The concentration may also be expressed in terms of parts per million (ppm) by volume:

$$ppm = mg/m^3 \times \frac{24.45}{M.W.} \times \frac{760}{P} \times \frac{T+273}{298}$$

where: 24.45 = molar volume (ℓ/mole) at 25°C and 760 mmHg.

M.W. = molecular weight

P = pressure (mmHg) of air sampled.

T = temperature (°C) of air sampled.

11. References

11.1 *"Selected Methods for the Measurement of Air Pollutants,"* Public Health Service Publication No. 999-AP-11, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio, 1965.

11.2 Saltzman, B.E., and A.F. Wartburg, "Absorption Tube for Removal of Interfering Sulfur Dioxide in Analysis of Atmospheric Oxidant,"Anal Chem.,37, 779 (1965).

11.3 Documentation of NIOSH Validation Tests, NIOSH Contract No. CDC-99-74-45.

PARATHION IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Parathion	Method No:	P&CAM 158
Matrix:	Air	Range:	5-250 $\mu g/M^3$
Procedure:	Collection via impinger/ ethylene glycol, extraction into hexane, glc analysis		
Date Issued:	11/3/72	Precision:	Unknown
Date Revised:	5/21/73	Classification:	C (Tentative)

1. Principle of Method

Parathion from the air is trapped in ethylene glycol contained in an impinger. The ethylene glycol solution is diluted with water and extracted with hexane. The resulting hexane solution of the parathion is concentrated and subjected to gas chromatographic analysis using a phosphorus flame photometric detector.

2. Range and Sensitivity

The linear range of the flame photometric detector is 0.5 ng to 25 ng of parathion. For a 50 liter air sample, carried through the following procedure to solution in 1 mℓ of hexane, 2 $\mu\ell$ of which is injected into the gas chromatograph, this is 5 to 250 $\mu g/m^3$. These limits can be lowered or raised by changing: (1) the volume of air sampled, (2) the volume of the final hexane solution, or (3) the size of the aliquot injected into the gas chromatograph.

3. Interferences

Phosphorus compounds having retention times close to that of parathion will interfere with the analysis. The equipment used must be scrupulously cleaned to remove any traces of phosphate detergents.

4. Precision and Accuracy

Unknown

5. Advantages and Disadvantages

5.1 The method is very sensitive, and the detector exhibits high specificity for phosphorus compounds. The analysis is performed directly on the compound of interest. Separation and quantitation are accomplished in a reasonable amount of time.

5.2 The cost of the equipment and supplies may tax the budget of some laboratories. The sensitivity of the equipment depends on careful adjustment of the operating parameters. Contamination can occur easily through equipment and/or reagents. This method presumes the presence of parathion. If interfering compounds are anticipated a lengthy clean-up procedure is required (Reference 11.3).

6. Apparatus

6.1 Sampling Equipment. The sampling unit for the impinger collection method consists of the following components:

6.1.1 A prefilter unit (if needed) which consists of the filter media and cassette filter holder.

6.1.2 A midget impinger containing the absorbing solution or reagent.

6.1.3 A pump suitable for delivering desired flow rates. The sampling pump is protected from splashover or water condensation by an adsorption tube loosely packed with a plug of glass wool and inserted between the exit arm of the impinger and the pump.

6.1.4 An integrating volume meter such as a dry gas or wet test meter.

6.1.5 Thermometer.

6.1.6 Manometer.

6.1.7 Stopwatch.

6.2 Filter cassette with glass-fiber filter, 8-μ, 37-mm

6.3 Forceps.

6.4 Glass stirring rods.

6.5 Separatory funnels, 60 mℓ and 125 mℓ with Teflon stopcocks.

6.6 Beakers, 100 mℓ.

6.7 Funnels, 65 or 75 mm (diameter at top).

6.8 Glass wool.

6.9 Hot water bath.

6.10 Kuderna-Danish evaporator-concentrator, consisting of a 125 mℓ Erlenmeyer-type flask, 3-ball Snyder column, and 10 mℓ receiver graduated in milliliters.

6.11 Glass beads, 3 mm.

6.12 Volumetric flasks for standards.

6.13 Tracor MT 220 gas chromatograph, or equivalent, with attendant equipment, including a phosphorus flame photometric detector. Alternatively, a gas chromatograph fitted with a KCl thermoionic detector may be used. (Reference 11.5).

6.14 Gas-chromatography column constructed from 6-ft x 4-mm I.D. borosilicate glass packed with one of the following:

A. 10% DC-200 (12,500 cst) on 80 to 100-mesh Gas Chrom Q (References 11.4 and 11.5).

B. 7.5% QF-1 (10,000 cst) /5% DC-200 (12,500 cst) on 80-100 Gas Chrom Q (References 11.4 and 11.5).

C. 2% diethylene glycol succinate (DEGS) (C6 stabilized) on 80-100 mesh Gas Chrom Q (References 11.4 and 11.5).

D. 4% SE-30/6% OV-210 on 80-100 mesh Chromosorb W, H.P. (Reference 11.3).

Columns A and B are conditioned by heating 2-4 days at 240-250°C under nitrogen flowing at 60 mℓ/min, then primed by repeated injections of standard parathion solution under the conditions of analysis given below. Column C is conditioned by heating 12 hrs at 225-230°C under nitrogen flowing at 60 mℓ/min. Column D is conditioned for at least 3 days at 245°C under nitrogen flowing at 60 mℓ/min. A column of 10% Carbowax 20M on 80-100 mesh silanized support (2-in. x 4-mm I.D. glass tubing) is then inserted before Column D and the assembly is heated at 230-235°C for 17 hrs under nitrogen flowing at 20 mℓ/min. The 10% Carbowax 20M column is subsequently removed.

6.15 Syringes, 5 or 10 μℓ and 100 μℓ.

7. **Reagents**

7.1 Ethylene glycol, chromatoquality; this is the absorbing solution.

7.2 Hexane, pesticide quality.

7.3 Distilled water, interference free.

7.4 Saturated aqueous NaCl, interference free.

7.5 Anhydrous Na_2SO_4.

7.6 Parathion of known purity.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware used must be scrupulously cleaned and given a final hexane rinse immediately before use.

8.2 **Collection and Shipping of Samples**

8.2.1 Pour 15 mℓ of the absorbing solution (Section 7) into the midget impinger, using a graduated cylinder to measure the volume.

8.2.2 Connect the impinger (via the adsorption tube) to the vacuum pump and the prefilter assembly (if needed) with a short piece of flexible tubing. The minimum amount of tubing necessary to make the joint between the prefilter and impinger should be used. The air being sampled should not be passed through any other tubing or other equipment before entering the impinger.

8.2.3 Turn on pump to begin sample collection. Care should be taken to measure the flow rate, time and/or volume as accurately as possible. The sample should be taken at a flow rate of 1-2 ℓpm. A sample size of not more than 30 liters and no less than 25 liters should be collected. The minimum volume of air sampled will allow the measurement at least 1/10 times the TLV, 0.01 mg/m^3 (760 mm Hg, 25°C).

8.2.4 After sampling, the impinger stem can be removed and cleaned. Tap the stem gently against the inside wall of the impinger bottle to recover as much of the sampling solution as possible. Wash the stem with a small amount (1-2 mℓ) of unused absorbing solution and add the wash to the

impinger. Then the impinger is sealed with a hard, non-reactive stopper (preferably Teflon). Do not seal with rubber. The stoppers on the impingers should be tightly sealed to prevent leakage during shipping. If it is preferred to ship the impingers with the stems in, the outlets of the stem should be sealed with Parafilm or other non-rubber covers, and the ground glass joints should be sealed (i.e., taped) to secure the top tightly.

8.2.5 Care should be taken to minimize spillage or loss by evaporation at all times. Refrigerate samples if analysis cannot be done within a day.

8.2.6 Whenever possible, hand delivery of the samples is recommended. Otherwise, special impinger shipping cases designed by NIOSH should be used to ship the samples.

8.2.7 A "blank" impinger should be handled as the other samples (fill, seal and transport) except that no air is sampled through this impinger.

8.2.8 Where a prefilter has been used, the filter-cassettes are capped and placed in an appropriate cassette shipping container.

One filter disc should be handled like the other samples (seal and transport) except that no air is sampled through, and this is labeled as a blank.

8.3 Analysis (References 11.3 – 11.5)

8.3.1 The sample in 17-20 mℓ of ethylene glycol is transferred to a 125-mℓ separatory funnel (#1). Wash the sample container with a measured amount of water and add the washings to the separatory funnel. Dilute the ethylene glycol with a total of 70 mℓ of water.

Note: All procedural instructions from here on assume the extraction of 20 mℓ of ethylene glycol. Should a different volume be extracted, the glassware sizes and reagent amounts should be scaled proportionately.

8.3.2 Transfer the glass-fiber filter to a 100-mℓ beaker with a clean pair of forceps. Add 12 mℓ of hexane and agitate with a glass rod. Quantitatively transfer the hexane to separatory funnel #1 and extract by shaking vigorously for 2 minutes. If an emulsion is formed, add 0.5 mℓ of saturated NaCl to the separatory funnel. After the layers have separated, transfer the aqueous layer to a second 125-mℓ separatory funnel (#2) and the hexane layer to a 60-mℓ separatory funnel (#3).

8.3.3 Extract the filter with a second 12-mℓ portion of hexane. Transfer the solution to separatory funnel #2 and extract for 2 minutes. After the

layers have separated, transfer the aqueous layer to separatory funnel #1 and the hexane layer to separatory funnel #3.

8.3.4 Extract the filter with a third 12-mℓ portion of hexane, transfer the solution to separatory funnel #1, and extract for 2 minutes. After the layers have separated, discard the aqueous layer and transfer the hexane layer to separatory funnel #3.

8.3.5 Add 10 mℓ of water to separatory funnel #3 and extract for 1 minute. Discard the aqueous layer and extract with a second 10-mℓ portion of water, again discarding the aqueous layer.

8.3.6 Dry the hexane solution by passing it through 2.6 g of anhydrous Na_2SO_4 contained in a funnel with a glass wool retaining plug at the top of the stem. Collect the eluate in a 125-mℓ Kuderna-Danish flask which has been fitted with a 10-mℓ receiving tube containing one 3-mm glass bead. When the extract has eluted, rinse the separatory funnel with three consecutive 2-mℓ portions of hexane, washing down the walls of the funnel. Allow each rinse to elute before adding the next. Finally, rinse the funnel and the Na_2SO_4 with two more 2-mℓ portions of hexane.

8.3.7 Place the Kuderna-Danish assembly in a boiling water bath and concentrate the extract to ca. 5 mℓ. Remove the assembly from the bath and, after it is cool, disconnect the receiving tube from the flask, rinsing the joint with a little hexane. Place the tube under a nitrogen stream at room temperature and further concentrate the extract to ca. 0.5 mℓ. Rinse down the wall of the tube with hexane, delivered from a 100-μℓ syringe, diluting the extract to exactly 1.0 mℓ, and stir.

8.3.8 Inject an aliquot of the hexane solution into the gas chromatograph and obtain a chromatogram. The chromatographic conditions are:

Column temperature	220°C for columns A and B 210°C for column C 200°C for column D
Injection port temperature	225°C
Detector temperature	200°C
Transfer line and switching valve	235°C
Carrier gas (N_2) flow	60 mℓ/min

The retention times (relative to parathion) at these conditions for parathion, related analytes, and interfering organophosphorus pesticides are tabulated below.

Column	A	B	C	D
Parathion	1.00 (4.4 min)	1.00 (8 min)	1.00 (3.4 min)	1.00
Paraoxon	0.77	1.13	1.23	1.10
Amino parathion	1.04	0.78		
Methyl parathion	0.73	0.78	1.18	0.75
Methyl paraoxon	0.56	0.88	1.41	0.83
Dursban	1.00			
Fenthion	0.97			
Ruelene		1.01		
Phosphamidon				1.02

The solvent-flush sample injection technique is recommended. Duplicate injections should be made. The hexane, which precedes the parathion, should be vented so the detector flame will not be extinguished. The conditions of the run should be such that no parathion is lost during the venting process.

8.3.9 By comparison to a standard curve for parathion, the average of the areas under the parathion peaks is converted to the amount (ng) of parathion seen by the detector. Paraoxon, or another analyte, can be quantitated by comparison of its peak area with a standard curve for the appropriate compound.

9. Calibration and Standards

9.1 Prepare at least three standard solutions in the concentration range 100 ng/mℓ to 10,000 ng/mℓ from a stock solution of parathion in hexane.

Note: Extreme care should be taken to avoid skin contact with parathion and parathion solutions.

9.2 Make duplicate injections of aliquots of each standard solution onto the gas chromatograph and determine the peak areas.

9.3 Plot the amount (ng) of parathion seen by the detector vs the peak area. Straight lines passing through the origin should result. If these conditions are not observed, either the linear range of the detector has been exceeded or a system malfunction has occurred.

9.4 Injections of standards should be interspersed among sample injections so a watch can be maintained on detector sensitivity.

10. **Calculations**

10.1 Determine the total amount in ng of parathion present in the sample.

$$\text{Total ng} = ng_o \times \frac{\text{Soln vol}}{\text{Inj vol}}$$

where:

ng_o = Nanograms of parathion determined from calibration curve based on peak area responses

Soln vol = volume in μℓ of the final hexane solution (usually 1000 μℓ)

Inj vol = volume in μℓ of the aliquot of the final hexane solution injected into the gas chromatograph

10.2 Convert the volume of air sampled to standard conditions of 25°C and 760 mm Hg.

$$V_s = V \times \frac{P}{760} \times \frac{298}{(T + 273)}$$

where:

V_s = volume of air in liters at 25°C and 760 mm Hg

V = volume of air in liters as measured

P = Barometric Pressure in mm Hg

T = Temperature of air in degrees centigrade.

10.3 The concentration of parathion can be expressed in ng per liter or μg per m^3.

$$\mu g/m^3 = ng/\text{liter}$$

$$\mu g/m^3 = \frac{\text{total ng}}{V_s} \quad \begin{matrix}\text{(Section 10.1)} \\ \text{(Section 10.2)}\end{matrix}$$

11. **References**

11.1 H.F. Enos, J.F. Thompson, J.B. Mann, and R.F. Moseman, "Determination of Pesticide Residues in Air," presented at the 163rd American Chemical Society National Meeting, Boston, Massachusetts, April 1972.

11.2 J.W. Miles, L.E. Fetzer, and G.W. Pearce, *Environmental Science and Technology*, 4, 420 (1970).

11.3 "Analysis of Pesticide Residues in Human and Environmental Samples," ed. F. Thompson, Perrine Primate Research Laboratories, EPA, Perrine, Florida, 1972, Section 4B and 8.

11.4 R.R. Watts and R.W. Sterkerr, *Journal of the Association of Official Analytical Chemists*, 52, 513 (1969).

11.5 "Pesticide Analytical Manual, Volume I," P.E. Cornelliussen, ed., Food & Drug Administration, USDHEW, Rockville, Md., 1972, Section 313.

OIL MIST IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Oil Mist, water insoluble	Method No.:	P&CAM 159
Matrix:	Air	Range:	5 – 500 μg of oil
Procedure:	Fluorescence, emission	Precision:	Unknown
Date Issued:	8/8/72		
Date Revised:	1/10/74	Classification:	D (Operational)

1. Principle of Method

   1.1 Air samples are collected using 37 mm membrane filters.

   1.2 Oil mist is extracted from the filters with chloroform.

   1.3 The chloroform solution is analyzed for oil content using fluorescence spectrophotometry.

2. Range and Sensitivity

   2.1 The sensitivity varies between oil types but is about 0.05 mg/m^3 for a 100 liter air sample.

   2.2 For a 100 liter air sample the working range is linear to at least 5 mg/m^3 and can easily be extended by dilution.

3. Interferences

   Highly fluorescent compounds may interfere in this analysis; however this type of compound is not generally found where oil mists are being used.

4. Precision and Accuracy

   The precision and accuracy of this method have not been completely determined at this time.

5. Advantages and Disadvantages of the Method

5.1 The method is simple and fast. It is specific for the oil used in the calibration and is relatively free from interference.

5.2 A disadvantage is that in all cases a bulk sample of the oil in question must be provided for use in the preparation of standards for the analysis. Some very light oils do not fluoresce readily and cannot be analyzed using this method.

6. Apparatus

6.1 **Sampling Equipment.** The sampling unit for the filter-collection method has the following components:

6.1.1 The filter unit, consisting of the filter media (Section 6.2) and appropriate cassette filter holder, either a 2- or 3-piece filter cassette.

6.1.2 A vacuum pump such as a personal sampling pump. This pump must be properly calibrated so the volume of air sampled can be measured as accurately as possible. The pump must be calibrated with a representative filter unit in the line. Flow rate, times, and/or volume must be known.

6.1.3 Thermometer.

6.1.4 Manometer.

6.1.5 Stopwatch.

6.2 0.8-μ cellulose membrane filters, or 5.0 μ PVC filters, 37 mm.

6.3 Fluorescence spectrophotometer with both excitation and analyzer scanning capabilities in the range of 250-550 mμ.

6.4 1 cm cells for fluorescence spectrophotometer.

6.5 Small glass stoppered bottles or vials for desorption of samples.

7. Reagents

7.1 **Chloroform** – Distilled reagent grade.

7.2 **Stock Solution, 100 mg/mℓ** – Weigh 10.00 g of oil into a 100 mℓ volumetric flask and dissolve with chloroform to volume.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 Wash in detergent solution and follow with tap water and distilled water rinses.

8.1.2 Rinse thoroughly with acetone, then with portions of the distilled chloroform, and allow to dry.

8.2 Collection and Shipping of Samples

8.2.1 Assemble the filter unit by mounting the filter disc in the filter cassette.

8.2.2 Connect the exit end of the filter unit to the pump with a short piece of flexible tubing.

8.2.3 Turn on pump to begin sample collection. The flow rate, times, and/or volume must be measured as accurately as possible. The sample should be taken at a flow rate of 2 liters per minute. A minimum sample of 100 liters should be collected. Larger sample volumes are encouraged, provided the filters do not become loaded with dust to the point that loose material might fall off or the filter become plugged.

8.2.4 The sample cassettes should be shipped in a suitable container designed by NIOSH to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample is dislodged from the filter, nor that the sample surface be disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the cassette on top of the sample filter.

8.2.5 With each batch of samples, one filter, labeled as a blank, must be submitted. No air should be drawn through this filter.

8.2.6 A bulk sample of the oil being used must be collected and sent to the laboratory for use as a standard material in the analysis. To prevent contamination, the bulk should not be shipped in the same container as the samples.

8.3 Analysis of Samples

8.3.1 Transfer sample and blank filters into separate small glass stoppered bottle or vial containing 5 mℓ of chloroform. The chloroform must completely cover the filter.

8.3.2 Seal each bottle and allow to stand for at least 30 minutes with occasional agitation. At this point the oil is in solution and the samples are ready for analysis.

8.3.3 Determine the proper excitation wavelength for the analysis using an oil solution containing 100 μg/mℓ. This solution is prepared by proper dilution of the stock solution with chloroform.

8.3.4 Transfer the sample and blank solutions to 1 cm cells and record a fluorescence spectrum for each solution using the previously determined excitation wavelength.

8.3.5 The peak-height of the largest fluorescent peak is used to measure the oil concentration from the calibration curve prepared from the bulk samples (Section 8.2.6).

9. Calibration and Standards

9.1 Appropriate dilutions of the stock solution with chloroform are made to obtain working standards in the range of 1.0 μg/mℓ to 100 μg/mℓ.

9.2 The working standards are transferred to 1 cm cells and a fluorescence spectrum is obtained for each standard. A calibration curve is constructed by measuring the peak height of the largest fluorescent peak and plotting this value vs. concentration in μg/mℓ.

10. Calculations

10.1 Blank values, if any, should first be subtracted from each sample.

10.2 The concentration of oil in the air sampled can be calculated as mg of oil per cubic meter or μg oil per liter.

$$\text{mg oil/m}^3 = \mu\text{g oil/ℓ}$$

$$\text{mg oil/m}^3 = \frac{\mu\text{g oil/mℓ}}{V_s} \times \text{mℓ solution}$$

where:

μg oil/mℓ = concentration of oil derived from the proper calibration curve (Section 9.2)
mℓ solution = final volume of sample analyzed including any dilution factor
V_s = volume of air sampled in ℓ at 25°C and 760 mm Hg.

11. References

11.1 Ray, E.M., American Industrial Hygiene Journal, 31, 472 (1970).

11.2 Lippmann, M., et al, Arch. Environ. Health, 21, 591 (1970).

11.3 Houghton, J.A. and G. Lee, "Data on Ultraviolet Absorption and Fluorescence Emission," Am. Ind. Hyg. Assoc. J., 21, 219-230 (1960).

SULFUR DIOXIDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Sulfur Dioxide	**Method No:**	P&CAM 160
Matrix:	Air	**Range:**	0.003-5.0 ppm SO_2
Procedure:	Collection via Impinger/ Colorimetric	**Precision**	±5%
Date Issued:	11/22/72		
Date Revised:	4/4/77	**Classification:**	C (Tentative)

1. **Principle of the Method**

1.1 Sulfur dioxide is absorbed by aspirating a measured air sample through a solution of potassium or sodium tetrachloromercurate (TCM). This procedure results in the formation of a dichlorosulfitomercurate complex, which resists oxidation by the oxygen in the air.

1.2 Ethylenediaminetetraacetic acid disodium salt (EDTA) is added to this solution to complex heavy metals that can interfere by oxidation of the sulfur dioxide before formation of the dichlorosulfitomercurate. After the absorption is completed, any ozone in the solution is allowed to decay.

1.3 The sample is first treated with 0.6% sulfamic acid to destroy any nitrite anions formed from the absorption of oxides of nitrogen in the air.

1.4 Next the sample is treated with formaldehyde and specially purified acid – bleached pararosaniline containing phosphoric acid to control pH. This forms the intensely colored pararosaniline methyl sulfonic acid which behaves as a pH indicator.

1.5 The pH of the final solution is adjusted to 1.6 ± 0.1 by the addition of 3M phosphoric acid.

1.6 Maximum absorbance is read spectrophotometrically at the 548 nm wavelength.

2. Range and Sensitivity

2.1 The lower limit of detection of sulfur dioxide in 10 mℓ of potassium or sodium tetrachloromercurate is 0.3 μℓ (based on twice the standard deviation), representing a concentration of 0.01 ppm (26 $\mu g/m^3$) of SO_2 in an air sample of 30 liters.

2.2 Beer's Law is followed through the working range from 0.005 to 1.0 absorbance units (0.2 to 35.0 μg in 25 mℓ of final solution). One cannot extrapolate beyond these ranges by changing volumes of atmosphere sampled unless the absorption efficiency of the particular system is known or determined at the volumes and concentrations under study.

2.3 Atmospheric sulfur dioxide concentrations of interest usually range from a few pphm to a few ppm. Higher concentrations (5 to 500 ppm) employed in special studies must be analyzed by using smaller gas samples, by analyzing an aliquot of the sample, or by diluting the sample with absorbing solution.

2.4 Collection efficiency falls off rapidly below 0.01 ppm and varies with the geometry of the absorber, the size of the gas bubbles, and the contact time with the solution.

3. Interferences

3.1 Interferences by oxides of nitrogen are minimized by the addition of sulfamic acid.

3.2 Interference from ozone is eliminated by a 20 minute delay prior to analysis, permitting any ozone present to decay.

3.3 Heavy metal concentrations in the atmosphere should not produce significant interference. However, addition of EDTA is recommended when low levels of sulfur dioxide are to be measured or when contamination by heavy metals is unusually high. A minimum EDTA concentration of 7 times the anticipated amount of metal interference should be added. At least 60 μg of Fe (III), 10 μg of Mn (II), and 10 μg of Cr (III) in 10 mℓ of absorbing reagent can be tolerated in the procedure. No significant interference was found with 10 μg of Cu (II) and 22 μg of V (V).

4. Precision and Accuracy

Standard deviation at the 95% confidence level is 5%.

5. Advantages and Disadvantages of the Method

5.1 The method is selective, sensitive, reproducible, and suitable for field use. However, many critical variables such as pH, temperature, impurities in reagents and in water must be carefully controlled for reliable results.

5.2 Solutions of bisulfite or sulfite are unstable as calibration standards. Careful standardization and immediate dilution with potassium tetrachloromercurate (TCM) are required for accurate results. Care must be taken with this reagent because it is very poisonous.

6. Apparatus

6.1 Sampling Equipment. The sampling unit for the impinger collection method consists of the following components:

6.1.1 A prefilter unit (if needed) which consists of the filter media and cassette filter holder.

6.1.2 A midget impinger containing the absorbing solution or reagent.

6.1.3 A pump suitable for delivering desired flow rates. The sampling pump is protected from splashover or water condensation by an adsorption tube loosely packed with a plug of glass wool and inserted between the exist arm of the impinger and the pump.

6.1.4 An integrating volume meter such as a dry gas or wet test meter.

6.1.5 Thermometer.

6.1.6 Manometer.

6.1.7 Stopwatch.

6.2 Volumetric flasks – 25, 100, 250, and 1000 mℓ capacity.

6.3 Pipets and Graduates.

6.4 Spectrophotometer suitable for measurement of color at 548 nm, preferably with a spectral band width less than 16 nm.

6.5 Glass cuvettes with 1 cm path length.

7. Reagents

All chemicals except for the dye are analytical reagent grade. The pararosaniline dye should meet the specifications as outlined below:

7.1 Double distilled water, free from oxidants.

7.2 **Absorbing Reagent, 0.04M Potassium Tetrachloromercurate (TCM).** Dissolve 10.86 g of mercuric chloride and 5.96 of potassium chloride in water and bring to volume in a 1-liter volumetric flask.

Caution: highly poisonous. If spilled on skin, flush with water immediately.

If excessive heavy metal interferences are expected add 0.07 g of ethylenediaminetetraacetic acid disodium salt (EDTA). The absorbing solution is normally stable for six months but if a precipitate forms, discard the solution. To prevent deterioration, the absorbing reagent must be shielded from sunlight during storage, and during and after sampling by covering the containers with a suitable wrapping, such as aluminum foil.

7.3 **Sulfamic Acid (0.6%).** Dissolve 0.6 g of sulfamic acid in 100 mℓ of water. This reagent can be kept for a few days if protected from air.

7.4 **Hydrochloric Acid (1M).** Dilute 86 mℓ of concentrated hydrochloric acid (HCl, sp gr 1.19) to 1 liter.

7.5 **Phosphoric Acid (3M).** Dilute 205 mℓ of concentrated phosphoric acid (H_3PO_4, sp gr 1.69) to 1 liter.

7.6 **Purified Pararosaniline** (99% or better), 0.2% (nominal) stock solution. Specifically purified pararosaniline dye 99.0 percent is used.* Weigh 0.200 g and completely dissolve the dye by shaking with 100 mℓ of 1M HCl in a 100-mℓ graduated cylinder that is glass-stoppered.

7.7 **Pararosaniline Reagent.** To a 250-mℓ volumetric flask add 20 mℓ of stock pararosaniline reagent. Add an additional 0.2 mℓ of stock for each percent the stock assays below 100 percent. Then add 25 mℓ of 3M H_3PO_4 and dilute to volume with water. These reagents are stable for at least 9 months.

7.8 **Formaldehyde (0.2%).** Dilute 5 mℓ of 36 to 38% formaldehyde to 1 liter with water. Prepare this solution daily.

*Specially purified PRA·HCl for SO_2 determined available from J.T. Baker Chemical Company

7.9 Reagents for Standardization

7.9.1 **Stock Iodine Solution (0.1N).** Place 12.7 g of iodine in a 250-mℓ beaker, add 40 g of KI and 25 mℓ of water. Stir until all is dissolved, then dilute to 1 liter with water.

7.9.2 **Working Iodine Solution (0.01N).** Prepare approximately 0.01N iodine solution by diluting 50 mℓ of the stock solution to 500 mℓ with distilled water.

7.9.3 **Starch Indicator Solution.** Triturate 0.4 g of soluble starch and 0.002 g of mercuric iodide (preservative) with a little water, and add the paste slowly to 200 mℓ of boiling water. Continue boiling the solution until it is clear; cool and transfer it to a glass stoppered bottle.

7.9.4 **Sodium Thiosulfate, Standard Solution (0.1N).** Dissolve 25 g of sodium thiosulfate ($Na_2S_2O_3-5H_2O$) in 1 liter of freshly boiled, cooled distilled water and add 0.1 g of sodium carbonate to the solution. Allow the solution to stand for one day before standardizing. To standardize, weigh 1.5 g of potassium iodate, primary standard grade, that was dried at 180°C and dilute to volume in a 500-mℓ volumetric flask. To a 500-mℓ iodine flask, pipet 50 mℓ of the iodate solution. Add 2 g of potassium iodide and 10 mℓ of a 1 + 10 dilution of concentrated hydrochloric acid. Stopper the flask. After 5 minutes titrate with thiosulfate to a pale yellow color. Add 5 mℓ of starch indicator and complete the titration. Calculate the normality of sodium thiosulfate, N, as follows:

$$N = [\text{weight (g } KIO_3) \times 10^3 \times 0.1]/(\text{mℓ of titer} \times 35.67)$$

7.9.5 **Standard 0.01N Sodium Thiosulfate.** Dilute 50.0 mℓ of standard 0.1N sodium thiosulfate to 500 mℓ with distilled water and mix. This 0.01N solution is not stable, and must be prepared fresh on the day it is used by diluting the standard 0.1N sodium thiosulfate.

7.9.6 **Standard Sulfite Solution.** Dissolve 0.4 g of sodium sulfite (Na_2SO_3) or 0.3 g of sodium metabisulfite ($Na_2S_2O_5$) in 500 mℓ of recently boiled and cooled distilled water. (Double distilled water that has been deaerated is preferred.) This solution contains from 320 to 400 μg/mℓ as SO_2. The actual concentration in the standard solution is determined by adding excess iodine and back titrating with sodium thiosulfate that has been standardized against potassium iodate or dichromate (primary standard). Sulfite solution is unstable.

Back titration is performed in the following manner: Add 25 mℓ of distilled water to a 500-mℓ iodine flask and pipet 50 mℓ of the 0.01N iodine solution into the flask designated flask A (blank). Pipet in order 50 mℓ of the 0.01N iodine and 25 mℓ of the standard sulfite solution into a second 500-mℓ iodine flask designated B (sample).
Stopper the flasks and allow to react for 5 minutes. By means of a buret containing standard 0.01N thiosulfate solution, titrate each flask in turn to a pale yellow color. Then add 5 mℓ of starch solution and continue the titration to the disappearance of the blue color. Calculate the concentration of SO_2 in the standard solution as follows:

$$SO_2, \mu g/m\ell = (A - B)\,NK\,/V$$

where:

A = milliliters of thiosulfate solution required for titration of the blank

B = milliliters of thiosulfate solution required for titration of the sample

N = normality of the thiosulfate solution

K = micro-equivalent weight for SO_2 = 32,030

V = milliliters of sample taken

8. Procedure

8.1 Cleaning of Equipment. All glassware should be cleaned initially using the following procedure:

8.1.1 Wash in detergent – tap water and follow with tap and distilled water rinses.

8.1.2 Soak in chromic acid cleaning solution for at least 30 minutes and follow with tap and distilled water rinses.

8.1.3 Soak in concentrated nitric acid for at least 30 minutes and follow with thorough tap, distilled, and double distilled water rinses, making sure no trace of acid remains.

8.2 Collection and Shipping of Samples

8.2.1 Pour 10 mℓ of the absorbing solution (Section 7) into the midget impinger, using a graduated cylinder to measure the volume.

8.2.2 Connect the impinger (via the absorption tube) to the vacuum pump and the prefilter assembly (if needed) with a short piece of flexible tubing. The minimum amount of tubing necessary to make the joint between the prefilter and impinger should be used. The air being sampled should not be passed through any other tubing or other equipment before entering the impinger.

8.2.3 Turn on pump to begin sample collection. Care should be taken to measure the flow rate, time and/or volume as accurately as possible. The sample should be taken at a flow rate of 0.2-1 ℓpm. A sample size of not more than 5 liters and no less than 4 liters should be collected. The minimum volume of air sampled will allow the measurement at least 1/10 times the TLV, 1.3 mg/m^3 (760 mm Hg, 25°C).

8.2.4 After sampling, the impinger stem can be removed and cleaned. Tap the stem gently against the inside wall of the impinger bottle to recover as much of the sampling solution as possible. Wash the stem with a small amount (1-2 mℓ) of unused absorbing solution and add the wash to the impinger. Then the impinger is sealed with a hard, non-reactive stopper (preferably Teflon). Do not seal with rubber. The stoppers on the impingers should be tightly sealed to prevent leakage during shipping. If it is preferred to ship the impingers with the stems in, the outlets of the stem should be sealed with Parafilm or other non-rubber covers, and the ground glass joints should be sealed (i.e., taped) to secure the top tightly.

8.2.5 Care should be taken to minimize spillage or loss by evaporation at all times. Refrigerate samples if analysis cannot be done within a day.

8.2.6 A "blank" impinger should be handled as the other samples (fill, seal and transport) except that no air is sampled through this impinger.

8.2.7 Where a prefilter has been used, the filter cassettes are capped and placed in an appropriate cassette shipping container. One filter disc should be handled like the other samples (seal and transport) except that no air is sampled through, and this is labeled as a blank.

8.3 Analysis of Samples

8.3.1 Transfer the collected sample quantitatively to a 25-mℓ volumetric flask, using about 5 mℓ of water for rinsing. Aliquots may be taken at this point if the concentration or volume of reagent is large.

8.3.2 If the presence of ozone is suspected, delay analysis for 20 minutes after sampling to allow the ozone to decompose.

8.3.3 For each set of determinations, prepare a reagent blank by adding 10 mℓ of the unexposed absorbing reagent to a 25-mℓ volumetric flask.

8.3.4 To each flask add 1 mℓ of 0.6% sulfamic acid and allow to react for 10 minutes to destroy the nitrite from oxides of nitrogen.

8.3.5 Accurately pipet in 2 mℓ of the 0.2% formaldehyde, then 5 mℓ of pararosaniline reagent.

8.3.6 Start a laboratory timer that has been set for 30 minutes. Bring all flasks to volume with freshly boiled distilled water.

8.3.7 After 30 minutes, determine the absorbances of the sample and of the blank at the wavelength of maximum absorbance, 548 nm. Use water (not the reagent blank) in the reference cell. Do not allow the colored solution to stand in the absorbance cell or a film of dye will be deposited.

8.3.8 If the absorbance of the sample solution ranges between 1.0 and 2.0, the sample can be diluted 1 to 1 with a portion of the reagent blank and read within a few minutes. Solutions with high absorbance can be diluted up to sixfold with the reagent blank in order to obtain on-scale readings within 10% of the true absorbance value.

9. **Calibration and Standards**

9.1 Immediately after standardization of the sulfite solution, pipet 2 mℓ of the freshly standardized solution into a 100-mℓ volumetric flask and bring to mark with 0.04M TCM. This solution is stable for 30 days if stored at 5°C.

9.2 Pipet graduated amounts of the diluted sulfite solution (such as 0, 1, 2, 3, 4, and 5 mℓ) into a series of 25-mℓ volumetric flasks.

9.3 Add sufficient 0.04M TCM to each flask to bring the volume of its contents to approximately 10 mℓ.

9.4 Add the remaining reagents as described in the procedure. For greater precision, a constant-temperature bath is preferred. The temperature of calibration should not differ from the temperature of analysis by more than a few degrees.

9.5 Plot total absorbance of these solutions (as ordinates) against the total micrograms of SO_2. A linear relationship is obtained.

9.6 The absorbance should be read on the samples and standards in the same cell; if more than one cell is used, the cells should be matched spectrophotometrically.

9.7 The intercept with the vertical axis of the line best fitting the points is usually within 0.2 absorbance unit of the blank (zero standard) reading. Under these conditions the plot need be determined only once to evaluate the calibration factor (reciprocal of the slope of the line). This calibration factor can be used for calculating results provided there are no radical changes in temperature or pH. At least one control sample is recommended per series of determinations to ensure the reliability of this factor.

10. Calculations

10.1 Calculate the concentration of SO_2 in the sample as follows:

$$SO_2, \text{ ppm} = (A - Ao)\ 0.382B/V$$

where:

A = sample absorbance

Ao = reagent blank absorbance

0.382 = volume ($\mu\ell$) of 1 μg of SO_2 at 25°C, 760 mm Hg

B = calibration factor, μg/absorbance unit

V = sample volume in liters corrected to 25°C, 760 mm Hg by PV = nRT

10.2 The SO_2 concentration can also be expressed in mg/m^3 of air which is numerically equal to $\mu g/\ell$ of air.

$$mg/m^3 = \mu g\ SO_2/\ell$$
$$= (A - Ao)\ B/V$$

11. References

11.1 Scaringelli, F.P., Saltzman, B.E., and Prey, S.A., "Spectrophotometric Determination of Atmospheric Sulfur Dioxide," *Analytical Chemistry, Vol.*, 39, No. 14, 1967, p. 1709.

11.2 "Sulfur Dioxide Content of the Atmosphere," ASTM Standard D2914, 1971.

SULFUR DIOXIDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Sulfur Dioxide	Method No:	P&CAM 163
Matrix:	Air	Range:	0.01-5 mg
Procedure:	Collection via Impinger by Peroxide Absorption/Titration with Barium Perchlorate	Precision:	4% RSD at 2.5 ppm
Date Issued:	1/26/73		
Date Revised:	4/4/77	Classification:	D (Operational)

1. Principle of the Method

   1.1 Sulfur dioxide in the air is absorbed and oxidized in 0.3N hydrogen peroxide (H_2O_2) reagent.

   1.2 The pH of the sample solution is adjusted with dilute perchloric acid. After isopropyl alcohol is added, bringing the alcohol concentration to approximately 80% by volume, the resulting solution is titrated with 0.005M barium perchlorate using Thorin as the indicator. There is a sharp change from yellow to pink at the end point.

2. Range and Sensitivity

   This method is sensitive to 0.1 mg SO_2 per cubic meter assuming a 100-liter air sample. This level corresponds to approximately 0.25 ppm SO_2 in air. The upper limit is the amount of SO_2 absorbed in the hydrogen peroxide reagent and is at least 5 mg.

3. Interferences

   3.1 Trapped particulate sulfates, and sulfuric acid in the air sample would give erroneously high sulfur dioxide values. However, these can be eliminated by placing a 0.8μ cellulose filter upstream of the impinger in the sampling train.

3.2 Metal ion interferences can be eliminated either by the use of a prefilter or by passing the solution through an ion exchange column.

3.3 Concentrations of phosphate ions greater than the sulfate ion concentration cause appreciable interference. Phosphate can be removed by precipitation with magnesium carbonate. The use of a prefilter should also remove phosphates.

4. Precision and Accuracy

At 2.5 ppm, the accuracy is 5% with a relative standard deviation of 4%. At 25 ppm, accuracy and relative standard deviation can be improved to about 1%.

5. Advantages and Disadvantages

5.1 The samples are easily collected and conveniently shipped to the laboratory for analysis in glass vials.

5.2 The sulfuric acid formed is stable and nonvolatile, making this manner of collection of SO_2 desirable.

5.3 The analysis is relatively rapid and simple.

6. Apparatus

6.1 Sampling Equipment. The sampling unit for the impinger collection method consists of the following components:

6.1.1 A prefilter unit (if needed) which consists of the filter media and cassette filter holder.

6.1.2 A midget impinger containing the absorbing solution or reagent.

6.1.3 A pump suitable for delivering desired flow rates. The sampling pump is protected from splashover or water condensation by an adsorption tube loosely packed with a plug of glass wool and inserted between the exit arm of the impinger and the pump.

6.1.4 An integrating volume meter such as a dry gas or wet test meter.

6.1.5 Thermometer.

6.1.6 Manometer.

6.1.7 Stopwatch.

6.2 Millipore 0.8μ cellulose AA filters and cassettes.

6.3 Necessary glassware.

6.4 **Burette.** A burette of 10 milliliter capacity graduated in 0.05 milliliter subdivisions.

6.5 Daylight fluorescent lamp aids in identifying the end point.

6.6 **Ion Exchange Resin.** Dowex 50 x 8, 20-50 mesh, hydrogen form ion exchange columns may be constructed using glass burettes or tubing. A column with an inside diameter of 8 mm and 7 inches of resin has a capacity of approximately 25 milliequivalents.

7. **Reagents**

7.1 **Alcohol,** Isopropanol, reagent grade.

7.2 **Barium Perchlorate, 0.005M.** Dissolve 2.0 g of barium perchlorate trihydrate in 200 mℓ of water and add 800 mℓ of isopropanol. Adjust apparent pH to about 3.5 with perchloric acid. Standardize against 0.005M H_2SO_4.

7.3 **Thorin.** Prepare a 0.1% to 0.2% solution in distilled water.

7.4 **Standard Sulfate Solution.** Prepare a 0.005M solution of H_2SO_4 and standardize by titration with 0.02N sodium hydroxide or dissolve 0.7393 g anhydrous Na_2SO_4 in distilled water and dilute to one liter (1 mℓ = 0.5 mg SO_4). The sodium is removed by passage of the standard through the ion exchange column.

7.5 **Hydrochloric Acid, 4N.** Add 300 mℓ concentrated HCl to 600 mℓ of distilled water. Needed only to regenerate the column if the ion exchange procedure is used.

7.6 **Absorbing Solution – Hydrogen Peroxide, 0.3N.** Dilute 17 mℓ of 30% H_2O_2 solution to one liter with distilled water.

7.7 **Perchloric Acid, 1.8%.** Dilute 25 mℓ of reagent grade perchloric acid (70-72%) to one liter with distilled water.

8. **Procedure**

8.1 **Cleaning of Equipment.** The glassware should be chemically clean. Wash in detergent, and rinse thoroughly with tap water and distilled water.

8.2 Collection and Shipping of Samples

8.2.1 Pour 15 mℓ of the absorbing solution (Section 7) into the midget impinger, using a graduated cylinder to measure the volume.

8.2.2 Connect the impinger via the absorption tube to the vacuum pump, and the prefilter assembly (if needed) with a short piece of flexible tubing. The minimum amount of tubing necessary to make the joint between the prefilter and impinger should be used. The air being sampled should not be passed through any other tubing or other equipment before entering the impinger.

8.2.3 Turn on pump to begin sample collection. Care should be taken to measure the flow rate, time and/or volume as accurately as possible. The sample should be taken at a flow rate of 1-2 ℓpm. A sample size of not more than 100 liters and no less than 10 liters should be collected. The minimum volume of air sampled will allow the measurement at least 1/10 times the TLV, 1.3 mg/m^3 (760 mm Hg, 25°C).

8.2.4 After sampling, the impinger stem can be removed and cleaned. Tap the stem gently against the inside wall of the impinger bottle to recover as much of the sampling solution as possible. Wash the stem with a small amount (1-2 mℓ) of unused absorbing solution and add the wash to the impinger. Then the impinger is sealed with a hard, non-reactive stopper (preferably Teflon). Do not seal with rubber. The stoppers on the impingers should be tightly sealed to prevent leakage during shipping. If it is preferred to ship the impingers with the stems in, the outlets of the stem should be sealed with Parafilm or other non-rubber covers, and the ground glass joints should be sealed (i.e., taped) to secure the top tightly.

8.2.5 Care should be taken to minimize spillage or loss by evaporation at all times. Refrigerate samples if analysis cannot be done within a day.

8.2.6 Whenever possible, hand delivery of the samples is recommended. Otherwise, special impinger shipping cases designed by NIOSH should be used to ship the samples.

8.2.7 A "blank" impinger should be handled as the other samples (fill, seal and transport) except that no air is sampled through this impinger.

8.2.8 Where a prefilter has been used, the filter cassettes are capped and placed in an appropriate cassette shipping container. One filter disc should be handled like the other samples (seal and transport) except that no air is sampled through, and this is labeled as a blank.

8.3 **Ion Exchange Procedure.** (Used to purify Standard Sulfate Solution and for samples only when filters were not used in front of the impinger.)

8.3.1 When about two-thirds of the resin's capacity has been exhausted (deterioration in sharpness of the end point), regenerate the resin by passing 30 mℓ of 4N hydrochloric acid through the column.

After thorough washing with distilled water, the column is ready for use.

8.3.2 Since small volumes of sample solution are passed through the ion exchange column, care must be taken not to dilute the sample with distilled water that remains on the resin. One way this can be accomplished is by blowing some air through the resin with a squeeze bulb to remove most of the distilled water from the ion exchange resin. One or two milliliters of sample are passed through the column and discarded after air is again blown through the resin. The remainder of the sample is then passed through the ion exchange column, and an aliquot is titrated according to the general procedure (Section 8.4.3).

8.3.3 The column is flushed with distilled water between samples to prevent contamination from the previous sample.

8.4 **Analysis of Samples**

8.4.1 Measure the volume of the sample solution or dilute it to a given volume.

8.4.2 Although all samples may be passed through the ion exchange column, it is necessary to do so only when the metal concentration exceeds that of the sulfate. The ion exchange procedure is detailed in Section 8.3. Use of a prefilter upstream from the impinger should remove most metal ions, however, and thus eliminate this entire step.

8.4.3 To a 10-mℓ aliquot, add 50 mℓ isopropanol. If necessary, adjust the pH, to between 3.0 and 3.5 with perchloric acid. Add 1-3 drops Thorin indicator and titrate with barium perchlorate, taking the change from yellow or yellow-orange to pink as the end-point.

8.4.4 Analyze the standard and absorbing solution blank in the same manner.

9. **Calibration and Standards**

9.1 The barium perchlorate solution is standardized by adding 25 ml isopropanol to a 5-ml aliquot of the standardized H_2SO_4 (0.005M) and titrating to an end-point with Thorin as indicator. The molarity of the solution is calculated as follows:

$$M_{Ba(ClO_4)_2} = \frac{m\ell_{H_2SO_4} \times M_{H_2SO_4}}{m\ell_{Ba(ClO_4)_2}}$$

The molarity of the $Ba(ClO_4)_2$ solution should be checked periodically following this same procedure.

9.2 If anhydrous sodium sulfate is used to standardize the barium perchlorate, it must first be ion exchanged since sodium obscures the end point. A 5-mℓ aliquot of the 0.5 mg/mℓ sulfate solution is ample for standardization.

10. Calculations

10.1 The analytical results are computed on the basis of the following reactions:

$$SO_2 + H_2O_2 \longrightarrow H_2SO_4$$

$$H_2SO_4 + Ba(ClO_4)_2 \longrightarrow BaSO_4 \downarrow + 2\ HClO_4$$

10.2 The concentration can be expressed as mg SO_2/m^3,

$$\frac{\text{mg } SO_2}{m^3} = \frac{m\ell_s \times M_{Ba(ClO_4)_2} \times MW_{SO_2}}{Vm^3} \times \frac{V}{V_{aliq}}$$

where:

$m\ell_s$ = mℓ of $Ba(ClO_4)_2$ solution needed to titrate the sample aliquot minus the blank value.

$M_{Ba(ClO_4)_2}$ = molarity of the $Ba(ClO_4)_2$ solution

MW_{SO_2} = molecular weight of SO_2 = 64

Vm^3 = Volume of air sampled, in cubic meters at 760 mm Hg, 25°C (1 m^3 = 1000 ℓ)

V_{aliq} = Volume of sample aliquot used for the titration, in mℓ

V = Original volume of sample in impinger, in mℓ

10.3 The SO_2 concentration of the sample can also be expressed in ppm,

$$\text{ppm } SO_2 \text{ by volume} = \frac{m\ell_s \times M_{Ba(ClO_4)_2} \times 24{,}450}{V_t} \times \frac{V}{V_{aliq}}$$

where:

V_1 = Volume of air sampled in liters at 25°C and 760 mm Hg.

24,450 = mℓ occupied by one mole of ideal gas at 25°C and 760 mm Hg.

11. References

11.1 Fritz, J.S., and Yamamura, S.S., *Anal. Chem.*, 27, 1461 (1955).

11.2 Leithe, W., "The Analysis of Air Pollutants," Ann Arbor Science Press, 1970.

11.3 Kundig, Chem. Rundechau 18, 123 (1965).

11.4 Fielder & Morgan, *Anal. Chem. Acta*, 23, 538 (1960).

11.5 Fielder & Morgan, *Z. Anal. Chem.*, 183, 455 (1961).

MERCURY IN URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Mercury	**Method No.:**	P&CAM 165
Matrix:	Urine	**Range, Urine:**	0.003 – > 0.3 mg/ℓ
Procedure:	Flameless Atomic Absorption	**Precision:**	± 3%
		Classification:	B (Accepted)
Date Issued:	2/13/73		
Date Revised:	1/15/74		

1. **Principle of the Method**

   1.1 After initial decomposition of the urine sample with nitric acid, the mercury is reduced to its elemental state with stannous chloride.

   1.2 Mercury vapor is bubbled through an absorption cell attached to a model MAS-50 (Mercury Analyzer System) or equivalent and the absorption is measured at the 253.7 nanometer line.

   1.3 The absorption signal is recorded and used to determine the concentration of mercury present.

2. **Range and Sensitivity**

   2.1 For a 1.0 mℓ urine sample the sensitivity is 0.003 mg/ℓ or below. This corresponds to an absolute sensitivity of 3 nanograms of mercury.

   2.2 The range extends up to 0.3 mg/ℓ for a 1.0 mℓ aliquot. The range can be extended beyond 0.3 mg/ℓ by taking an aliquot of urine that is less than 1.0 mℓ.

3. **Interferences**

   3.1 Metals such as gold, platinum and copper will interfere since they form an alloy with the reduced Hg.

3.2 Certain organic solvents, such as benzene, that absorb 253.7 nanometer radiation would interfere if present in significant amounts as would any substance with broad absorption such as dust, water droplets, etc.

4. **Precision and Accuracy**

4.1 Comparisons of bubbler recoveries from water standards and urine samples spiked with identical amounts of radio or stable mercury indicate that the urine samples give a 10% lower signal (Figure 1). Although the use of water standards may reduce the accuracy of the method, results may be acceptable for routine analyses. Where more accurate values are needed a multiple addition technique using the same urine spiked with Hg standard is recommended.

4.2 Absolute deviations from the means between sets of identical samples varied from 0.000 to 0.005 mg/ℓ. The average relative standard deviation (1 σ) was ± 3%.

4.3 The temperature of the water used to dilute the sample should be controlled to within ±1°C.

4.4 At least 5 mℓ of nitric acid per 1 mℓ sample should be used to insure the release of metabolized mercury.

4.5 Only aliquots of urine less than one mℓ should be used if water standards are used. *Larger* aliquots of urine would increase the difference in transfer efficiency between water standards and urine (Figure 1).

5. **Advantages of the Method**

A trained technician can do 20-40 samples a day.

6. **Apparatus**

6.1 **Mercury Evolution Train**

6.1.1 **Bubbler Flask** – 300 mℓ BOD sample bottle as supplied with Coleman Model MAS-50 or equivalent.

6.1.2 **Connecting Tubing** – minimum lengths of either glass or Tygon.

6.1.3 **Drying Tube** – approximately 8 cm length (optional)

6.2 **Analytical Equipment**

6.2.1 Coleman Mercury Analyzer Model MAS-50 or equivalent.

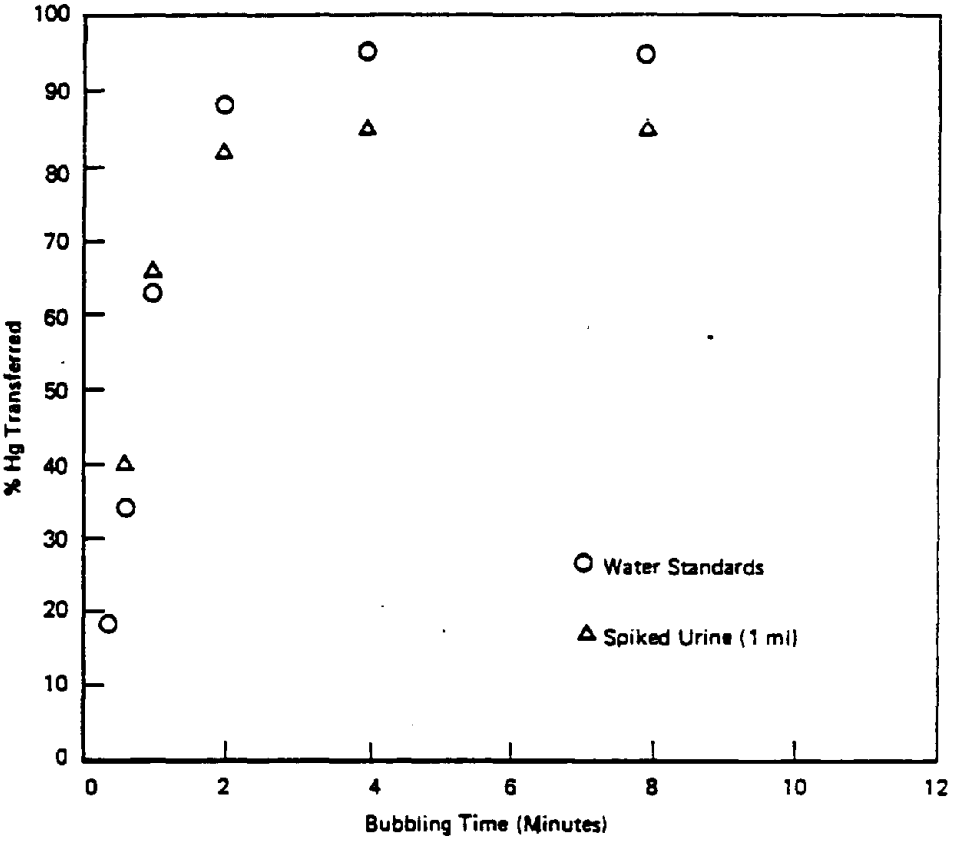


FIGURE 1 TRANSFER EFFICIENCY IN THE BUBBLER SYSTEM

6.2.2. Pipets

7. **Reagents**

All reagents used must be analytical reagent grade.

7.1 Double distilled water.

7.2 **Nitric Acid**, concentrated

7.3 **Stannous Chloride Solution**, 20% (w/v) in 6 N HCl – freshly prepared.

7.4 **Standard Stock Mercury Solution.** Add 0.1000 g metallic mercury or 0.1708g mercuric nitrate, $Hg(NO_3)_2 \cdot H_2O$, into a clean, dry 100 mℓ volumetric flask. Add 10 mℓ concentrated HNO_3, dissolve the mercury, and then dilute to the mark with double distilled water. Pipet exactly 10.0 mg of Hg into a one-liter volumetric flask. Add 50 mℓ concentrated HNO_3 and dilute to the mark with water. The final concentration is 10 μg/mℓ. This solution is stable for 6 months.

7.5 **Working Standard Mercury Solution.** Transfer 10.0 mℓ of the standard stock solution to a one-liter volumetric flask. Add 50 mℓ concentrated HNO_3 and dilute to the mark with double distilled water. The final concentration is 0.1 μg mℓ. This solution should be prepared fresh daily.

7.6 **Potassium Persulfate**, low nitrogen.

7.7 **Magnesium Perchlorate** – anhydrous.

8. **Procedure**

8.1 **Cleaning of Equipment.** Acid-clean all glassware before use. This can be done by using the following procedure:

8.1.1 Wash in detergent-tap water solution and follow with tap water.

8.1.2 Soak in concentrated HNO_3 for 30 minutes and follow with tap, distilled, and double-distilled water rinses.

8.2 **Collection and Shipping of Samples**

8.2.1 Urine samples must be collected in acid cleaned borosilicate bottles. At least 25 mℓ should be collected.

8.2.2 The samples must be preserved by the addition of 0.1 g potassium persulfate per 100 mℓ of urine. Urine treated with potassium persulfate is stable at room temperature for 2 weeks.

8.3 **Analysis of Samples**

8.3.1 Transfer a 1.0 mℓ urine sample to the BOD bottle. If a sediment is present in the collection bottle, shake well immediately before removing the aliquot. Add 5 mℓ of concentrated HNO_3 to the BOD bottle and allow the sample to stand at room temperature for 3 minutes.

8.3.2 Dilute to 100 mℓ with distilled water at room temperature and gently blow out any mist that may form above the liquid. Add 1.0 mℓ of freshly prepared $SnCl_2$ solution and immediately connect the flask to the generating train shown in Figure 2.

8.3.3 The system is run until maximum signal is attained as shown on a recorder. Peak heights are used in the calculations.

8.3.4 The drying tube in the mercury transfer train results in the loss of some mercury. However, the drying tube will give lower background signals. If a drying tube is used it must be repacked with fresh magnesium perchlorate after 20 analyses.

8.3.5 Remove the BOD bottle and allow the signal to return to zero.

9. **Calibration and Standards**

9.1 Standards for instrument calibration are analyzed exactly as samples except that known amounts of mercury are added to the sample.

9.2 The calibration curve for the multiple addition method is constructed by subtracting the reading of the unspiked urine from that of the spiked aliquots.

9.3 For routine analysis a calibration curve may be constructed using water standards instead of spiked urine; however, this will result in loss of accuracy.

9.4 The calibration is repeated with appropriate quantities of mercury to cover a working range.

10. **Calculations**

10.1 Subtract the background signal, if any, from the sample signal.

10.2 Micrograms of mercury are determined directly from the calibration curve using the corrected signal peak height obtained for each sample.

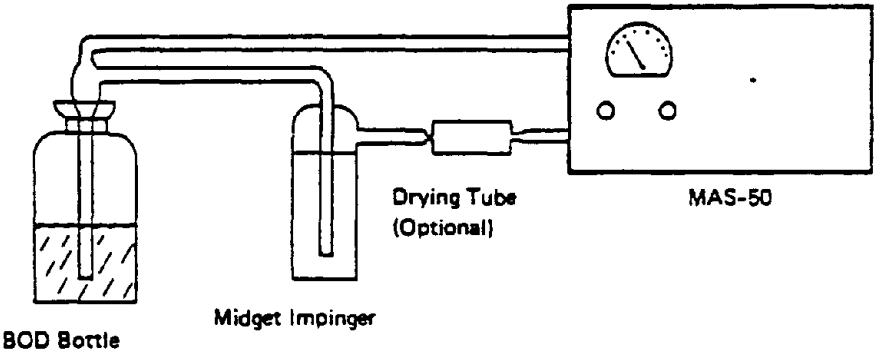


FIGURE 2 ANALYTICAL APPARATUS

10.3 The mercury concentration is calculated by dividing the micrograms of mercury by the sample volume analyzed to give μg Hg/liter.

11. References

11.1 Rathje, Arnold O., "A Rapid Ultraviolet Absorption Method for the Determination of Mercury in Urine," Am. Ind. Hyg. Assoc. J. 30. 126-132 (1969).

MERCURY IN BLOOD

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Mercury	Method No:	P&CAM 167
Matrix:	Blood	Range:	0.005-4.5 μg/mℓ Not verified
Procedure:	Flameless Atomic Absorption	Precision:	Unknown
Date Issued:	2/13/73	Classification:	D(Operational)
Date Revised:	1/3/77		

1. Principle of the Method

   1.1 Whole blood samples are digested by treating with H_2SO_4 at 54 (±1)°C followed by potassium permanganate solution.

   1.2 After reduction of excess oxidant, elemental mercury is liberated by reduction with $SnCl_2$ solution, and measured by flameless atomic absorption at 2537 Å.

2. Range and Sensitivity

   2.1 No actual range or sensitivity has been established. However, the absolute sensitivity is 0.005 μg.

   2.2 The range may be extended by increasing sample size, and varying dilution and aliquot size.

3. Interferences

   3.1 Since Hg forms very stable organic compounds, adequate digestion of the blood is needed to decompose organic mercurials.

   3.2 Acetone and other organic vapors absorb highly at the Hg line and must be absent.

4. Precision and Accuracy

   The accuracy and precision of the method have not been reported up to this time. No collaborative tests have been performed.

5. Advantages and Disadvantages of the Method

5.1 Flameless Atomic Absorption is a more sensitive method than either flame spectrometry or colorimetric methods.

5.2 Total Hg is measured. There is no distinction made between organic and inorganic Hg.

6. Apparatus

6.1 5 mℓ mercury-free heparinized vacutainers.

6.2 Rocking constant temperature water bath.

6.3 Coleman MAS-50 Mercury Analyzer System or similar Flameless Atomic Absorption System.

6.4 Mercury Evolution Train.

6.4.1 Bubbler Flask – 300 mℓ BOD bottle or equivalent.

6.4.2 All components are connected with minimum lengths of Tygon tubing.

6.5 Eppendorf pipets or equivalent.

6.6 Absorption cell, 155 mm length made of Pyrex glass (12 mm I.D.) with quartz glass windows.

6.7 Equipment for Generating Train and Absorption Sampling Tube.

6.7.1 Vacuum pump

6.7.2 Needle valve

6.7.3 Flowmeter 0-5 ℓpm

6.7.4 150 mℓ glass bubbler flask with glass joints

6.7.5 25 mℓ burette

6.7.6 All-glass midget impinger

7. Reagents

All reagents used must be ACS Reagent Grade or better. Allow all reagents and solutions to reach room temperature before using.

7.1 Double distilled water.

7.2 Concentrated sulfuric acid.

7.3 **Potassium permanganate solution** – Saturated (ca. 6%).

7.4 **Hydroxylamine-Hydrochloride solution** – 20% (w/v) in 6 N HCl or in 5% H_2SO_4 (v/v).

7.5 **Stannous Chloride solution** – 20% (w/v) in 6 N HCl or in 5% H_2SO_4 (v/v), freshly prepared.

7.6 **Standard Stock Mercury Solution:** Dissolve 100 mg of metallic Hg or 0.1708 g mercuric nitrate, $Hg(NO_3)_2 \cdot H_2O$, in 50 mℓ of concentrated nitric acid in a clean acid-washed 1 liter volumetric flask. Dilute to 1 liter with double-distilled water. This solution may be stored as long as six months.

7.7 **Hg working standard** (1 μg/mℓ) – Prepare *daily*, by diluting 1 mℓ of the standard stock solution to 100 mℓ with 2 mℓ concentrated HNO_3 and double-distilled water.

8. Procedure

8.1 **Cleaning of Equipment.** Acid clean all glassware before use. This can be accomplished by using the following procedure:

8.1.1 Wash in a detergent-tap water solution and follow with tap water rinses.

8.1.2 Soak in 1:1 or concentrated nitric acid for 30 minutes and follow with tap, distilled, and double-distilled water rinses.

8.2 **Collection and Shipping of Samples**

8.2.1 Collect *blood* in Hg-free heparinized vacutainers equipped with sterilized stainless steel needles. The vacutainers should be heparinized before the collection of the blood. Heparinized vacutainers are available commercially from most laboratory supply houses. Adding a preservative after collecting blood can result in error due to volume changes. Exercise caution during handling to prevent any external contamination.

8.2.2 Keep samples as cool as possible (do not freeze) during storage and shipment and ship to the laboratory as quickly as possible. Heparinized, refrigerated blood samples are stable for two weeks.

8.2.3 Two milliliters (2 mℓ) of whole blood are adequate for this determination.

8.3 Sample Preparation

8.3.1 Pipet 2 mℓ of blood into a 300-mℓ BOD bottle or 125 mℓ Erlenmeyer flask with stopper. Dilute blood with 5 mℓ double distilled water.

Caution: BOD bottles are not made of "Pyrex" and should not be subjected to rapid temperature changes.

8.3.2 Add 5 mℓ conc. H_2SO_4, Swirl, and Cap.

8.3.3 Heat in a rocking-constant water bath to 54°C until tissue is dissolved (3–4 hours). Add 1 mℓ water or more to provide enough liquid to mix. Digested tissue is a clear, dark reddish solution.

8.3.4 Remove digest from water bath. Cool in ice bath while slowly adding 17 mℓ 6% $KMnO_4$.

8.3.5 Cap and let stand overnight.

8.3.6 Add 2 mℓ 20% Hydroxylamine-Hydrochloride. The solution should turn clear.

8.3.7 Dilute to 50 mℓ volume with water

8.4 Analysis of Sample

8.4.1 Pipet 25 mℓ of the digest (Section 8.3.7) into the bubbler flask.

8.4.2 Add 1 mℓ 20% $SnCl_2$.

8.4.3 Immediately connect the flask to the generating train of the apparatus shown in Figure 1.

8.4.4 Recycle the evolved Hg until the signal on the recorder reaches a maximum. Use peak height as a measure of the concentration.

8.4.5 Repeat Steps 1 to 4 and average the result of the two determinations.

9. Calibration and Standards

9.1 Standards for a calibration curve are analyzed exactly as samples except that known amounts of mercury are added to the sample aliquot.

9.2 The calibration curve is constructed by subtracting the reading from the unspiked aliquot from that of the spiked aliquots.

9.3 The calibration is repeated with appropriate quantities of mercury to cover a convenient working range.

10. Calculations

10.1 The micrograms of Hg in the sample aliquot analyzed are determined from the calibration curve using peak heights after subtracting the peak height obtained from the reagent blank from the peak height of the sample aliquot.

10.2 Divide by the volume of the sample aliquot (μg/mℓ).

10.3 Multiply by 50 (or the volume of the sample digest) to obtain μg Hg/sample.

10.4 Divide by 2 (or volume of sample used) to obtain μg Hg/mℓ blood.

11. References

11.1 Applications Data Sheet, Coleman Instruments Division, January 22, 1971.

11.2 Coleman MAS-50 Manual, December 1970.

11.3 Uthe, J.F., Armstrong, F.A.J. and Stainton, M.P., "Mercury Determination in Fish Samples by Wet Digestion and Flameless Atomic Absorption Spectrophotometry." Journal Fisheries Research Board of Canada, 27, 805-11 (1970).

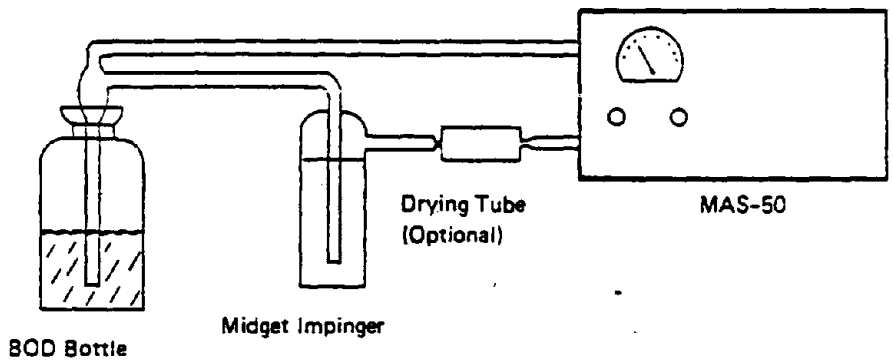


FIGURE 1 APPARATUS FOR ANALYTICAL PROCEDURE

260

AROMATIC AMINES[1] IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Aromatic Amines[1]	**Method No.:**	P&CAM 168
Matrix:	Air	**Range:**	0.01-14 mg/ sample
Procedure:	Adsorption on silica gel; elution by ethanol; GC analysis		
Date Issued:	11/1/73	**Precision:**	$2\sigma = \pm 9\%$
Date Revised:	7/15/74	**Classification:**	D (Operational)

1. **Principle of the Method**

   1.1 A known volume of air is drawn through a tube containing silica gel to trap the aromatic amines present.

   1.2 The silica gel in the tube is transferred to a glass-stoppered tube and treated with ethanol.

   1.3 An aliquot of the desorbed aromatic amines in ethanol is injected into a gas chromatograph.

   1.4 Peak areas are determined and compared with calibration curves obtained from the injection of standards.

2. **Range and Sensitivity**

   2.1 The lower limit of this method using a flame ionization detector is 0.01 mg/sample of any one compound when the analyte is desorbed with 5 ml ethanol and a 10-µl aliquot is injected into the gas chromatograph. Sensitivity for p-nitroaniline is 5 times less.

   2.2 The upper limit is at least 14 mg/sample. This is a minimum amount of aniline which the large section (700 mg) and the center section (150 mg silica gel) of the sampling tube will retain before 2% of the sample is found on the third (150 mg) section after 8 hours of sampling (200 ml/min high humidity air containing 150 mg/m³ aniline). The corresponding upper limit for sampling in the reverse direction through the tube is 3.5 mg/sample. The less volatile substituted anilines have higher upper limits than aniline.

3. **Interferences**

   3.1 The most common possible sampling interference is water vapor. The sampling tube has been designed so that 96 liters of high humidity air can be sampled over an 8-hour period at 200 ml/min without displacement of the collected aromatic amines by water vapor.

   3.2 Any compound which has nearly the same retention time as one of these aromatic amines at the gas chromatograph analytical conditions described in this method is an interference. This type of interference often can be overcome by changing the operating conditions of the gas chromatograph or selecting another column. Retention time data on a single column, or even on a number of columns, cannot be considered as conclusive proof of chemical identity

[1]The list of aromatic amines for which this method has been specifically developed is given in Table 1.

261

in all cases. For this reason it is important that whenever practical a sample of the bulk compound or mixture be submitted at the same time as the sample tube (but shipped separately) so that chemical identification can be made by other means.

4. **Precision and Accuracy**

4.1 The accuracy of the method depends upon collection efficiencies and desorption efficiencies. If a negligible amount of aromatic amine is detected on the backup section, the collection efficiency of the tube must be essentially 100%. Desorption efficiencies for the range of 1-8 mg have been found to be 100% within experimental error of $\pm$ 5%.

4.2 Precision of the analysis is quite dependent upon the precision and sensitivity of the technique used to quantitate gas chromatographic peaks of samples and standards. Electronic digital integrators with baseline correction capabilities can be used to maximize analytical precision, particularly at lower concentrations.

4.3 Precision of preparing chemical standards can be $\pm$ 1%.

4.4 The precision of the overall method, $2\sigma = \pm$ 9%, has been determined from eight consecutive identical samples taken with a personal sampling pump.

4.5 Analytical precision can be improved by reducing or eliminating the error associated with syringe injection into the gas chromatograph. This is best accomplished by the addition of internal standard to the ethanol used to both prepare standards and elute samples from the silica gel. Therefore, it is recommended that about 0.1% solution of n-heptanol in ethanol be used. For isothermal analyses at temperatures above 120°C, n-octanol may be preferable.

5. **Advantages and Disadvantages of the Method**

5.1 The sampling method uses a small, portable device involving no liquids. Taking the effect of humidity into account, a sample of up to 8 hours can be taken for an average work day concentration or a 15-minute sample can be taken to test for excursion concentrations. Desorption of the collected sample is simple and is accomplished with a solvent with low toxicity. The analysis is accomplished by a quick instrumental method. Most analytical interferences which occur can be eliminated by altering gas chromatographic conditions. Several aromatic amines can be collected and analyzed simultaneously; this is useful where the composition of the aromatic amine vapors may not be known.

5.2 A major disadvantage of the method is the limitation on its precision due to the use of personal sampling pumps currently available. After initial adjustment of flow any change in the pumping rate will affect the volume of air actually sampled. Furthermore, if the pump used is calibrated for one tube only, as is often the case, the precision of the volume of air sampled will be limited by the reproducibility of the pressure drop across the tubes.

6. **Apparatus**

6.1 One or more personal sampling pumps whose flow can be set at and maintained at 1 liter/min for 15 min or 200 ml/min for 8 hours.

6.2 Pyrex glass sampling tubes of the dimensions shown in Figure 1 packed with three sections of 45/60 mesh silica gel. The weights of the sections in order are 700 mg, 150 mg, and 150 mg. The silica gel should be the equivalent of Silica Gel D-08, Chromatograph Grade, Activated and Fines Free, 45/60 Mesh, from Applied Science Laboratories, Inc., State College, Pa. Plugs of 100 mesh stainless steel screen are used to contain the silica gel sections. These plugs of negligible pressure drop are prepared from 11-mm diameter discs pushed into an

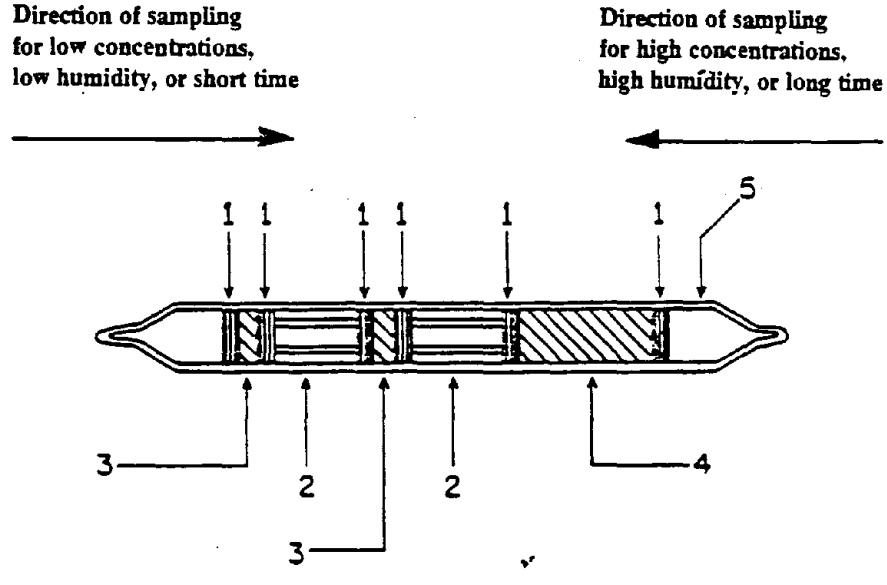


FIGURE 1. Silica gel sampling tube for aromatic amines. (1) 100 mesh stainless steel screen plugs; (2) 12-mm glass tube separator; (3) 150-mg silica gel section, 45/60 mesh; (4) 700-mg silica gel section, 45/60 mesh; (5) 8-mm I. D. glass tube.

8-mm I.D. tube with a 7-mm O.D. rod. Pieces of Pyrex tubing 7 mm O.D. by 12 mm long and located between the sorbent sections greatly reduce migration of the sample throughout the tube prior to analysis. The ends of each tube should be flame sealed after packing to prevent contamination before sampling. The pressure drop of such tubes should not exceed 13 in of water at 1 liter/min or 2.5 in of water at 200 ml/min air flows.

6.3 Gas chromatograph equipped with a flame ionization detector. Linear temperature programming capability is desirable but not essential.

6.4 Column (4 ft × ⅛ in O.D., stainless steel) packed with Silicone OV-25 liquid phase, 10% on 80/100 mesh Supelcoport (or equivalent support). A column (2 ft × ⅛ in O.D.) packed with Chromosorb 103 (80/100 mesh) can be used for all the amines except p-nitroaniline.

6.5 Recorder and some method for determining peak height or area.

6.6 Glass-stoppered tubes or flasks, 2 ml and 10 ml.

6.7 Syringes, 10 μl.

6.8 Pipettes and volumetric flasks for preparation of standard solutions.

7. Reagents

7.1 Ethanol, 95%.

7.2 n-Heptanol and n-octanol, reagent grade.

7.3 Reagent grade aromatic amines standards.

7.4 Bureau of Mines Grade A helium.

7.5 Prepurified hydrogen.

7.6 Filtered compressed air.

8. Procedure

8.1 **Cleaning of equipment:** All glassware used for the laboratory analysis is detergent washed followed by tap and distilled water rinses.

8.2 **Calibration of personal pumps:** Each pump must be calibrated with a representative tube in the line to minimize errors associated with uncertainties in the sample volume collected.

8.3 **Collection and Shipping of Samples**

8.3.1 Immediately before sampling, the ends of the tube should be broken so as to provide an opening at least one half the internal diameter of the tube.

8.3.2 The desired sampling direction is chosen, the initial section marked with a permanent marker, and tubing from the sampling pump attached to the other end. For low concentrations, low humidity, and short-term samples, the smaller (150 mg) section is used for the initial section. For expected high concentrations, high humidity, and long-term samples, the largest (700 mg) section is used for the initial section. See Table 1 footnotes. Sampled air should not pass through any hose or tubing before entering the sampling tube.

8.3.3 The atmosphere is sampled at the desired flow rate for the desired period of time. Recommended sampling volumes based on sensitivity and breakthrough studies are given in Table 1. The flow rate and sampling time or the volume of sampled air must be measured as accurately as possible.

8.3.4 The temperature, pressure, and humidity of the atmosphere being sampled is measured and recorded.

8.3.5 The sampling tubes are capped with the supplied plastic caps immediately after sampling. Under no circumstances should rubber caps be used.

8.3.6 One tube should be handled in the same manner as the sample tube (break, seal, and transport), except that no air is sampled through this tube. This tube is labeled as a blank.

8.3.7 Capped tubes should be packed tightly before shipping to minimize tube breakage during shipping.

8.3.8 Samples of the bulk liquids or solids from which the aromatic amine vapors arise should be submitted to the laboratory also, but **not** in the same container as the air samples or blank tubes.

8.3.9 Storage: Tubes after sampling should be tightly capped and not subjected to extremes of high temperature or low pressure, if avoidable.

8.4 **Analysis of Samples**

8.4.1 Preparation of samples: By removing and discarding the stainless steel plugs and glass spacers the silica gel sections are transferred to separate glass-stoppered tubes or flasks (2 ml for the smaller sections and 10 ml for the larger section). Note or mark which tubes contain the initial section, the backup section, and the third section. These are analyzed separately.

8.4.2 Desorption of samples: Prior to analysis, ethanol containing 0.1% n-heptanol internal standard is pipetted into each flask — 5 ml for the large section and 1 ml for all other sections. Tests indicate that desorption is complete in 30 min if the sample is stirred or shaken occasionally.

8.4.3 Gas chromatograph conditions: Typical operating conditions for the gas chromatograph. are: carrier flow (25 ml He/min); injection port (150°C); flame ionization detector (250°C, 50 ml/min H_2, 470 ml/min air); oven temperature program (100°C for 4 min, then increase at 8°C/min to 225°C).

8.4.4 An aliquot of the sample is injected into the gas chromatograph. With an internal standard in the eluent, direct injection of up to 10 μl with a microliter syringe is acceptably precise. At least duplicate injections of the same sample or standard are recommended.

8.4.5 The areas of the sample peak and the internal standard peak are measured by an electronic integrator or some other suitable method of area measurement. The ratio of these areas is calculated and used to determine sample concentration in the eluent by using a standard curve prepared as discussed below.

9. **Calibration and Standards**

9.1 For accuracy in the preparation of standards, it is recommended that one standard be prepared in a relatively large volume and at a high concentration. Aliquots of this standard can then be diluted to prepare other standards. The solvent and diluent used must be the same ethanol/n-heptanol mixture used for the elution of the samples. For example, to prepare a 100-ml standard corresponding to a 96-liter sample of air containing 95 mg/m^3 of aniline (density = 1.022 g/ml) desorbed with 5 ml,

$$\frac{(95\ \text{mg/m}^3)\ (0.096\ \text{m}^3)}{5\ \text{ml}} \times \frac{100\ \text{ml}}{1.022\ \text{mg/}\mu\text{l}} = 178\ \mu\text{l}$$

of aniline is added to a 100-ml volumetric flask and diluted to the mark. The resulting concentration is

$$\frac{(178\ \mu l)\ (1.022\ mg/\mu l)}{100\ ml} = 1.82\ mg/ml$$

If 2 ml of this solution is diluted to 10 ml in a volumetric flask with the ethanol/n-heptanol mixture, the resulting concentration is

$$(1.82\ mg/ml) \times \frac{2\ ml}{10\ ml} = 0.365\ mg/ml$$

When microliter pipettes are used instead of microliter syringes, it is better to prepare standards using a round number of microliters (e.g., 200 μl aniline instead of 178 μl). For solids, the amount of compound used for the first standard should be weighed on an analytical balance. A series of standards is prepared varying in concentration over the range of interest.

9.2 The standards prepared as above should be analyzed under the same GC conditions and during the same time period as the unknown samples. This will minimize the effect of day-to-day variations of the flame ionization detector response.

9.3 A standard curve is prepared for each compound by plotting ratios of peak areas of the compound to the internal standard against the concentration of the compound. From the resulting curve the concentration of an eluted sample is determined. This concentration (in mg/ml) is then converted to total sample weight by multiplying by the amount of ethanol used for that section (1 or 5 ml).

10. Calculations

10.1 Corrections for the blank must be made for each sample.

$$\text{Correct mg} = mg_a - mg_b$$

where:

mg_a = mg found in front section of sample tube

mg_b = mg found in front section of blank tube

A similar procedure is followed for the backup sections.

10.2 Add the corrected amounts present in the front and backup sections of the same sample tube to determine the total measured amount (w) in the sample.

10.3 Convert the volume of air sampled to standard conditions of 25°C and 760 mm Hg.

$$V_s = V \times \frac{P}{760} \times \frac{298}{T + 273}$$

where:

V_s = volume of air in liters at 25°C and 760 mm Hg

V = volume of air in liters as calculated (sampling time × correct flow rate)

P = barometric pressure in mm Hg

T = temperature of air in degrees centigrade

10.4 The concentration of the organic solvent in the air sampled can be expressed in mg per m^3, which is numerically equal to μg per liter of air

$$mg/m^3 = \mu g/l = \frac{w\ (mg) \times 1000\ (\mu g/mg)}{V_s}$$

10.5 Another method of expressing concentration is ppm, defined as μl of compound vapor per liter of air

$$ppm = \mu l \text{ of vapor}/V_s$$

$$ppm = \frac{\text{"v } (\mu g)}{V_s} \times \frac{24.45 \text{ l/mole}}{MW}$$

where:

24.45 l/mole = molar volume at 25°C and 760 mm Hg

MW = molecular weight of the compound (g/mole)

11. Rererence

11.1 Campbell, E. E., G. O. Wood, and R. G. Anderson, Los Alamos Scientific Laboratory Progress Reports LA-5104-PR, LA-5164-PR, LA-5308-PR, LA-5389-PR, LA-5484-PR, and LA-5634-PR, Los Alamos, New Mexico, November 1972, January 1973, June 1973, August 1973, December 1973, and June 1974.

TABLE I

Aromatic amines for which the method has been tested

	OSHA standard[a] (ppm)	OSHA standard[a] (mg/m³)	Recommended sample volumes (liters) Minimum[b]	Recommended sample volumes (liters) Maximum[c]
Aniline	5	19	5	150
N,N-Dimethylaniline	5	25	5	190
o-Toluidine	5	22	5	300
2,4-Xylidine	5	25	5	430
o-Anisidine	0.1	0.5	250	35,800
p-Anisidine	0.1	0.5	250	33,000
p-Nitroaniline	1	6	80	12,100

[a]Taken or calculated from Federal Register, 37: #202, 22139 (18 October 1972).

[b]Based on analytical sensitivity and 0.1 OSHA standard detection when using the large section as the initial section (see 2.1). When the smaller section is used as the initial section, divide these minimum volumes by 5.

[c]Based on breakthrough studies at high humidities and a concentration of 5 times the OSHA standard when using the large section as the initial section. When the smaller section is used as the initial section, divide these maximum volumes by 5.

CHROMIC ACID MIST IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Chromic Acid Mist	Method No.:	P&CAM 169
Matrix:	Air	Range:	0.004 to 0.2 mg CrO_3/m^3
Procedure:	PVC Filter-Colorimetric	Precision:	~ 4% RSD
Date Issued:	5/1/73		
Date Revised:	1/10/74	Classification:	D (Operational)

1. Principle of the Method

   1.1 Chromic acid mist is collected on a polyvinyl chloride (PVC) filter.

   1.2 The filter is washed in 0.5N H_2SO_4, diphenylcarbazide is added, and additional acid is added to bring the volume to 15 mℓ.

   1.3 The absorbance of the solution at 540 nm is read and compared to the absorbance of the standards.

2. Range and Sensitivity

   2.1 When using 2 cm cells and a 15 mℓ final volume, a 1% absorption occurs with about 0.05 μg of hexavalent chromium. For a 100 liter air sample, this translates to a sensitivity of about 0.001 mg CrO_3/m^3 for the sampled air. It is not recommended to work at this low concentration due to poor precision.

   2.2 The range for the colorimetric method is useful from 0.2 μg Cr(VI) up to about 10 μg Cr(VI) which is from 0.004 to 0.2 mg CrO_3/m^3 for a 100 liter sample of air. Dilutions are easily made.

3. Interferences

   Possible interferences for the diphenylcarbazide method include many of the heavy metals. The ones likely to be encountered at appreciable levels are iron, copper, nickel, and vanadium. Tests show that 10 μg of any of these give less than 0.5% absorbance which is equivalent to about 0.04 μg CrO_3.

4. **Precision and Accuracy**

Ten filters spiked with 1.0 μg of hexavalent chromium (a 10 μℓ droplet of 100 ppm Cr(VI) was placed on each filter and allowed to dry) gave recoveries of 93% with a relative standard deviation of 3.2%. Twenty-two filters exposed in a chromic acid mist generator had about 5 μg Cr(VI) per filter and the pooled results showed a precision of 4.3% RSD. No collaborative tests have been performed on this method.

5. **Advantages and Disadvantages**

5.1 The method is simple, specific, and sensitive. The samples collected on PVC filters are stable. There is no significant sample loss when stored for up to two weeks, with sample recoveries still in the order of 96%. Storage for longer periods results in lower recoveries, i.e., after nine weeks the average recovery is about 76%.

5.2 Samples made by spiking PVC filters are not very stable and give poor recoveries. It is, therefore, not recommended to use spiked filters for standards or any other purpose.

6. **Apparatus**

6.1 **Sampling Equipment.** The sampling unit for the filter-collection method has the following components:

6.1.1 The filter unit, consisting of the filter media (Section 6.2) and appropriate cassette filter holder, either a 2- or 3-piece filter cassette.

6.1.2 A vacuum pump such as a personal sampling pump capable of sampling at a rate of 2 ℓpm. This pump must be properly calibrated so the volume of air sampled can be measured as accurately as possible. The pump must be calibrated with a representative filter unit in the line. Flow rate, times, and/or volume must be known.

6.1.3 Thermometer.

6.1.4 Manometer.

6.1.5 Stopwatch.

6.2 VMI (Gelman) – PVC filters or equivalent.

6.3 Spectrophotometer set to operate at 540 nm.

6.4 Matched cuvettes, 2 cm path length.

6.5 All polypropylene or Teflon forceps.

6.6 Assorted laboratory glassware.

7. **Reagents**

All reagents must be analytical reagent grade or better.

7.1 **Sulfuric Acid, 0.5N.** Add 13.9 mℓ of concentrated H_2SO_4 to some water in a 1ℓ volumetric flask and dilute to mark. For uniformity of results it is suggested that the same solution be used for a complete test – samples, blanks and standards. After thorough mixing, it is convenient to transfer part of the solution to a small plastic wash bottle.

7.2 **Diphenylcarbazide.** Dissolve 0.50g of *sym*-diphenylcarbazide in a mixture of 100 mℓ of acetone and 100 mℓ of double distilled water. Store in a dark bottle in the refrigerator for up to one month.

7.3 **Chromium-Six Standard.** Dissolve 0.2829g of $K_2Cr_2O_7$ in water in a 1000 mℓ volumetric flask and dilute to mark. This solution is 100 ppm (by weight) in chromium (VI). The chromium (VI) concentration can also be expressed as 0.1 μg per μℓ.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 Wash all containers in hot, soapy water and follow with tap and distilled water rinses.

8.1.2 Soak in concentrated nitric acid (10% nitric acid for plastics) for 30 minutes. Rinse thoroughly with tap water, distilled water, and double distilled water.

8.2 Collection and Shipping of Samples

8.2.1 Chromic acid mist is collected on 5 micron PVC filters mounted into a 3-piece filter cassette. It is important that the cassette be assembled properly to assure a tight seal at the face of the filter, otherwise some chromic acid will end up on the backup pad and will be lost from the analysis.

8.2.2 Draw air through the filter by means of a personal sampling pump at the rate of 2 liters per minute. A 100 liter sample is recommended. Some

minimum sampling volumes (for 0.1 absorbance in a 2 cm cell) are as follows:

Concentration to be Measured (mg CrO_3/m^3)	Minimum Required Sample Size (liters)
.01	200
.05	40
.1 (TLV)	20
.2	10

8.2.3 With each batch of samples, one filter, labeled as a blank, should be submitted. This filter is subjected to exactly the same handling as the samples except that no air is drawn through it.

8.2.4 The cassettes in which samples are collected should be shipped in a suitable container, designed to prevent damage in transit.

8.3 **Analysis of Samples**

8.3.1 Pipette 15 mℓ of water into each cuvette to be used. Put a piece of tape on the cuvette so that its bottom edge matches the meniscus. Rinse the cuvettes.

8.3.2 Blank filters are folded and placed directly into 2 cm cuvettes. Sample filters are folded and placed in cuvettes or large test tubes.

8.3.3 Add six or seven mℓ of 0.5N H_2SO_4 to each tube and shake the tube to assure that all surfaces of the filter are washed. Remove the filters from the tubes with small forceps carefully washing all surfaces with an additional mℓ or two of 0.5N H_2SO_4. The washed filters are discarded.

8.3.4 To remove suspended dust, solutions from actual samples should be filtered in transferring them from the original tubes to 2 cm cuvettes. A No. 5, 2-hole stopper, if altered by enlarging one hole, can accommodate a small Buchener funnel and vacuum line and will fit a 2 cm cuvette. After the solution has filtered through, wash the funnel and filter with several mℓ of the 0.5N H_2SO_4. Standards should be set up along with each set of samples being analyzed as in Section 9 below.

8.3.5 Add 0.5 mℓ of the diphenylcarbazide solution to each cuvette. Then add additional 0.5N H_2SO_4 until the meniscus matches the bottom edge of the tape. Shake the cuvette to mix and wipe the outside clean with absorbent tissue.

8.3.6 Read the absorbance of the solutions on a spectrophotometer at 540 nm with 0.0 absorbance being set with the 0 μg standard described below.

9. Calibration and Standards

9.1 Transfer 6 or 7 mℓ of 0.5N H_2SO_4 into each of 6 of the 2 cm cuvettes. Pipette 0, 5, 10, 20, 50, and 100 μℓ of the 100 ppm standard into the 6 cuvettes respectively. Add 0.5 mℓ of the diphenylcarbazide solution and sufficient 0.5N H_2SO_4 to bring to the 15 mℓ mark. Shake and wipe clean. These standards are respectively the 0 μg standard, 0.5 μg standard ... etc. The zero microgram standard is used to set the zero absorbance reading of the spectrophotometer at 540 nm. The absorbance of the other standards is read and recorded along with that of the samples.

9.2 A calibration curve is drawn by plotting the absorbance of the standards against μg of chromium-six.

10. Calculations

10.1 Blank absorbance values, if any, should be subtracted from each sample absorbance value.

10.2 The concentration of CrO_3 in air can be expressed as mg CrO_3 per cubic meter or μg CrO_3 per liter.

$$\text{mg } CrO_3/m^3 = \mu g\ CrO_3/\ell$$

$$\text{mg } CrO_3/m^3 = \frac{\mu g\ Cr\ (VI)}{V_s} \times \frac{CrO_3}{Cr}$$

where:

μg Cr (VI)	=	concentration of Cr (VI) derived from calibration curve
V_s	=	volume of air sampled in ℓ at 25°C, 760 mm Hg
CrO_3	=	molecular weight of CrO_3, 100
Cr	=	molecular weight of Cr, 52.

11. References

11.1 Snell and Snell, *Colorimetric Methods of Analysis*, Volume IIA, D. Van Nostrand Co., pp. 212-215, 19

11.2 Jacobs, M.B., *The Analytical Toxicology of Industrial Inorganic Poisons*. Interscience Publishers, pp. 396-402, 19

11.3 Abell, M.T., and J.R. Carlberg, "A Simple Reliable Method for the Determination of Airborne Hexavalent Chromium," Amer. Ind. Hyg. Assoc. J., 35, 229 (1974).

GENERAL PROCEDURE FOR METALS

Measurements Research Branch

Analytical Method

Analyte:	Trace Metals (Tables 1 and 2)	**Method No.:**	P&CAM 173
Matrix:	Air	**Range:**	Varies with analyte (Table 2)
Procedure:	Filter collection, atomic absorption analysis	**Precision:**	3% RSD (Analytical)
Date Issued:	9/17/73		
Date Revised:	3/11/77	**Classification:**	D (Operational)

1. **Principle of the Method**

   1.1 This procedure describes a general method for the collection, dissolution and determination of trace metals in industrial and ambient airborne material. The samples are collected on membrane filters and treated with nitric acid to ash the organic matrix and to dissolve the metals present in the sample. The analysis is subsequently made by atomic absorption spectrophotometry (AAS).

   1.2 Samples and standards are aspirated into the appropriate AAS flame. A source of characteristic radiation energy is necessary for each metal. The absorption of this characteristic energy by the atoms of interest in the flame is related to the concentration of the metal in the aspirated sample. The flames and operating conditions for each element are listed in Table 1.

2. **Range and Sensitivity**

   The sensitivity, detection limit, and optimum working range for each metal are given in Table 2. The sensitivity is defined as that concentration of a given element which will absorb 1% of the incident radiation (0.0044 absorbance units) when aspirated into the flame. The detection limit is defined as that concentration of a given element which produces a signal equivalent to two times the standard deviation of the blank signal for aqueous solutions. Detection limits and blank values for real samples may be greater than those given in Table 2 since the blanks resulting from the reagents and the filter material have not been taken into account. The working range for an analytical precision better than 3% is generally defined as those sample concentrations which will absorb greater than 10% of the incident radiation and are in the linear region of the calibration curve. The values for the sensitivity and detection limits are instrument dependent and may vary from instrument to instrument.

3. **Interferences**

   3.1 In atomic absorption spectrophotometry, the occurrence of interference is less common than in many other analytical determination methods. Interferences can occur, however, and when encountered are corrected for as indicated in the following sections. The known interfer-

ences and correction methods for each metal are indicated in Table 1. The methods of standard additions and background monitoring and correction (11.1-11.4) are used to identify the presence of an interference problem. Insofar as possible, the matrix of the samples and standards are matched to minimize the possible interference problems.

3.1.1 Background or non-specific absorption can occur from particles produced in the flame which can scatter the incident radiation causing an apparent absorption signal. Light scattering problems may be encountered when solutions of high salt content are being analyzed. Light scattering problems are most severe when measurements are made at the lower wavelengths (i.e., below about 250 nm). Background absorption may also occur as the result of the formation of various molecular species which can absorb light. The background absorption should be accounted for by the use of background correction techniques (use of D_2 or H_2 continuum or a nearby non-absorbing wavelength) (11.1, 11.6).

3.1.2 Spectral interferences are those interferences which occur as the result of an atom different from that being measured absorbing a portion of the incident radiation. Such interferences are extremely rare in atomic absorption. In some cases, multi-element hollow cathode lamps may cause a spectral interference by having closely adjacent radiation lines from two different elements. In such instances, multi-element hollow cathode lamps should not be used, or the use of more narrow spectral slits or alternate wavelengths may be used to alleviate the problem.

3.1.3 Ionization interferences can occur when easily ionized atoms are being measured. The degree to which such atoms are ionized is dependent upon the atomic concentration and presence of other easily ionized atoms in the sample. Ionization interferences can be controlled by the addition to the sample of a high concentration of another easily ionized element which will buffer the electron concentration in the flame. Typically, 1000 to 2000 µg/ml of an alkali metal (K, Na, Cs) salt is added to sample and standard solutions.

3.1.4 Chemical interferences occur in atomic absorption spectrophotometry when species present in the sample cause variations in the degree to which atoms are formed in the flame. Such interferences may be corrected for by controlling the sample and standard matrix, by using the method of standard additions, or by use of a higher temperature flame (11.2, 11.6).

3.1.5 Physical interferences may result if the physical properties of the samples vary significantly. Changes in viscosity and surface tension can affect the sample aspiration rate and thus cause erroneous results. Sample dilution and/or the method of standard additions are used to correct such interferences. High concentrations of silicates in the sample can cause an interference for many of the elements and may cause aspiration problems. No matter what elements are being measured, if large amounts of silicates are extracted from the samples, the samples should be allowed to stand for several hours and centrifuged or filtered to remove the silicates.

3.2 This procedure describes a generalized method for sample preparation which is applicable to the majority of samples of interest. There are, however, some relatively rare chemical forms of a few of the elements listed in Table 1 which will not be dissolved by this procedure. If such chemical forms are suspected, results obtained using this procedure should be compared with those obtained using an appropriately altered dissolution procedure. Alternatively, the results may be compared with values obtained utilizing a non-destructive technique which does not require sample dissolution (e.g., X-ray fluorescence, activation analysis).

4. **Precision and Accuracy**

4.1 The relative standard deviation of the analytical measurement is approximately 3% when measurements are made in the ranges listed in Table 2. The overall relative standard deviation will be somewhat larger than this value due to errors associated with the sample collection and preparation steps.

4.2 No data are presently available on the accuracy of this method for actual air samples.

5. **Advantages and Disadvantages of the Method**

5.1 The sensitivity is adequate for all metals in air samples provided an adequate volume of air is sampled.

5.2 A disadvantage of the method is that approximately 2 ml of solution is necessary for each metal determination. Also, the necessary dilution limits the detection of small quantities of analyte or replicate analysis of several elements per sample.

6. **Apparatus**

6.1 Sampling Equipment. The sampling unit for the collection of personal air samples has the following components:

6.1.1 The filter unit, consisting of the filter media (6.2) and appropriate cassette filter holder, either a 2- or 3-piece filter cassette (Millipore Filter Corporation, Bedford, Mass., or equivalent).

6.1.2 A personal sampling pump of sufficient capacity to maintain a face velocity of 2.5 cm/sec (1-2 lpm using a 37-mm filter). This pump must be calibrated so the volume of air sampled can be measured to an accuracy of $\pm$ 5%. The pump must be calibrated with a representative filter unit in the line.

6.1.3 Thermometer.

6.1.4 Manometer.

6.1.5 Stopwatch.

6.1.6 Various clips, tubing, spring connectors, and belt for connecting sampling apparatus to worker being sampled.

6.2 Cellulose ester membrane filter, 0.8-μm pore size, 37-mm (Millipore Type AA or equivalent).

6.3 Glassware, borosilicate. Before use, all glassware must be cleaned in 1:1 diluted nitric acid and rinsed several times with distilled water.

6.3.1 125-ml Phillips or Griffin beakers with watch glass covers.

6.3.2 15-ml graduated centrifuge tubes.

6.3.3 10-ml volumetric flasks.

6.3.4 100-ml volumetric flasks.

6.3.5 1-liter volumetric flasks.

6.3.6 125-ml polyethylene bottles.

6.3.7 Additional auxiliary glassware such as pipettes and different size volumetric glassware will be required depending on the elements being determined and the dilutions required to have sample concentrations above the detection limit and in the linear response range (i.e., see Table 2). All pipettes and volumetric flasks required in this procedure should be calibrated class A volumetric glassware.

6.4 Hotplate (suitable for operation at 140°C).

6.5 **Equipment for Analysis**

6.5.1 Atomic absorption spectrophotometer, with burner heads for air-acetylene and nitrous oxide-acetylene flames.

6.5.2 Hollow cathode or electrodeless discharge lamps, for each metal and a continuum lamp (D_2 or H_2).

6.5.3 Two stage regulators, for air, acetylene, and nitrous oxide.

6.5.4 Heating tape and rheostat, for nitrous oxide regulator (second regulator stage and connecting hose to the instrument should be heated to approximately 60°C to prevent freeze-up).

6.6 **Supplies**

6.6.1 Acetylene gas (cylinder), of a grade specified by the manufacturer of the instrument employed. (Replace cylinder when pressure decreases below 100 psi.)

6.6.2 Nitrous oxide gas (cylinder).

6.6.3 Air supply, with a minimum pressure of 40 psi, filtered to remove oil and water.

7. **Reagents**

7.1 Purity. ACS analytical reagent grade chemicals or equivalent shall be used in all tests. References to water shall be understood to mean double distilled water or equivalent. Care in selection of reagents and in following the listed precautions is essential if low blank values are to be obtained.

7.2 Concentrated nitric acid (68-71%), redistilled, specific gravity 1.42.

7.3 Standard stock solutions (1000 μg/ml) for each metal in Table 1, commercially prepared or prepared per instrument manufacturer's recommendations.

7.4 Lanthanum nitrate [$La(NO_3)_3 \cdot 6H_2O$].

7.5 Cesium nitrate ($CsNO_3$).

8. **Procedure**

8.1 **Cleaning of Equipment**

8.1.1 Before initial use, glassware is cleaned with a saturated solution of sodium dichromate in concentrated sulfuric acid (Note: Do not use for chromium analysis) and then rinsed thoroughly with warm tap water, concentrated nitric acid, tap water, and deionized water, in that order, and then dried.

8.1.2 All glassware is soaked in a mild detergent solution immediately after use to remove any residual grease or chemicals.

8.1.3 For glassware which has previously been subjected to the entire cleaning procedure, it is not necessary to use the chromic acid cleaning solution.

8.2 **Collection and Shipping of Samples**

8.2.1 Ambient atmospheric particulate matter and industrial dusts and fumes are sampled with cellulose membrane filters. Sample flow rate is monitored with a calibrated rotameter (11.5) or the equivalent. The flow rate, ambient temperature, and barometric pressure are recorded at the beginning and the end of the sample collection period.

8.2.2 For personal sampling, 37-mm diameter filters in holders are used. The personal sampling pumps for this application are operated at 1.5 lpm. In general, a 2-hour

sample at 1.5 lpm will provide enough sample to detect the elements sought at air concentrations of 0.2 × TLV.

8.2.3 After sample collection is complete, plug the openings of the cassette and submit the sampling unit to the laboratory. Losses of sample due to overloading (>2 mg) of the filter must be avoided.

8.2.4 Filter samples should be sealed in individual plastic filter holders for storage and shipment.

8.3 **Preparation of Samples**

8.3.1 The samples and blanks (minimum of 1 filter blank for every 10 filter samples) are transferred to clean 125-ml Phillips or Griffin beakers and 6.0 ml HNO_3 is added. Antimony samples are ashed in 6.0 ml of a 5:1 mixture of HNO_3:H_2SO_4. Each beaker is covered with a watch glass and heated on a hot plate (140°C) in a fume hood until the sample dissolves and a slightly yellow solution is produced. Approximately 4 hours of heating will be sufficient for most air samples. However, subsequent additions of HNO_3 may be needed to completely ash and destroy high concentrations of organic material, and under these conditions longer ashing times will be needed. Once the ashing is complete as indicated by a clear solution in the beaker, the watch glass is removed and the sample is allowed to evaporate to near dryness (approximately 0.5 ml).

8.3.2 Remove the beaker from the hot plate; cool, and add 1 ml HNO_3 and 2-3 ml of distilled H_2O. For lead samples, concentrated HCl is used instead of HNO_3. The solution is quantitatively transferred with distilled water to a 10-ml volumetric flask. If any elements are being determined which require the ionization buffer, 0.2 ml of 50 mg/ml Cs is added to the volumetric flask (see Table 1, footnote d). If any elements requiring the releasing agent are being determined, 0.2 ml of 50 mg/ml La is added to each volumetric flask (see Table 1, footnote 3). The samples are then diluted to volume with water.

8.3.3 The 10-ml solution may be analyzed directly for any element of very low concentration in the sample. Aliquots of this solution may then be diluted to an appropriate volume for the other elements of interest present at higher concentrations. (Note: Approximately 2 ml of solution are required for each element being analyzed.) The dilution factor will depend upon the concentration of elements in the sample and the number of elements being determined by this procedure.

8.4 **Analysis of Samples**

8.4.1 Set the instrument operating conditions as recommended by the manufacturer. The instrument should be set at the radiation intensity maximum for the wavelength listed in Table 1 for the element being determined.

8.4.2 Standard solutions should match the sample matrix as closely as possible and should be run in duplicate. Working standard solutions, prepared fresh daily, are aspirated into the flame and the absorbance recorded. Prepare a calibration graph as described in Section 9.2.3. (Note: All combustion products from the AA flame must be removed by direct exhaustion through the use of a good separate flame ventilation system.)

8.4.3 Blank filters must be carried through the entire procedure each time samples are analyzed.

8.4.4 Aspirate the appropriately diluted samples directly into the instrument and record the absorbance for comparison with standards. Should the absorbance be above the calibration range, dilute an appropriate aliquot to 10 ml. Aspirate water between each sample. A mid-range standard must be aspirated with sufficient frequency (i.e.,

once every 5 samples) to assure the accuracy of the sample determinations. To the extent possible, all determinations are to be based on replicate analysis.

9. Calibration and Standards

9.1 Ionization and chemical interference suppressants

9.1.1 Lanthanum solution (50 mg/ml). Dissolve 156.32 g of lanthanum nitrate [$La(NO_3)_3 \cdot 6H_2O$] in 2% (v/v) HNO_3. Dilute to volume in a 1-liter volumetric flask with 2% (v/v) HNO_3. When stored in a polyethylene bottle, this solution is stable for at least one year.

9.1.2 Cesium solution (50 mg/ml). Dissolve 73.40 g of cesium nitrate ($CsNO_3$) in distilled water. Dilute to volume in a 1-liter volumetric flask with distilled water. When stored in a polyethylene bottle this solution is stable for at least one year.

9.2 Standard metal solutions

9.2.1 Dilute standards (100 μg metal/ml). Pipet 10 ml of the stock (1000 μg metal/ml; Section 7.3) into a 100-ml volumetric flask, add 10 ml HNO_3 and dilute to volume with distilled water. For antimony standards, 5 ml H_2SO_4 is added with the HNO_3. Lead standards require 10 ml HCl instead of 10 ml HNO_3. Prepare these standards fresh weekly. (Note: Silver standards must be stored in amber bottles away from direct light.)

9.2.2 Working standards. Working standards for each metal of interest are prepared by dilution of the dilute standards (9.2.1) or the stock standards (7.3) such that the final acid concentration is the same for the samples and standards (i.e., 10% v/v HNO_3 in most cases). Lanthanum or cesium is added to samples and standards as indicated in Table 1 such that the final concentration is 1000 μg La or Cs/ml. Concentrations of the working standards should cover the range for the metal of interest (Table 2). Prepare these solutions fresh daily.

9.2.3 The standard solutions are aspirated into the flame and the absorbance (or concentration) recorded. If the instrument used displays transmittance, these values must be converted to absorbance. A calibration curve is prepared by plotting absorbance (units) versus metal concentration. The best fit curve (calculated by linear least square regression analysis) is fitted to the data points. This line or the equation describing the line is used to obtain the metal concentration in the samples being analyzed.

9.2.4 To insure that the preparation procedure is being properly followed, clean membrane filters are spiked with known amounts of the elements being determined by adding appropriate amounts of the previously described standards and carried through the entire procedure. The amount of metal is determined and the per cent recovery calculated. These tests will provide recovery and precision data for the procedure as it is carried out in the laboratory for the soluble compounds of the elements being determined.

9.2.5 Analysis by the method of standard additions. In order to check for interferences, samples are initially and periodically analyzed by the method of standard additions and the results compared to those obtained by the conventional analytical determination. For this method the sample is divided into three 2-ml aliquots. To one of the aliquots an amount of metal approximately equal to that in the sample is added. To another aliquot twice this amount is added. (Note: Additions should be made by micropipetting techniques such that the volume does not exceed 1% of the original aliquot volume, i.e., 10 μl and 20 μl additions to a 2-ml aliquot.) The solutions are then analyzed and the absorbance readings are plotted against metal added to the

original sample. The line obtained from such a plot is extrapolated to 0 absorbance and the intercept on the concentration axis is taken as the amount of metal in the original sample (11.2). If the result of this determination does not agree to within 10% of the values obtained with the procedure described in section 9.2.3, an interference is indicated and standard addition techniques should be utilized for sample analysis.

10. Calculations

10.1 The uncorrected volume collected by the filter is calculated by averaging the beginning and ending sample flow rates, converting to cubic meters and multiplying by the sample collection time. The formula for this calculation is

$$V = \frac{(F_B + F_E)\,t}{2000}$$

where:

V = sample volume (m^3)

F_B = sample flow rate at beginning of sample collection (lpm)

F_E = sample flow rate at end of sample collection (lpm)

t = sample collection time (minutes)

10.2 After any necessary correction for the blank has been made, metal concentrations are calculated by multiplying the micrograms of metal per ml in the sample aliquot by the aliquot volume and dividing by the fraction which the aliquot represents of the total sample and the volume of air collected by the filter:

$$\mu g\ \text{metal}/m^3 = \frac{(C \times V_A) - B}{V \times F}$$

where:

C = concentration (μg metal/ml) in the aliquot

V_A = volume of aliquot (ml)

B = total μg of metal in the blank

F = fraction of total sample in the aliquot used for measurement (dimensionless)

V = volume of air sampled (m^3)

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11.10 Analytical Data for Elements Determined by Atomic Absorption Spectroscopy, Varian Techtron, Walnut Creek, California, 1971.

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Table 1. Instrument Parameters

Element	Type of Flame o = oxidizing r = reducing	Analytical Wavelength (nm)	Interferences[b]	Remedy[b]	References
Ag	Air-C_2H_2(o)	328.1	IO_3^-, WO_4^{-2}, Mn_4^{-2}	c	(5)
Al[a]	N_2O-C_2H_2(r)	309.3	Ionization, SO_4^{-2}, V, Fe, HCl, H_2SO_4	c,d,e	(4,6)
As	Air-C_2H_2(o)	193.7	Background absorption	g	(6)
Ba	N_2O-C_2H_2(r)	553.6	Ionization, large conc. of Ca	d,f	(1,4)
Be[a]	N_2O-C_2H_2(r)	234.9	Al, Si, Mn	c,g	(4)
Bi	Air-C_2H_2(o)	223.1		g	
Ca	Air-C_2H_2(r) N_2O-C_2H_2(r)	422.7	Ionization & chemical	d,e	(1,4)
Cd	Air-C_2H_2(o)	228.8		g	
Co[a]	Air-C_2H_2(o)	240.7		g	
Cr[a]	Air-C_2H_2(r)	357.9	Fe, Ni	c	(4)
Cu	Air-C_2H_2(o)	324.8			
Fe	Air-C_2H_2(o)	248.3	High Ni conc., Si	c,g	(1,4)
In	Air-C_2H_2(o)	303.9	Al, Mg, Cu, Zn, $H_2PO_4^{-3}$	c	(10)
K	Air-C_2H_2(o)	766.5	Ionization	d	(1,4)
Li	Air-C_2H_2(o)	670.8	Ionization	d	(11)
Mg	Air-C_2H_2(o)	285.2	Chemical	e	(1,4)
	N_2O-C_2H_2(o)		Ionization	d	
Mn	Air-C_2H_2(o)	279.5			
Mo	N_2O-C_2H_2(r)	313.3	Ca and other ions	h	(6)
Na	Air-C_2H_2(o)	589.6	Ionization	e	(1,4)
Ni	Air-C_2H_2(o)	232.0		g	
Pb	Air-C_2H_2(o)	217.0 283.3	Ca, High conc. $SO_4^{=}$	c,g	(7)
Pd	Air-C_2H_2(o)	247.6	Al, Co, Ni, Pt, Rh, Ru	e	(6)
Rb	Air-C_2H_2(o)	780.0	Ionization	d	(1,3)
Sb	Air-C_2H_2(o)	217.6	Pb	i,g	(6)
Si	N_2O-C_2H_2(r)	251.6	Avoided by not using multi-element lamp containing Fe		
Sr	Air-C_2H_2(r) N_2O-C_2H_2(r)	460.7	Ionization & chemical	d,e	(1,3)
Te	Air-C_2H_2(o)	214.3		g	(6)
Tl	Air-C_2H_2(o)	276.8			
V[a]	N_2O-C_2H_2(r)	318.4			
Zn	Air-C_2H_2(o)	213.9		g	

a. Some compounds of these elements will not be dissolved by the procedure described here. When determining these elements one should verify that the types of compounds suspected in the sample will dissolve using this procedure. (See Section 3.2)

b. High concentrations of silicates in the sample can cause an interference for many of the elements in this table and may cause aspiration problems. No matter what elements are being measured, if large amounts of silicates are extracted from the samples, the samples should be allowed to stand for several hours and centrifuged or filtered to remove the silicates.

c. Samples are periodically analyzed by the method of additions to check for chemical interferences. If interferences are encountered, determinations must be made by the standard additions method or, if the interferent is identified, it may be added to the standards.

d. Ionization interferences are controlled by bringing all solutions to 1000 μg/ml Cs (samples and standards).

e. 1000 μg/ml solution of La as a releasing agent is added to all samples and standards.

f. In the presence of very large Ca concentrations (greater than 0.1%) a molecular absorption from $Ca(OH)_2$ may be observed. This interference may be overcome by using background correction when analyzing for Ba.

g. Use D_2 or H_2 continuum for background correction.

h. Add 1000 μg/ml Al to both standards and samples.

i. Use alternate Sb line (231.2 nm).

Table 2. Sensitivity Data

Element	Sensitivity[a] µg/ml	Solution[a] Detection Limits µg/ml	Range[a,b] µg/ml	Range[a,b] µg/m³ [c,d]	Minimum[e,f] TLV µg/m³
Ag	0.06	0.002	0.5-4.0	21-170	10 (metal and soluble compounds)
Al	1.0	0.02	5-50	210-2100	NL
As	0.8	0.2	10-50	420-2100	50 (arsenic trioxide production)
					500 (arsenic and compounds as As)
Ba	0.4	0.008	1-25	42-1050	500 (soluble compounds)
Be	0.025	0.001	0.1-2.0	4.2-84	2
Bi	0.5	0.025	1-30	42-1250	NL
Ca	0.08	0.005	0.1-5.0	4.2-210	2000 (CaO)
Cd	0.025	0.001	0.1-2.0	4.2-84	50 (metal dust, soluble salts, cadmium oxide fume)
Co	0.15	0.01	0.5-5.0	21-210	50 (metal fume and dusts)
Cr	0.1	0.003	0.5-5.0	21-210	500 (soluble chromic, chromous salts)
					100 (chromic acid and chromate, as CrO_3)
					100 (chromite ore processing, as CrO_3)
Cu	0.09	0.002	0.5-5.0	21-210	1000 (dusts and mists)
					200 (fume)
Fe	0.12	0.005	0.5-5.0	21-210	5000 (iron oxide fume, as iron oxide
					1000 (soluble compounds)
In	0.7	0.02	5-50	210-2100	100 (metal and compounds)
K	0.04	0.002	0.1-2.0	4.2-84	2000 (as KOH)
Li	0.035	0.0003	0.1-2.0	4.2-84	25 (as lithium hydride)
Mg	0.007	0.0001	0.05-0.50	2.1-21	10,000 (as magnesium oxide fume)
Mn	0.055	0.002	0.5-3.0	21-125	5000 (metal and compounds)
Mo	0.5	0.02	15-40	625-1650	5000 (soluble, as Mo)
					10,000 (insoluble, as Mo)
Na	0.015	0.0002	0.05-1.0	2.1-42	2000 (as NaOH)
Ni	0.15	0.005	0.5-5.0	21-210	100 (metal and soluble compounds)
Pb	0.5	0.01	1-20	42-840	150 (inorganic compounds, fumes and dusts)
Pd	0.25	0.02	4-15	170-625	NL
Rb	0.1	0.002	0.5-5.0	21-210	NL
Sb	0.5	0.04	10-40	420-1650	50 (antimony trioxide production)
					500 (antimony trioxide handling and use, as Sb)
Si	1.8	0.02	50-150	2100-6300	10,000
Sr	0.12	0.002	0.5-5.0	21-210	NL
Te	0.5	0.03	5-25	210-1050	100
Tl	0.5	0.01	5-20	210-840	100 (soluble compounds)
V	1.7	0.04	10-150	420-6300	500 (V_2O_5 dust)
					50 (V_2O_5 fume)
Zn	0.018	0.001	0.1-1.0	4.2-42	5000 (ZnO fume)

a. Data from "Analytical Methods for Atomic Absorption Spectrophotometry," Perkin-Elmer Corp., Norwalk, Conn. 1976.

b. Data from "Analytical Methods for Flame Spectroscopy," Varian Techtron, Australia, 1972.

c. The atmospheric concentrations were calculated assuming a collection volume of 0.24 m³ (2 lpm for 2 hours) and an analyte volume of 10 ml for the entire sample.

d. For elements whose TLV is less than the lower limits of the range, a larger sampling volume will be required.

e. Threshold limit values of airborne contaminants and physical agents with intended changes adopted by ACGIH for 1976. All values listed are expressed as elemental concentrations except as noted.

f. NL signifies No Limit expressed for this element or its compounds.

MERCURY IN AIR

Measurements Research Branch

Analytical Method

Analyte:	Particulate Hg, Hg° and organic Hg vapors	**Method No.:**	P&CAM 175
Matrix:	Air	**Range:**	0.001-1.0 μg Hg
Procedure:	Collection in three-section solid sorbent, flameless AA	**Precision:**	0.073 RSD at 65 ng Hg vapor/liter
Date Issued:	12/15/73		
Date Revised:	3/9/77	**Classification:**	E (Proposed)

1. Principle of the Method

1.1 Particulate mercury is collected on a membrane prefilter ahead of a two-section, solid phase sampling tube. Organic mercury vapor is collected on the first section of the tube and metallic mercury vapor is amalgamated on the second section.

1.2 The membrane filter is removed from the sampler and digested in a Teflon decomposition vessel with nitric acid. Mercury in the digested filter is reduced to metallic mercury vapor and is transferred onto a tube containing a silver-coated substrate. The collected mercury is thermally desorbed from the silver through a flameless atomic absorption spectrophotometer (modified Coleman Model MAS-50 or equivalent) where the amount of mercury from the membrane is determined.

1.3 The two-section sampling tube used for absorbing organic and metallic mercury is purged by passing filtered air through the tube to remove any metallic mercury which may have remained in the first section.

1.4 The two-section tube is then broken between the sections and each section is analyzed separately. The first section represents mercury collected as organic mercury vapor and the second section represents metallic mercury. Each section is analyzed by thermally desorbing the mercury through the absorption cell of the flameless atomic absorption spectrophotometer (modified Coleman Model MAS-50 or equivalent). Absorption signals at the 2537Å line are recorded by strip chart recorders.

1.5 The recorder signals are compared to standard calibration curves covering the required concentration range and the concentration of the mercury in the two sections of the tube is calculated from the signals.

2. Range and Sensitivity

2.1 A dual optical cell system is used to extend the range of the method. The first cell is 15.5 cm in length and is used in the 0.001 μg to 0.20 μg range. The second cell is 2.5 cm long and is used for 0.005 μg to 1.0 μg of mercury.

285

2.2 The range and sensitivity of the method (as Hg) is the same for the three forms of mercury since the same analytical technique is used for each form.

2.3 The sensitivity of the method using the 0 to 100% transmittance scale for both optical cells is 0.001 μg Hg/sample/1% Abs. for the 15.5 cm cell and 0.005 μg for the 2.5 cm cell.

3. Interferences

3.1 Loading the prefilter with excess particulates will increase air flow resistance through the sampler. This may overload battery operated sampling pumps.

3.2 Excessive amounts of water vapor may interfere if water is condensed in the sampling tubes; however, small amounts of water are removed by purging the tubes with filtered air before analysis as described.

3.3 Strong oxidizing vapors and gases, particularly chlorine, which attack silver reduce the efficiency of the sampling tube but do not interfere in the analysis of the tube. These interferences should not be a problem under normal sampling conditions.

4. Precision and Accuracy

4.1 Standards are prepared by injecting known amounts of mercury as mercuric nitrate solution directly into tubes containing the silvered substrate, followed by drying the tubes at 50°C for at least 6 hours. Studies using labelled Hg have shown that there are no losses of Hg during a drying period of seven days.

4.2 Studies on the efficiency of the bubbler system used in the procedure for particulate mercury show that only 95 $\pm$ 3% of the mercury in aqueous solutions is transferred after 4 minutes of bubbling. Therefore, calibration curves must be prepared using bubbled standards.

4.3 The overall sampling and analytical precision of this revised method is 7.3% relative standard deviation (RSD) of 13 mercury vapor samples collected at 0.2 lpm in an atmosphere containing 65 ng Hg/l. On the average, 6% of the vapor is retained by the Carbosieve B section. This bias is corrected for in the calculation.

4.4 The precision and accuracy of the method for particulate and organic mercury has not been completely determined.

4.5 The pooled precision (n = 40) for the preparation and analysis of four groups of standards (20, 41, 81, and 122 ng Hg/tube) analyzed over a two-week period is 5.6% RSD.

5. Advantages and Disadvantages of the Method

5.1 The main advantage is the convenience of the sampling procedure and the ability to analyze particulate, metallic, and organic mercury.

5.1.1 The efficiency of the silvered substrate for collecting metallic mercury vapor and the efficiency of Carbosieve B for organic mercury vapor allows the use of small sampling tubes with low air flow resistance.

5.1.2 The sampling tubes contain no liquids and are easily stored and shipped without mercury losses.

5.1.3 Since each section of the sample is analyzed by the same technique, the need for separate analytical methods is eliminated.

5.1.4 Amalgamation of the metallic mercury released from each sample makes the method more selective than other methods.

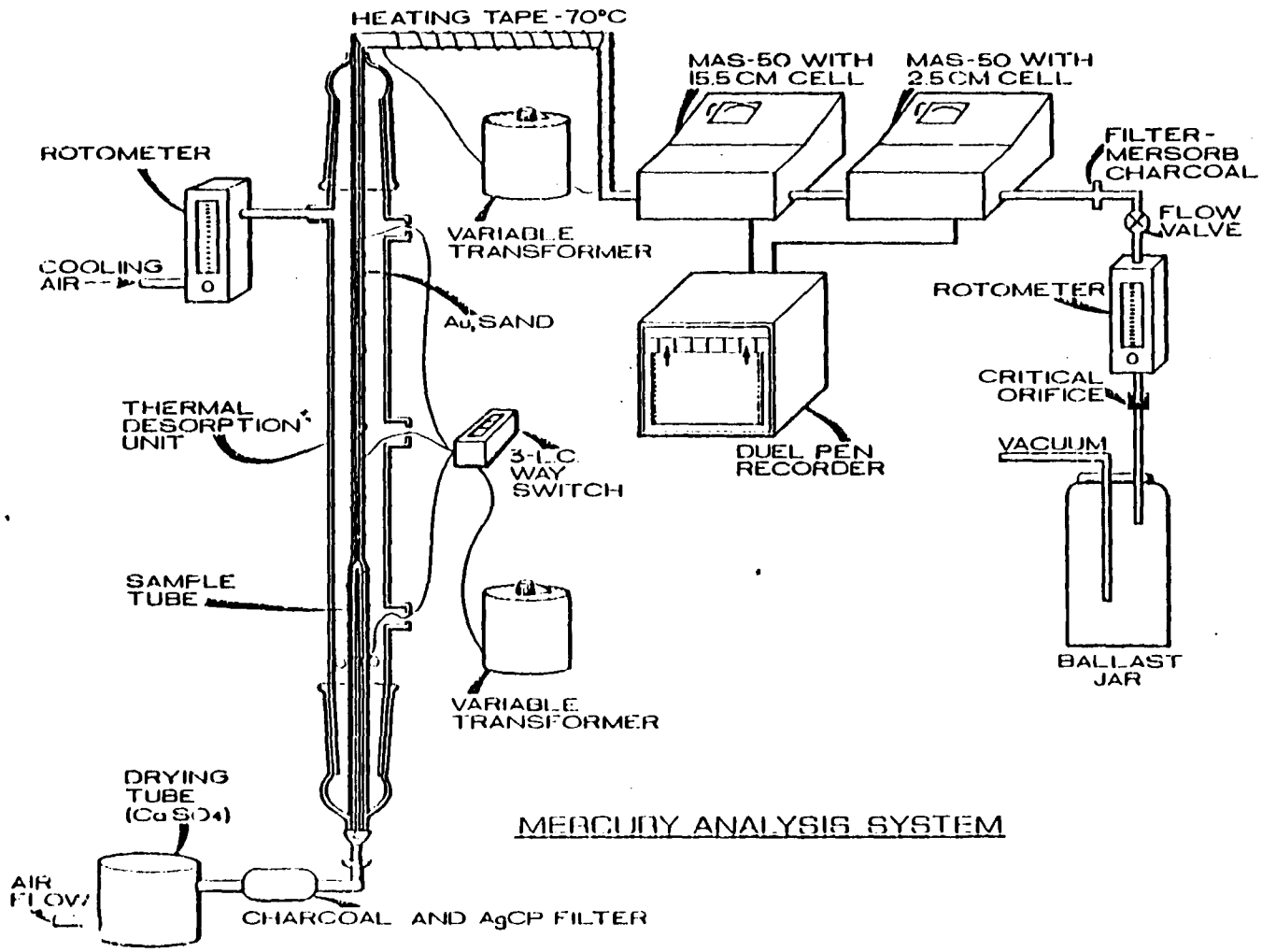


FIGURE 1. Mercury Analysis System

5.2 The main disadvantage of the method is the care necessary to avoid contamination of the outer surfaces of the sampling tubes. Although the method is satisfactory for total mercury below 0.001 μg, absolute separation of organic and metallic mercury becomes less certain for amounts below this level.

6. Apparatus

6.1 Air Sampling Tubes — Prepared as described or available on special order from SKC, Inc., 402 Greentree Rd., Pittsburgh, PA 15220.

6.2 Preparation of Silvered Substrate

6.2.1 Beaker: 1000 ml.

6.2.2 Erlenmeyer flask: 1000 ml capacity with glass stopper.

6.2.3 Stirrer: magnetic with stirring bar.

6.2.4 Separatory funnel or burrette with stopcock: 125 ml capacity.

6.3 Preparation of Air Sampling Tubes

6.3.1 Glass rod.

6.3.2 Gloves, white cotton or disposable laboratory wipers.

6.3.3 Thermal Desorption Analysis System: Figure 1.

6.4 Preparation of Mercury Standards: Direct Addition Method

6.4.1 Eppendorf pipettes or equivalent: 0.025 ml, 0.050 ml, 0.075 ml, and 0.100 ml.

6.4.2 Forceps (tweezers), fine tip.

6.4.3 Glass rod: approximately 3-mm diameter.

6.4.4 Thermal Desorption Analysis System: Figure 1.

6.5 Collection and Analysis of Air Samples

6.5.1 Sampling Equipment

1. A filter unit (for particulate mercury) which consists of the filter media (Section 6.5.1.8) and cassette filter holder.
2. Two stage mercury sampling tube (Figure 2).
3. A calibrated personal sampling pump, capable of maintaining an accuracy of ± 5% at the recommended flow rate (1 lpm).
4. An integrating volume meter such as a dry gas or wet test meter.
5. Thermometer.
6. Manometer.
7. Stopwatch.
8. Membrane filter, 0.8-μm, 37-mm, Type AA.

6.5.2 Analysis of Particulate Mercury

1. Air pump: capable of delivering air at 1 lpm through a bubbler flask.
2. Bubbler flask: 300-ml BOD bottle as supplied with Coleman MAS-50 or equivalent.
3. Connecting tubing: minimum lengths of either glass or Tygon.
4. Decomposition vessels: Teflon, 20 ml capacity, Parr Instrument Co. (211 Fifty-third St., Moline, IL 61265) or equivalent.
5. Oven, capable of maintaining 50°C, ± 5°C.

6.5.3 Analysis of Metallic and Organic Mercury.

Thermal Desorption Analysis System (see Figure 1), with the following components:

1. Flow meters: 0-2 liters/min and 0-20 liters/min.
2. Critical orifice, 1.0 lpm.
3. Variable transformers, 22 amp and 7.5 amp.
4. Vacuum pump.
5. Thermal desorption unit (see Figure 3).
6. Absorption cell #1: 15.5 cm length, made of Pyrex glass with quartz glass windows for Coleman MAS-50, 1.2 cm inside diameter.
7. Absorption cell #2: 2.5 cm length, made of Pyrex glass with quartz glass windows, 1.2 cm inside diameter.
8. Flameless atomic absorption mercury analyzers with recorders: Coleman Mercury Analysis System (MAS-50), or equivalent.

7. Reagents

All reagents used should be ACS Analytical Reagent Grade or equivalent.

7.1 Preparation of Silvered Substrate

7.1.1 Ammonium hydroxide (NH_4OH): concentrated.

7.1.2 Chromosorb P: non-acid washed, sieved to 30/60 mesh, (Johns-Manville).

7.1.3 Dextrose: powder.

7.1.4 Potassium hydroxide (KOH).

7.1.5 Silver nitrate ($AgNO_3$): crystals.

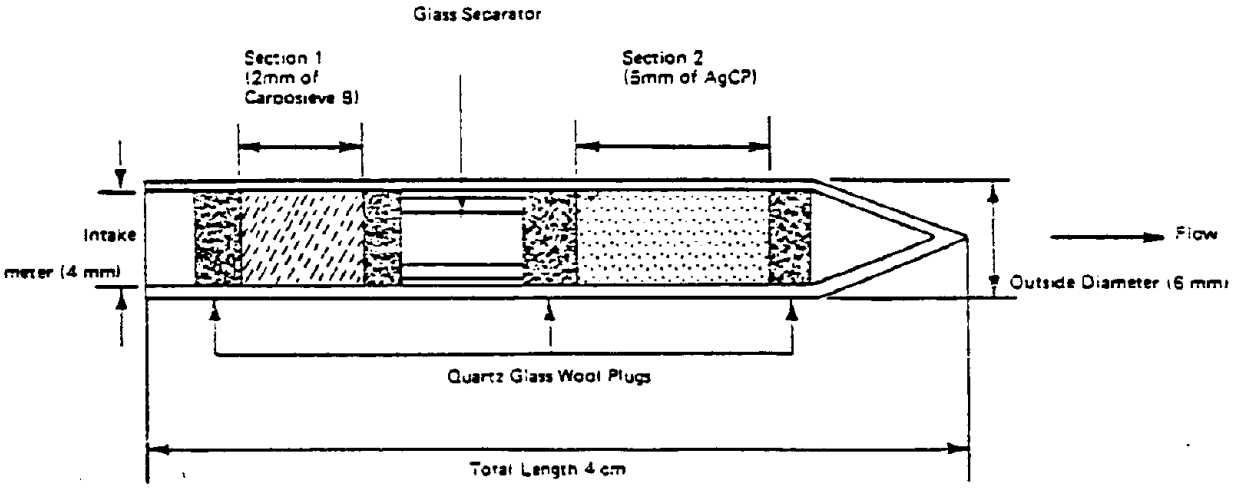
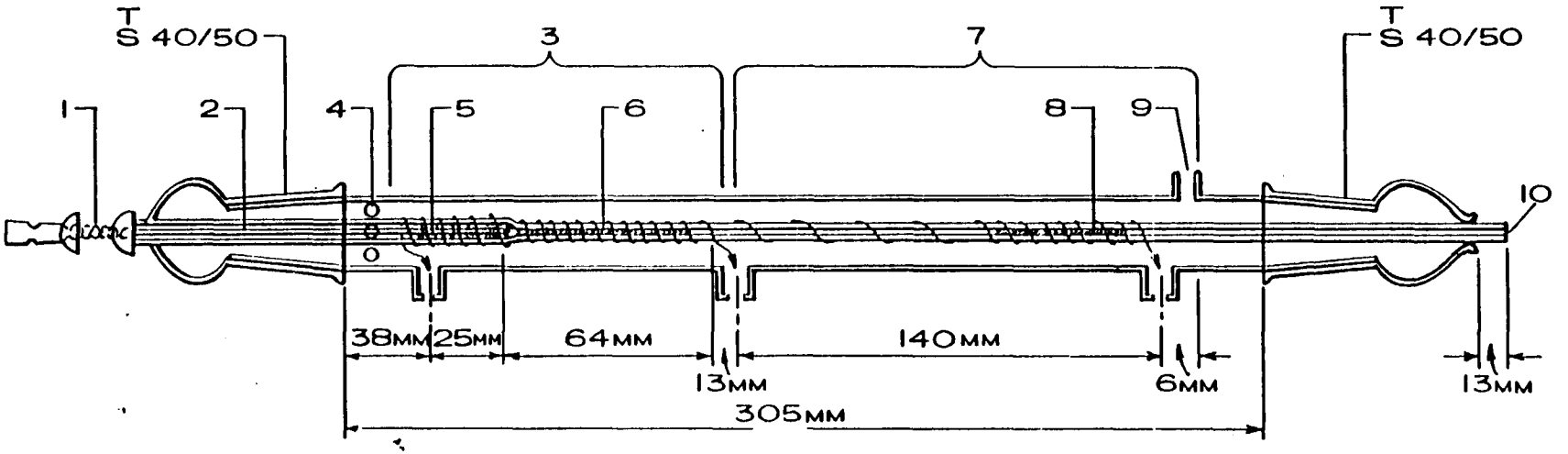


FIGURE 2. Two Stage Mercury Sampling Tube



1. LOADING MECHANISM
2. QUARTZ GLASS PLUNGER TUBE
3. SAMPLE DESORPTION SECTION
4. COOLING AIR VENT HOLES
5. SAMPLING TUBE
6. CUPRIC OXIDE (CuO)
7. SECOND DESORPTION SECTION
8. GOLD SECTION
9. COOLING AIR INTAKE
10. OUTLET TO SPECTROPHOTOMETER

FIGURE 3. Thermal Desorption Unit

7.2 **Preparation of Air Sampling Tubes**

7.2.1 Carbosieve B.: 45/60 mesh (Supelco, Inc.).

7.2.2 Glass tubes: Pyrex, 4 mm I.D., 4 cm length.

7.2.3 Glass separators: 1 cm glass tubes, approximately 3 mm O.D.

7.2.4 Quartz glass wool.

7.2.5 Silvered Chromosorb P (AgCP): see Section 8.2.

7.3 **Preparation of Mercury Standards: Direct Addition Method**

7.3.1 Silver Chromosorb P (AgCP) tubes: prepared as described in 8.3.1 and 8.3.2 but containing a 10-mm section of AgCP.

7.3.2 Stock solution: 100 μg Hg/ml prepared by dissolving 100 mg of metallic mercury in 50 ml concentrated nitric acid in a 1000 ml Pyrex volumetric flask and diluting to volume with double distilled H_2O. The solution may be stored in the flask for as long as 6 months. Conventional atomic absorption standards or a stable mercury salt may be used.

7.3.3 Standard working solution A: 1 μg/ml prepared by diluting 1.00 ml of the standard stock solution to 100 ml with 1 ml concentrated HNO_3 and double distilled water. Prepare fresh dilutions every 5 days.

7.3.4 Standard working solution B: 10 μg/ml, prepared as above except with 10 ml of stock solution. Prepare fresh dilutions every 5 days.

7.4 **Analysis of Air Samples**

7.4.1 Double distilled water.

7.4.2 Nitric acid, HNO_3: concentrated.

7.4.3 Standard working solutions A and B: previously described.

7.4.4 Stannous chloride solution: 20% w/v in 6 N HCl — freshly prepared.

7.4.5 Pure nitrogen gas: filtered through AgCP.

7.5 **Thermal Analysis System**

7.5.1 Calcium sulfate ($CaSO_4$) anhydrous.

7.5.2 Mersorb® charcoal.

7.5.3 Cupric oxide (CuO) rods.

7.5.4 Gold, granular (35/50 mesh).

7.5.5 Sea sand (20/40 mesh).

8. **Procedure**

8.1 **Cleaning of Equipment.** Acid clean all glassware and Teflon before use.

8.1.1 Wash in detergent tap water solution and follow with tap water rinse.

8.1.2 Soak in concentrated HNO_3 for 30 minutes, and follow with tap, distilled, and double distilled water rinses.

8.2 **Preparation of Silvered Substrate**

CAUTION: Use face shield and rubber gloves.

8.2.1 Dissolve 20 g $AgNO_3$ in 300 ml of distilled water (Solution A) in a 1000 ml beaker.

8.2.2 Place a magnetic stirring bar in the solution and stir on a magnetic stirrer.

8.2.3 Slowly add concentrated NH_4OH until a dark brown precipitate of Ag_2O forms.

8.2.4 While stirring, add concentrated NH_4OH drop by drop until the solution just clears. Disregard any small specks which may form.

8.2.5 Take another 2 g of $AgNO_3$ and dissolve in 30 ml distilled water in a separatory funnel (Solution B). Then add this solution drop by drop to the mixture in the 1000 ml beaker (Section 8.2.4) until the solution turns straw yellow.

8.2.6 Slowly add 100 ml of KOH solution (14 g KOH in 100 ml of H_2O) while constantly stirring.

8.2.7 Add concentrated NH_4OH drop by drop until the solution in 1000 ml beaker just clears.

8.2.8 Add more Solution B until a thin straw yellow or light brown precipitate forms. Again disregard any small specks.

8.2.9 Remove the 1000 ml beaker from the stirrer and filter the solution (Solution C) through glass wool.

8.2.10 Dissolve 7.8 g dextrose in 120 ml of distilled H_2O in a 1000 ml Erlenmeyer flask and add 40 grams of Chromosorb P to the dextrose solution.

8.2.11 Swirl the Chromosorb P in the dextrose solution until the surfaces are wet and then carefully decant any excess dextrose solution.

8.2.12 Slowly add the silver solution (Solution C — Section 8.2.9) to the Chromosorb P, stopper flask and shake vigorously until all the surfaces come in contact with the silver solution.

8.2.13 Decant and discard the excess silver solution.

8.2.14 Rinse and decant the silvered Chromosorb P (AgCP) three times with distilled water.

8.2.15 Dry the AgCP in a drying oven at 60°C.

Note: Dry in an atmosphere free of sulfides.

8.3 **Preparation of Air Sampling Tubes**

8.3.1 Insert a glass wool plug into the end of a glass tube and lightly pack with the glass rod.

8.3.2 Add enough AgCP to the tube to make a 5 mm section of AgCP and pack lightly with a second glass wool plug (Figure 2).

8.3.3 Insert a glass separator on top of the second wool plug and lightly pack a third quartz glass wool plug over the separator.

8.3.4 Add enough Carbosieve B to make a 2 mm section and pack the Carbosieve with a fourth glass wool plug (Figure 2).

8.3.5 Place the tube inside the thermal desorption unit.

8.3.6 Purge the tube in the desorption unit with pure air at a flow rate of approximately 1 liter/minute and heat the tube for 45 seconds (~500°C).

8.3.7 Remove the tube from the thermal desorption unit and allow to cool.

CAUTION: The tubes are hot on removal from the desorption unit and should be dropped directly into a small flask or vial for cooling.

8.3.8 Seal and store the sampling tube in the bottle or vial until used.

8.4 **Description and Installation of the Two-Stage Desorption Unit**

8.4.1 Description. With the exception of the electrical components and the loading spring, the entire thermal desorption unit is made of either quartz or Pyrex glass. A diagram of the unit giving the critical dimensions is shown in Figure 3. Each important part of the thermal desorption unit is numbered in the diagram and the numbers represent the following:

1-2. Loading mechanism (Figure 4) — Made from an 18/7 female glass joint with a steel spring (#1), and a plunger cut to reach the sample desorption section.

3. Sample desorption section — Made from 8-mm I.D. and 5-mm I.D. quartz tubing. The junction between the two sizes of tubing is tapered on the inside so that sampling tube tips fit snugly against the junction. Twenty-eight coils of 18 gauge Nichrome wire are wrapped around the first desorption section to heat the sampling tubes during the thermal desorption step.

4. Cooling vent holes — About seven 4-mm holes are placed around the outer jacket of the unit to allow cooling air to flow from the second heating coil to the first coil.

5. Sampling tube — Sampling tubes are positioned inside the first desorption section during mercury desorption. The spring on the loading mechanism is adjusted to press the sampling tubes lightly in place.

6. Cupric oxide (CuO) — A 40-mm section of rod-shaped CuO is placed just downstream from the sampling tubes. When heated, the CuO oxidizes organic vapors desorbed from the sampling tube. The CuO is held by a quartz glass wool plug which fits against a crimp in the glass tubing. Although the plugs should be large enough to hold the materials, they should not be packed too tightly.

7. Second desorption section — This section is made from the 5-mm I.D. quartz glass tubing extending from the first desorption section. The contents are held in place by a crimp in the glass tube, and quartz glass wool plugs.
Nichrome wire coils — Thirty coils of size 20 Nichrome wire are wrapped around the second desorption section. The coils are wrapped to allow most of the generated heat to concentrate over the gold granules of the second desorption section.

8. Gold section — This section consists of a 25-mm length of 35/50 mesh granular gold mixed one-to-one with 20/40 mesh sea sand. The sand is added to the gold to prevent fusing of the gold granules and to allow better air flow through the section. This section must be at least 15 mm below the entrance of the cooling air.

9. Cooling air intake — A 6-mm I.D. piece of glass tubing is used for connecting plastic tubing from the cooling air supply to the cooling jacket. The Pyrex glass cooling jacket not only directs the flow of cooling air, but also acts as electrical insulation for the heating coils. Wires to the heating coils enter the cooling jacket through 5-mm holes at the ends of glass nipples on the side of the jacket. The solderless connectors between the wires and the coils are placed inside the jacket to prevent exposing uninsulated wire outside the cooling jacket. The insulated wires to the connectors are sealed in place with a heat resistant sealer.

10. Outlet — The outlet from the desorption unit is butt connected with a Tygon overseal to a glass tube which leads to a flameless atomic absorption spectrophotometer (MAS-50). The end of the quartz tube slides through an opening at the end of the cooling jacket. The opening is kept to a minimum to limit the escape of cooling air.

8.4.2 Installation. A diagram of the installed thermal desorption unit system is shown in Figure 1.

1. Power connections — The wires to the heating coils should be heavy enough to carry the current necessary to heat the coils (about #12). Power to the heating coils is controlled by a three position switch with an off position, an on position for coil #1 and an on position for coil #2. Power to the switch comes from a 120 VAC input, 22 A variable transformer set at 28 VAC.
2. Air supply — The air which passes through the heated sections of the thermal desorption unit is drawn from the room by a vacuum pump. The pump is attached to a ballast tank to prevent fluctuations in air flow.
   Before entering the desorption unit, room air passes through a drying tube containing anhydrous $CaSO_4$. The dimensions of the drying tube and all subsequent filters may vary but should not interfere with the air flow through the system. The $CaSO_4$ should be changed periodically depending on the humidity of the work area. The dried air passes through a filter containing activated charcoal and 30/60 mesh silvered Chromosorb P to remove organic vapors and metallic mercury. The dry, filtered air then passes through the thermal desorption unit, through the flameless atomic absorption optical cells and into a filter containing Mersorb charcoal which collects any mercury desorbed by the desorption unit. Air from

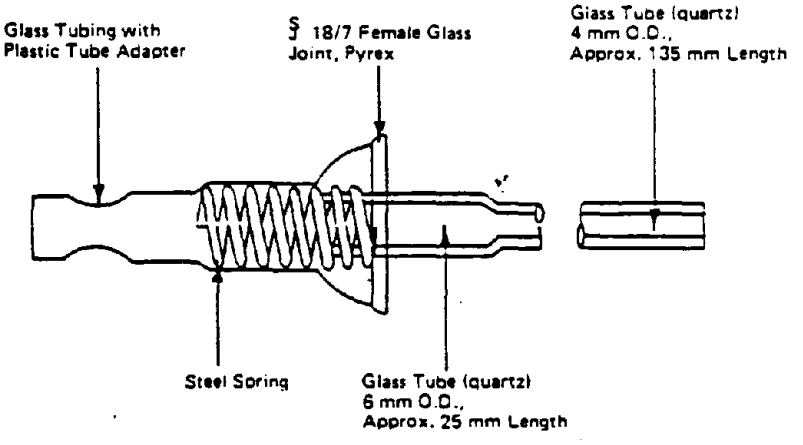


FIGURE 4. Loading Mechanism

this charcoal filter then passes through a flow valve and a flowmeter and into a 1.0 lpm critical orifice and the ballast tank. A "T" with a flow valve is located between the ballast tank and the vacuum pump. The valve on the "T" is closed during normal operation. When shutting down the system, the valve prior to the flowmeter is closed and the valve on the "T" is opened before the vacuum pump is turned off.

3. Cooling air — Cooling air for the heating coils is supplied by an air compressor. A flowmeter controls the flow rate at 15 lpm. The air enters the desorption unit from a plastic tube into the air intake near the second desorption section.
4. Detection System — Mercury desorbed from the gold section of the thermal desorption system enters the optical cell of a flameless atomic absorption spectrophotometer through a glass tube. The glass tube connecting the desorption unit with the optical cell is maintained at 70°C using a heating tape. Power for the heating tape is supplied by a 120 VAC, 3 A variable transformer.

   Any reliable flameless atomic absorption spectrophotometer analysis system set to absorb the 2537 Å wavelength of mercury may be used to detect the mercury vapor released from the desorption unit. A dual optical cell system made from two modified Coleman Mercury Analyzer Systems (MAS-50) is shown. The first MAS-50 contains a 15.5-cm optical cell made of Pyrex glass with quartz glass windows. The output signal from the first instrument is displayed on a 0-10 mV recorder. After the mercury vapor is detected by the first MAS-50, the vapor passes directly into the optical cell of a second modified MAS-50. This MAS-50 contains a 2.5 cm glass optical cell with quartz glass windows. Signals from the second MAS-50 are displayed by a second recorder. (A dual pen recorder may be used, if available.) The tubing in both the MAS-50's was changed to glass and was rerouted to bypass the air pumps which came with the MAS-50's. Both air pumps were turned off and not used. If a conventional atomic absorption spectrophotometer is used in place of the MAS-50, the optical cell must be wrapped with heating tape maintained at 70°C.

8.5 Collection and Shipping of Air Samples

8.5.1 After the sampling tubes have been prepared and stored, they should be stable indefinitely. White gloves or laboratory wipers should be used when handling the tubes.

8.5.2 The sampler should be equipped with a holder for a 37-mm diameter, 0.8-µm membrane filter preceding the sampling tube to collect particulate mercury.

8.5.3 Draw air through the filter and sampling tube at a flow rate of 1 lpm. For ceiling measurements, sample for 10 minutes. Sample for one hour for TWA measurements.

8.5.4 After sampling, disconnect the filter cassette from the sampling tube. Cap the openings of the cassette and submit the entire holder with the filter to the laboratory for analysis.

8.5.5 Immediately connect a second sampling tube to the original tube with a short piece of clean, uncontaminated tubing. Turn on the pump and using the second tube as a filter, purge the sample tube for 10.0 minutes at the maximum flow rate of the pump (up to 5 lpm). Turn off the pump and discard the tube that was used as a filter for the original sampling tube.

8.5.6 Remove the sampling tube from the sampler, and replace inside the glass vial. Insert cotton into the vial to prevent sampling tube breakage. Gloves or wipers should be used when handling the sampling tube.

8.5.7 Appropriate "blank" samples should be handled and submitted together with the samples.

8.6 **Analysis of Filters for Particulate Mercury**

8.6.1 Remove the filter from the holder or glass vial and place inside the decomposition vessel.

8.6.2 Add 5 ml of concentrated HNO_3 and cap the vessel securely.

8.6.3 Place the vessel in an oven at 150°C for 1.5 hours.

8.6.4 Remove the vessels from the oven and cool to room temperature in a water bath.

8.6.5 Open the vessel and quantitatively transfer the contents into a bubbler flask with double distilled water.

8.6.6 Bring the total volume of liquid in the flask to 75 ml with double distilled water.

8.6.7 Connect a blank sampling tube containing a single section of AgCP to the outlet end of the bubbler system.

8.6.8 Add one ml of stannous chloride solution to the contents of the bubbler flask and immediately connect the flask to the bubbler system.

8.6.9 Turn on the pump and bubble the contents of the flask for 4 min at 1 to 2 liters/min.

8.6.10 Turn off the pump and remove the AgCP tube from the bubbler system.

8.6.11 Purge the AgCP tube for 2 min at 5 liters/min with pure, dry nitrogen gas and analyze the contents of the tube using the procedure for metallic mercury. (Section 8.7).

8.6.12 The signals obtained from this AgCP tube represent total particulate mercury.

8.7 **Analysis of Air Sampling Tubes for Organic and Metallic Mercury**

8.7.1 All operations requiring the handling of the sample tubes should be done using clean white cotton gloves or laboratory wipers.

8.7.2 Turn on both MAS-50 analyzers and allow to stabilize.

Note: The time required for stabilization varies with individual instruments.

8.7.3 Turn on the heating tape.

8.7.4 After the MAS-50's have stabilized, turn on the vacuum pump and open the shutoff valve.

8.7.5 Observe the rotometer to insure flow through the critical orifice has not been interrupted.

8.7.6 Turn on the cooling air and adjust cooling air flowmeter to 15 l/min.

8.7.7 Turn on the recorders and allow to stabilize.

8.7.8 Adjust the MAS-50's and recorders to the desired 0 and 100% T settings.

8.7.9 Purge the system before analyzing samples by heating the first desorption section of the desorption unit for 45 seconds and then heating the second section for 20 seconds.

8.7.10 Allow the desorption unit to cool for 7 minutes before analyzing samples.

8.7.11 Cut the sampling tube between the second and third glass wool plugs and separate the two tube sections.

8.7.12 Remove the clamp between the loading mechanism and desorption unit and insert the Carbosieve B section of the sampling-tube.

8.7.13 Push the sampling tube into the first desorption section of the desorption unit using the plunger of the loading mechanism and replace the clamp.

8.7.14 Turn on the first section of the thermal desorption unit and heat the sampling tube for 45 seconds.

8.7.15 After the first section is heated, wait 1.0 minute before switching the 3-way power switch to heat the second desorption section of the thermal desorption unit. This section is usually heated for about 25 seconds.

Note: At this time both recorders (or the dual recorder) will record the absorption signal of any mercury desorbed from the sampling tube.

8.7.16 Turn the 3-way switch to the off position.

8.7.17 Allow the thermal desorption unit to cool for about 30 seconds and open the clamp on the loading mechanism to unload the used sampling tube. Replace the loading mechanism and clamp.

Caution: The sampling tube is *very hot* when it comes out of the desorption unit. Do not touch the tube with bare hands. Drop the hot tube into a dry beaker to cool.

Note: If the used sampling tube does not drop when the plunger is removed, a narrow spatula may be used to loosen the tube.

8.7.18 Reload the desorption unit with the AgCP section of the sampling tube after allowing a minimal 7-minute cooling period.

8.7.19 Repeat steps 8.7.14 through 8.7.18 for the analysis of additional tubes.

9. Calibration and Standards

9.1 Organic and Metallic Mercury

9.1.1 Remove the glass wool plug from the open end of a blank AgCP tube using gloved hands and tweezers.

9.1.2 Inject the required amount (10-100 µl) of mercury solution (A or B) directly on top of the exposed AgCP using an Eppendorf pipette while holding the tube in the upright position.

9.1.3 Replace the glass wool plug and repack the tube using a glass rod.

9.1.4 Place the wet tubes upright in a small beaker and dry the tubes for at least 6 hours at 50°C in a drying oven.

9.1.5 The absorbances determined using the standard tubes are used to prepare a calibration curve. A minimum of 5 concentrations replicated 3 times at each concentration should be used in preparing the calibration curve. Analyze the standards at the same time as samples.

9.2 Particulate Mercury

9.2.1 Calibration curves for particulate mercury must be prepared from bubbled standards.

9.2.2 Prepare these standards by adding the appropriate amount (10-100 µl) of Solution A or B to 5 ml of the concentrated HNO_3 in the bubbler flask (8.6.5) and proceeding with steps 8.6.6 through 8.6.11.

9.2.3 The calibration curve should be prepared from a minimum of 5 concentrations (ng Hg/tube) with a minimum of 3 replicates for each concentration.

10. Calculations

10.1 To determine total micrograms of mercury as particulate, organic and metallic mercury, the absorption signals for the filters and sampling tubes are compared with the appropriate calibration curve.

10.2 Calculate the mercury concentration in micrograms per cubic meter (µg Hg/m^3) for each type of mercury,

$$\mu g\ Hg/m^3 = \frac{\mu g\ Hg}{V_s} \text{ (from Section 10.1)}$$

where:

V_s = volume of air sampled in cubic meter at standard conditions of 25°C and 760 mm Hg. (1000 liter = 1 m^3; volume = flow rate × time).

10.3 Since part of the elemental mercury is retained by Carbosieve B, a correction must be made. This is done for the metallic mercury by multiplying the quantity of mercury found on the AgCP section by 1.06 to obtain the total metallic mercury. The difference between the total metallic mercury and the quantity of mercury found on the AgCP section must be subtracted from the amount found on the Carbosieve B section to obtain the total organic mercury.

11. References

11.1 Campbell, E. E., P. E. Trujillo, and G. O. Wood, "Development of a Multistage Tandem Air Sampler for Mercury", Quarterly Report No. LA-5340-PR, LA-5467-PR, LA-5188-PR, LA-5105-PR, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, March 31, 1973, September 30, 1973, December 31, 1973, and September 30, 1972.

11.2 Trujillo, P. E. and E. E. Campbell, "Development of a Multistage Air Sampler for Mercury", Anal Chem 47:1629, 1975.

11.3 Haartz, J. C., R. D. Hull, and M. Bolyard, "Analytical Evaluation of a Multistage Sampling Device for Mercury", to be published.

TIN IN ATMOSPHERIC PARTICULATE MATTER AND BIOLOGICAL MATERIALS

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Tin	Method No.: P&CAM 176
Matrix: Air and biological materials	Range: 0.5 to 10 μg (0.1 to 2 mg/m³ tin in a 5-liter air sample)
Procedure: Colorimetry	
Date Issued: 1/29/76	Precision (CV, analytical) 0.087 at a level of 0.50 μg and 0.098 at a level of 1.00 μg
Date Revised: 4/4/77	
	Classification: D (Operational)

1. Principle of the Method

Tin compounds, collected on a filter from the air or in biological materials, are oxidized and solubilized by digestion with a mixture of nitric and sulfuric acids to yield a solution of Sn (IV). Difficulty in the dissolution with acids of tin dioxide, especially calcined tin dioxide, necessitates a more vigorous treatment such as peroxide or bisulfate fusion. (See Reference 11.1.) Therefore, the procedure described here is not appropriate for the measurement of tin occurring as tin dioxide.

After digestion, the tin is converted to its tetraiodide by addition of an acid solution of potassium iodide. The tin tetraiodide is extracted into hexane; Sn (IV) is then extracted back into an aqueous solution with citric and sulfuric acids. Finally, pyrocatechol violet is added to the aqueous solution. Its chelation with tin is sensitized with cetyltrimethylammonium bromide, and the absorbance maximum at 660 nm is measured with a spectrophotometer.

2. Range and Sensitivity

2.1 The limit of detection of the analytical method including the digestion, the extractions, the chelation, and the absorbance measurement is 0.5 μg of tin. In a 5-liter air sample, this would correspond to a tin concentration of 0.1 mg/m³.

2.2 The calibration curve is linear at tin concentrations as high as 10 μg in the final 50-mℓ solution described in the procedure. In a 5-liter air sample, this would correspond to a tin concentration of 2 mg/m³

3. Interferences (See Reference 11.1.)

3.1 If present in the color-producing step, many metals will cause positive interferences. However, it has been shown that the iodide extraction procedure limits the effect of these interferences. The error is not greater than 0.01 μg of tin when the level of the interfering metal is less than the amount shown below:

(1) 100 mg: Ca

(2) 10 mg: Au, Cd, Cr (III), Cr (VI), Cu, Fe (III), Fe (II), Hg, Ni, Pb, Ti, Zn

(3) 2 mg: Ba, Be, Bi, Sr, Te, W

(4) 1 mg: Mo, Sb

(5) 0.1 mg: Ge

3.2 Several anions will produce negative interferences by the complexation of tin. The indicated amounts of the following anions can be tolerated after the sulfuric acid-nitric acid digestion with a resulting error that is not greater than 0.01 μg of tin:

(1) 100 mg: PO_4^{3-}

(2) 50 mg: Cl^-

(3) 1 mg: BF_4^-

(4) 0.2 mg: F^-

3.3 No interference in the analytical method was found for 100 mg of silicon dioxide.

4. Precision and Accuracy

4.1 The determination by this procedure of known amounts of tin in aqueous solution in the range 0.5 to 10 μg yielded coefficients of variation ranging from 0.030 to 0.057.

4.2 The coefficients of variation for the determination of 0.50-μg and 1.00-μg amounts of tin added to NBS standard bovine liver samples were 0.087 and 0.098, respectively.

4.3 The average recoveries of 0.50-μg to 10.00-μg amounts of tin added to NBS standard bovine liver samples ranged from 98 to 106%.

4.4 A ruggedness test during the analyses of 1-μg and 9-μg amounts of tin has indicated that 10% variations in reagent concentrations do not seriously affect results. The most significant effect was the variation in concentration of pyrocatechol violet, which can easily be controlled. (See Reference 11.1.)

5. Advantages and Disadvantages of the Method

5.1 The double extraction procedure renders the method selective for tin.

5.2 The method can be applied to organic as well as inorganic compounds with the exception of tin dioxide, which requires a more vigorous treatment for dissolution.

5.3 The accuracy and precision of the method are not significantly affected by 10% variations in reagent concentrations.

5.4 One disadvantage is the large amount of time required by the digestion and separation procedures.

6. Apparatus

6.1 Air Sampling Equipment. The sampling unit for the filter collection of particulate samples has the following components:

6.1.1 A Filter Unit. The unit consists of the filter medium and an appropriate filter holder.

6.1.2 A Millipore 37-mm AA Type Filter. This filter has a pore size of 0.8 μm. It is easily digested in the oxidizing acids.

6.1.3 A Properly Calibrated Personal Sampling Pump. The pump should be calibrated with a representative filter unit in the sampling line. A wet or dry test meter or a glass rotameter capable of measuring the appropriate flow rate (1.7ℓ/min) to within ±5% may be used in the calibration.

6.2 Polyethylene containers for collection and storage of biological materials.

6.3 Erlenmeyer flasks (250 mℓ).

6.4 Separatory funnels (250 mℓ) with a support rack.

6.5 Volumetric flasks (50 ,100 ,250 , and 500 mℓ).

6.6 Spectrophotometer and 10-cm cells.

7. Reagents

All reagents must be analytical reagent grade. Doubly distilled or distilled deionized water should be used in the preparation of reagent solutions and in the analytical procedure.

7.1 Sulfuric acid, concentrated.

7.2 Nitric acid, concentrated.

7.3 Potassium iodide solution (20% w/v in water).

7.4 **Acid-Iodide Wash Solution.** Mix one volume of sulfuric acid with two volumes of water and cool. Add one volume of potassium iodide solution (20% w/v). Prepare fresh solutions as needed.

7.5 *n*-Hexane.

7.6 Sulfuric acid (5.0% w/v) -citric acid (2.5% w/v) solution (in water).

7.7 **Ascorbic Acid** (5% w/v). Dissolve 2.5 g of ascorbic acid in 50 mℓ of water. Prepare fresh daily.

7.8 **Cetyltrimethylammonium Bromide (CTAB) Solution** (11 mg/mℓ). Dissolve 1.1 g of CTAB in 100 mℓ of water.

7.9 **Sensitized Pyrocatechol Violet Solution** (0.18 mg/mℓ). Dissolve 45 mg of pyrocatechol violet in a small amount of water in a 250-mℓ volumetric flask, Add 5 mℓ of CTAB solution and dilute to 250-mℓ with water. Prepare fresh daily.

7.10 Tin, granular.

7.11 Hydrochloric acid, concentrated.

7.12 **Tin, Standard Solution "A" (1 mg/mℓ).** Dissolve 0.5000 g of pure tin in 150 mℓ of concentrated hydrochloric acid in a 500-mℓ volumetric flask. Dilute to 500 mℓ with water.

7.13 **Tin, Standard Solution "B" (100 μg/mℓ in Sulfuric Acid** (20% w/v) and Citric Acid (10% w/v)). Transfer 50 mℓ of standard tin solution "A" with a volumetric pipette to a 250-mℓ graduated beaker. Add 50 mℓ of concentrated sulfuric acid and 5 mℓ of concentrated nitric acid. Heat until all the nitric acid is eliminated and white fumes of sulfur trioxide appear. Add concentrated sulfuric acid until the total volume of the solution is 55 mℓ. Transfer the solution quantitatively to a 500-mℓ volumetric flask. Rinse the beaker with several portions of water, and add each rinse slowly and carefully to the volumetric flask until the total volume is 200 to 250 mℓ. Cool the solution to room temperature and add to it all of a solution of 50 g of citric acid in 100 mℓ of water. Dilute with water to 500 mℓ. Prepare fresh daily.

7.14 **Tin, Standard Solution "C" (1 μg/mℓ in Sulfuric Acid** (5% w/v) and Citric Acid (2.5% w/v)). Transfer 1 mℓ of standard tin solution "B" with a volumetric pipette to a 100-mℓ volumetric flask. Dilute to 100 mℓ with the sulfuric acid (5.0% w/v)- citric acid (2.5% w/v) solution. Prepare fresh daily.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All glassware may be sufficiently cleaned with a chromic acid – sulfuric acid solution and concentrated nitric acid. It should then be rinsed thoroughly with doubly distilled water.

8.1.2 Polyethylene equipment should be cleaned with concentrated nitric acid and then rinsed with doubly distilled water.

8.2 Collection and Shipping of Samples

8.2.1 **Air Samples**

1. Weigh the filter disc. Assemble the filter unit by mounting the filter disc in the holder.

2. Connect the exit end of the filter unit to the pump with a short piece of flexible tubing.

3. Sample the air at a flow rate of 1.7 lpm. Measure the flow rate and time or volume as accurately as possible. A minimum sample of 5 liters should be collected. Larger volumes are desirable, provided the filter does not become loaded with dust to the point that loose material could fall off or the filter become plugged.

4. The sample filter holders should be shipped in a suitable container designed by NIOSH to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample is dislodged from the filter nor that the sample surface be disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the holder on top of the sample filter.

5. With each batch of samples, one filter, labeled as a blank, must be submitted. No air should be drawn through this filter.

8.2.2 **Biological Materials.** Polyethylene containers may be used for sampling transfer, and storage of most biological materials. Cooling the samples to 0°C or freezing them will prevent deterioration.

8.3 Analysis of Samples

8.3.1 Transfer a weighed sample, either a filter or a specimen of biological material, to a 250-mℓ Erlenmeyer flask. If preliminary estimates can be made, the sample should contain at least 0.5 μg but not more than 10 μg of tin.

8.3.2 Add 10 mℓ of concentrated sulfuric acid, 15 mℓ of nitric acid, and a few glass beads to the flask.

8.3.3 Heat gently to prevent excess frothing. Occasionally, add small amounts of distilled water to the flask to release trapped gas.

8.3.4 Add additional aliquots (5-mℓ) of concentrated nitric acid until the organic matter is destroyed.

8.3.5 After the sample has been solubilized, heat the solution until white fumes of sulfur trioxide appear.

8.3.6 Cool the solution to room temperature and add 20 mℓ of water.

8.3.7 Add 10 mℓ of the acid-iodide wash solution to the sample solution.

8.3.8 Transfer the resulting solution to a 250-mℓ separatory funnel. Rinse the Erlenmeyer flask three times with 10-mℓ aliquots of *n*-hexane and add the washings to the separatory funnel.

8.3.9 Shake the funnel for 1 min, allow the phases to separate for 5 to 10 min, and discard the aqueous layer.

8.3.10 Transfer the *n*-hexane layer to a 125-mℓ separatory funnel by pouring from the neck of the 250-mℓ separatory funnel. Rinse the first funnel with 10 mℓ of *n*-hexane, and transfer the washings to the second funnel.

8.3.11 To the 125-mℓ separatory funnel add 15 mℓ of the sulfuric acid (5.0% w/v)-citric acid (2.5% w/v) solution and 5 mℓ of the standard tin solution "C" (1 μg/mℓ in 5.0% w/v sulfuric acid and 2.5% citric acid).

8.3.12 Shake the funnel for 1 min, allow the phases to separate for 5 to 10 min, and transfer the aqueous layer to a 50-mℓ volumetric flask.

8.3.13 Wash the *n*-hexane layer twice with 10-mℓ portions of water. Allow the phases to separate for 5 min. Add the washings to the 50-mℓ volumetric flask.

8.3.14 Allow the solution to stand in the volumetric flask for 2 to 3 hr or overnight before developing the color.

8.3.15 After the indicated amount of time, add 2 mℓ of freshly prepared ascorbic acid solution to the sample solution in the volumetric flask.

8.3.16 Then add 5 mℓ of the sensitized pyrocatechol violet solution, dilute to 50 mℓ and mix.

8.3.17 After 30 min, measure the absorbance of the sample solution at 660 nm in a 10-cm cell. All absorbance measurements should be taken against a reference solution containing 5 μg of tin. Prepare the reference solution by adding 5 μg of tin (5 mℓ of standard tin solution "C"), 15 mℓ of the sulfuric acid (5.0% w/v)-citric acid (2.5% w/v) solution, 20 mℓ of water, 2 mℓ of the ascorbic acid solution, and 5 mℓ of the sensitized pyrocatechol violet solution to a 50-mℓ volumetric flask in that order. Dilute to 50 mℓ with water, mix the solution, and let it stand for 30 min prior to its use.

8.3.18 If air particulate samples are being analyzed, digest and analyze the blank filter according to the described procedure.

8.3.19 If biological samples are being analyzed, a reagent blank should be carried through the entire analytical procedure. The absorbance of this blank should be subtracted from the absorbances of the sample solutions.

9. Calibration and Standards

9.1 Preparation of Standards. Five or six standards are necessary to establish the calibration curve. The preparation of each standard is similar to the preparation of the reference standard described in step 8.3.17 of the analytical procedure. However, in addition to the 5 μg of tin transferred to the 50-mℓ volumetric flask, 0.5 to 10 μg of tin (0.5 to 10 mℓ of tin standard solution "C") should also be added to the flask. Consequently, the volume of the sulfuric acid (5.0% w/v)-citric acid (2.5% w/v) solution added should be reduced from 15 mℓ by the increased volume of tin standard solution added. Make the other additions as specified. Again, let the final solution stand for 30 min prior to the absorbance measurement, which is taken against the reference standard solution described above.

9.2 Construction of the Calibration Curve. Plot the absorbance of each standard solution, measured against the reference standard solution, as a function of the amount (in μg) of tin in the standard solution excluding the 5 μg present in all standard solutions. The amount of tin (in μg) in a sample can be read directly from the curve.

10. Calculations

10.1 Air Samples

10.1.1 Read from the calibration curve the weight (in μg) of tin in a sample.

10.1.2 Any amount of tin found in the unexposed filter (blank filter) should be subtracted from sample values.

10.1.3 The concentration of the tin in the air sampled may be expressed in mg/m^3, which is numerically equal to $\mu g/\ell$ in air:

$$mg/m^3 = \frac{\text{weight (in } \mu g\text{) of tin in sample}}{V_S\ (\ell)}$$

where: V_S = volume of air sampled.

10.2 Biological Materials

10.2.1 The concentration of the tin in the material sampled may be expressed as follows:

$$\mu g/g = \frac{\text{weight (in } \mu g\text{) of tin in sample}}{\text{weight (in g) of sample}}$$

10.2.2 If the material is a fluid, such as blood or urine, the concentration of tin in the fluid may be expressed as follows:

$$mg/\ell = \frac{\text{weight (in } \mu g\text{) of tin in sample}}{\text{volume (in m}\ell\text{) of sample}}$$

11. Reference

Corbin, H.B., "Rapid and Selective Pyrocatechol Violet Method for Tin."Anal. Chem.,45. 534 (1973).

GALLIUM IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Gallium	Method No.: P&CAM 177
Matrix: Air	Range: 0.0002 to 0.01 mg/m^3 in 100 liters of air
Procedure: Collection on a membrane filter; anodic stripping voltammetry	Precision (CV): 0.15 (analytical) at 0.002 mg/m^3
Date Issued: 4/15/73	Classification: E (Proposed)
Date Revised: 1/29/76	

1. Principle of the Method

Particulate matter containing gallium is collected from air on a membrane filter. The filter and trapped particulate material are wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The residue is dissolved in an acetate buffer solution and the buffered sample solution is analyzed by anodic stripping voltammetry (ASV) with a composite-mercury-graphite electrode (CMGE). In principle, a hanging mercury drop electrode (HMDE) can also be used. However, optimum analysis parameters may vary significantly and must be established for each electrode system. The method as presented is designed for use with the CMGE.

2. Range and Sensitivity

2.1 The potential required to plate gallium on the CMGE from samples containing less than 20 ng is sufficiently cathodic to cause the evolution of hydrogen. Consequently, the ASV response for gallium is not a linear function of the weight of gallium present below the operational detection limit of 20 ng.

2.2 The linear range of the electrode response extends from the detection limit to 1 μg of gallium. However, at amounts near 1 μg, the percent error in estimating values from the calibration curve will be about 10%. The range 20 ng to 1 μg corresponds to 0.0002 to 0.01 mg/m^3 in a 100-liter sample of air. The range may be extended by diluting the sample digestate with acetate buffer and taking an aliquot of the diluted solution for the ASV determination.

3. Interferences

3.1 Even small amounts of copper pose a severe and variable interference; copper forms an interamalgam complex with gallium that hinders the stripping of the gallium from the electrode in the ASV procedure. The interference can be diminished by changing the plating potential from -1300 mV to -1400 mV, which yields a higher gallium response in the presence of copper, and by concomitantly using the method of standard additions for the analysis of each sample. However, this approach may lead to interference from iron (see Section 3.2). Another approach is to remove the copper by electrolytically plating it at a potential of -600 mV on one electrode prior to the plating of gallium on a second electrode. This procedure has been shown to markedly improve the ASV response to gallium in the presence of copper.

3.2 At a potential of -1400 mV or more, iron, if present at levels higher than 10 μg in the sample digestate, will be plated on the electrode and will mask the electrode processes for all metals reduced at more anodic potentials.

3.3 If present at levels higher than 10 μg in the sample aliquot, zinc will cause a serious interference in the determination of gallium. However, zinc may be removed from the sample digestate by solvent extraction prior to the ASV analysis. (See Reference 11.2.)

4. Precision and Accuracy

4.1 Several processes determine the reproducibility of the overall analytical method: (1) sampling, (2) shipping, (3) preparation of samples, and (4) analysis. Variations introduced by sampling from workroom atmospheres and by shipping the collected samples to a distant laboratory prior to analysis have not been evaluated. The coefficient of variation of the preparation and analysis steps has been empirically determined to be 0.15 over the range of 100 ng to 1 μg, which corresponds to 0.001 to 0.01 mg/m^3 of gallium in a 100-liter sample of air.

4.2 The estimated percent error in a measurement, neglecting any contribution from sampling and shipping, is less than 15% of the "true" value in the range of 100 ng to 1 μg.

5. Advantages and Disadvantages of the Method

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 One disadvantage is that copper, iron, and zinc may pose serious interferences in the ASV determination of gallium with a CMGE.

6. Apparatus

6.1 **Air Sampling Equipment.** The sampling unit for the collection of particulate samples has the following components:

6.1.1 **Filter Unit.** The unit consists of the filter and an appropriate filter holder such as the Millipore Aerosol Analysis Monitor Catalog No. MAWP 0.37 AO, or equivalent. This unit is a plastic disposable holder preassembled with a filter in place. The recommended filter is the Millipore Type AA, a mixed cellulose ester, with a pore size of 0.8 μm and a diameter of 37 mm.

6.1.2 **Adaptor.** An adaptor such as the Millipore Aerosol Adaptor, Catalog No. XX62 000 04, is inserted into the filter unit to allow a hose to be attached.

6.1.3 **Personal Sampling Pump.** The pump should be calibrated with a representative filter unit in the sampling line. A wet or dry test meter or glass rotameter capable of measuring the appropriate flow rate (1.5 to 2 ℓ/min) to within ± 5% may be used in the calibration.

6.2 Pyrex test tubes (20 x 100 mm) for digesting filter samples and subsequent use as analysis cells.

6.3 Digestion racks for analysis cells.

6.4 Micro pipettes, Eppendorf or equivalent (10, 100, 200, and 300 $\mu\ell$).

6.5 Model SA 2011 Single-Cell Anodic Stripping Voltammeter with composite mercury-graphite electrode, Environmental Sciences Associates, Inc., Burlington, Mass., or equivalent. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations. Instructions for the preparation and reconditioning of the CMGE are included in the instruction manual.

6.6 Potentiometric recorder (100-mV range).

6.7 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric pipettes, and volumetric flasks of appropriate sizes.

7. Reagents

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in gallium. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 **Acid Ashing Solution.** Prepare the acid ashing solution by adding 24 volumes of concentrated nitric acid to 24 volumes of perchloric acid (70%) and 1 volume of concentrated sulfuric acid.

7.5 **Acetate Buffer Solution (0.5 *M* NaAc, 4 *M* LiCl, pH 4 to 5).** To prepare 1 liter of this reagent, dissolve 41 g of sodium acetate, 170 g of lithium chloride, and 15 to 35 mℓ of 70% $HClO_4$ in water in a 1-liter volumetric flask and fill to the mark with water. Remove trace metals from the resulting solution electrolytically with a system such as the Model 2014PM Reagent Cleaning System, Environmental Sciences Associates, Inc., or equivalent. The acetate buffer maintains the pH of the resulting sample solution between 4 and 5, a pH range favorable for the determination of gallium.

7.6 **Aqua Regia.** Prepare aqua regia by mixing 1 volume of concentrated nitric acid (doubly distilled) with 4 volumes of concentrated hydrochloric acid.

7.7 **Gallium (Ga(III)), Standard Solution "A" (10 mg/mℓ).** Weigh accurately an amount of pure gallium metal near 1 g and transfer the weighed gallium to a 100-mℓ volumetric flask. Dissolve the metal in 10 mℓ of aqua regia and dilute to the mark with water.

7.8 **Gallium (Ga(III), Standard Solution "B" (100 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "A" to a 100-mℓ volumetric flask containing 10 mℓ of concentrated hydrochloric acid and dilute to the mark with water. Prepare fresh daily to prevent loss of gallium or contamination.

7.9 **Gallium (Ga(III)), Standard Solution "C" (1 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "B" to a 100-mℓ volumetric flask containing 10 mℓ of concentrated hydrochloric acid and dilute to the mark with water. Prepare fresh daily to prevent loss of gallium or contamination.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any gallium from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 220 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove and allow to cool. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom. Allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water.

8.2 Collection and Shipping of Samples

8.2.1 Connect the exit end of a filter unit to the adaptor. Connect the adaptor to the pump with a short piece of flexible tubing.

8.2.2 Sample the air at a flow rate of 1.5 to 2 ℓ/min. Measure the flow rate and time, or volume, as accurately as possible. A minimum sample of 100 liters should be collected. Larger volumes are desirable, provided the filter does not become loaded with dust to the point that loose material could fall off or the filter become plugged.

8.2.3 The filter holders should be shipped in a suitable container to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample is dislodged from the filter nor that the sample surface is disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the holder on top of the sample filter.

8.2.4 Unexposed filters in filter holders should be labeled as blanks and shipped with the samples.

8.3 Analysis of Samples

8.3.1 Carefully remove the filter from the filter unit by opening the unit, gently pushing up the filter and its backing, and grasping the unexposed edge of the filter with stainless steel forceps. Fold the filter in half, then into quarters, and subsequently into eighths without creasing or cracking the filter material. Insert the filter into an analysis cell and push it down into the cell with a glass rod.

8.3.2 Pipette 300 μℓ of the acid ashing solution into the analysis cell containing a filter. Wet the filter as thoroughly as possible by adding the acid slowly. Place the cell in a digestion rack on a hot plate with a surface temperature of 220 to 240°C. Carry out the digestion in a hood. The filter sample will clear in about 10 min, and a reflux line will be established about halfway up the cell. At the end of 20 min, there will be approximately 100 to 200 μℓ of acid left in the cell, with most of the nitric acid having evaporated. The digestion should be continued for an additional 20 to 30 min until the acid is almost completely evaporated.

8.3.3 Remove the sample from the hot plate, allow it to cool, and add 4.5 mℓ of the acetate buffer solution.

8.3.4 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the buffered solution under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -1300 mV
- Auto-hold cutoff potential, 1150 mV
- Plating time, 30 min
- Sweep rate, +60 mV/sec
- Current range, 0.1 to 2 mA (full scale)

The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of gallium plated on the electrode may occur. As the sample is stripped, a zinc peak (-1150 mV) will occur first, followed by the gallium peak (-1050 mV), then a cadmium peak (-720 mV). A baseline is drawn by connecting with a straight line the flat portion of the curve before and after the gallium peak. The peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outline in this section should be followed for the analyses of the following four kinds of solutions: (1) filter and reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solutions of gallium in acetate buffer; (4) filter standards prepared as described below in Section 9.1. The sequence in which these four kinds of solutions are analyzed is as follows; (1) a set of standard solutions of gallium in acetate buffer; (2) sample solutions, with filter and reagent blanks and filter standards interspersed at the rate of one blank and one filter standard for every ten samples; and (3) a second set of standard solutions of gallium in acetate buffer. The standard solutions of gallium in acetate buffer are used for calibration; the results for the samples are corrected for the amount of gallium found in the reagent blank; and the filter standards are used to determine the efficiency of recovery of known amounts of gallium from filters. The procedure described is for use with a four-cell ASV unit.

9.1 **Preparation of Gallium Filter Standards.** Prepackaged filters of the type used for sampling are removed from their plastic cases and separated from their support mats. The filters are placed on the center ring supports of the plastic cases so that only the edge of the filter is touching the rim of its holder. Gallium standard solution "B" is appropriately diluted to provide 50-, 100-, 200-, and 500-ng amounts in 10-$\mu\ell$ volumes. These solutions are placed on the centers of the filters and allowed to evaporate and air-dry for approximately 30 min. Each filter is then placed back in its holder. These filter standards are analyzed along with the calibration standards as outlined in Section 9.3. The results obtained with the filter standards are used to estimate the percent recovery of gallium.

9.2 **Initial Analysis of Four Standards.** Four standards, each containing 200 ng of gallium, are prepared by adding 200 $\mu\ell$ of standard solution "C" to 4.5 mℓ of acetate buffer solution. Each of the four standards should be analyzed with a different electrode. At the level of 200 ng of gallium, the contributions of the reagent blank should be negligible, and a single point for the gallium calibration curve usually is sufficient.

9.3 **Analysis of Samples and Controls.** With every ten samples, a filter standard (Section 9.1) and a reagent blank are analyzed by the ASV procedure given in Section 8.3. At least one reagent blank and one filter standard should be analyzed with each electrode that is used for analysis of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 200 ng of gallium will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA. The overall response factor is the amount of gallium in the standard (200 ng) divided by the average of the eight peak heights (in μA). However, it will be necessary to calculate an individual response factor for an electrode if the average of the two peak height values for an individual electrode differs from the overall average of the eight peak heights by more than 10%. For example, if the average for the eight peak heights is 210 μA, and the average of the two values for one of the electrodes is 185 μA, then the response factor to be used for that electrode is 200 ng/185 μA, or 1.08 ng/μA. The overall response factor in this example is 200 ng/210 μA, or 0.95 ng/μA.

10. Calculations

10.1 The amount of gallium in a sample is calculated from the gallium peak height (in μA), as measured according to Section 8.3.4, and the response factor in the following manner:

$$\text{Amount (in ng)} = \text{peak height (in } \mu\text{A)} \times \text{response factor (in ng/}\mu\text{A)}$$

Any amount of gallium found in the reagent and filter blank should be subtracted from the sample value to give the correct amount (in ng) of gallium in the sample. The average recovery of gallium from filter standards is usually greater than 90%. However, it may be necessary to include a factor in the above equation to correct for incomplete recovery.

10.2 The concentration of gallium may be expressed in mg/m^3, which is numerically equal to $\mu g/\ell$:

$$mg/m^3 = \frac{\text{corrected weight (in ng) of gallium in sample}}{V_s\,(\ell) \times 1000\,(ng/\mu g)}$$

where: V_s = volume of air sampled.

11. References

11.1 Environmental Sciences Associates, Inc. Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Division of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

11.2 Davis, P.H., and E.D. Moorhead, "Phase-Selective Anodic Stripping Analyses of Gallium in Spiked Bovine Tissue," **Anal. Lett.**, 8, 387 (1975).

VINYL CHLORIDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Vinyl Chloride	Method No.: P&CAM 178
Matrix: Air	Range: 0.008 to 5.2 mg/m³ in a 5 - liter air sample
Procedure: Adsorption on activated carbon, desorption with carbon disulfide, gas chromatography	Precision (CV_T): 0.08 at levels of 7 and 71 mg/m³
Date Issued: 9/3/74	Classification: B (accepted)
Date Revised: 1/29/76	

1. Principle of the Method

A known volume of air is drawn through two small sorbent tubes in series containing activated carbon (made from coconut shells), which adsorbs the vinyl chloride present in the air sample. The collected vinyl chloride is then desorbed with carbon disulfide, and the resulting solutions are analyzed by gas chromatography with a flame ionization detector. The areas under the resulting peaks are compared with areas obtained from the injection of standards.

2. Range and Sensitivity

2.1 The minimum detectable amount of vinyl chloride was found to be 0.2 ng per injection at a 1 x 1 attenuation on a gas chromatograph. This corresponds to an estimated concentration of 0.008 mg/m³ in a 5-liter air sample analyzed by this method. However, the desorption efficiency from activated carbon of amounts of vinyl chloride as small as 40 ng (0.008 μg/ℓ x 5 liters) has not been determined. Therefore, the detection limit of the overall method may be somewhat higher than 0.008 mg/m³.

2.2 At the recommended sampling flow rate of 50 mℓ/min, the total volume to be sampled should not exceed 5 liters. This value is based upon data which indicated that more than 10 liters of air containing 2.6 μg/ℓ (1 ppm) of vinyl chloride could be sampled on activated carbon before 5% breakthrough was observed. This indicates that 5 liters of air containing no more than 5.2 mg/m³ may be sampled without significant breakthrough. (The sorbent tube consists of two sections of activated carbon separated by a section of urethane foam. [See Section 6.2.]) If a particular atmosphere is suspected of containing a high concentration of contaminants or a high humidity is suspected, the sampling volume should be reduced by 50%. A safety factor has been included in the recommended 5-liter volume and the capacity of the first tube should be within these limits except under the most extreme conditions.

3. Interferences

3.1 When the amount of water in the air is so great that condensation actually occurs in the tube, organic vapors will not be trapped effectively. Experiments indicate that high humidity severely decreases the capacity of activated carbon for organic vapors.

3.2 When two or more substances are known or suspected to be present in the air, such information, including their suspected identities, should be transmitted with the sample since these compounds may interfere with the analysis for vinyl chloride.

3.3 Any compound that has the same retention time as vinyl chloride at the operating conditions described in this method is an interference. Hence, retention time data on a single column, or even on a number of columns, may not provide proof of chemical identity. Often, operating conditions can be modified to eliminate interferences. Samples should be analyzed by an independent method when overlapping gas chromatographic peaks cannot be resolved.

4. Precision and Accuracy

4.1 The coefficients of variation resulting from the analysis of two sets of sorbent tubes, one set of 27 tubes exposed to a vinyl chloride concentration of 7.2 mg/m^3 in air and another set of 29 tubes exposed to a concentration of 71.3 mg/m^3, were 0.076 and 0.075, respectively. These values reflect total sampling and analytical error as well as desorption efficiency correction errors.

4.2 Experiments were performed to obtain some indication of the accuracy, although accuracy was difficult to evaluate in the absence of a primary standard. These experiments generally involved six sorbent tube samples exposed to a synthetic atmosphere. The calculated value was the concentration expected based on the measured amounts of vinyl chloride and air mixed to prepare the synthetic atmosphere. Therefore the calculated value was not the "true" value, since it was subject to experimental error. The value found from analysis of each sorbent tube, after correction for desorption efficiency, was also compared to that found by the direct injection of gas samples from the same synthetic atmosphere used in loading the tubes. The results of these experiments are shown in the table below. It should be noted that average concentrations determined by analysis of sorbent tubes were within 6% of the average concentrations determined by analysis of gas samples.

Experiment No.		Concentration, calculated, mg/m³	Concentration, experimental, mg/m³	Estimated error, %[a]
I	Gas samples	64	71.2 ± 0.7[b]	
	Sorbent tubes	64	69.8 ± 1.5	-2
II	Gas samples	13	14.5 ± 0.5	
	Sorbent tubes	13	13.6 ± 0.4	-6
III	Gas samples	2.6	2.88 ± 0.07	
	Sorbent tubes	2.6	2.91 ± 0.13	+1
IV	Gas samples	1.3	-	
	Sorbent tubes	1.3	1.27 ± 0.09	–

a. The estimated error is the average of concentrations determined from sorbent tubes minus the average of concentrations determined from gas samples, divided by the average of concentrations determined from gas samples, multiplied by 100.

b. The number given is the mean value plus or minus the 95% confidence level. The 95% confidence level is defined as the standard deviation multiplied by Student's *t* at the 0.05 significance level, divided by the square root of the number of samples.

5. Advantages and Disadvantages of the Method

5.1 The sampling device is small, portable, and involves no liquids. Interferences are minimal, and most of those that do occur can be eliminated by altering chromatographic conditions. The tubes are analyzed by means of a rapid instrumental method. The method can also be used for the simultaneous determination of two or more components suspected to be present in the same sample by changing gas chromatographic conditions from isothermal to a temperature-programmed mode of operation.

5.2 One disadvantage of the method is that the amount of sample that can be taken is limited by the amount of vinyl chloride that the tube will hold before it becomes overloaded. When the sample value obtained for the backup section of the sorbent tube exceeds 20% of that found on the front section, the possibility of sample loss exists. During storage, volatile compounds such as vinyl chloride will migrate throughout the tube until equilibrium is reached. At this time, 33% of these compounds will be found in the backup section. This may lead to some confusion as to whether sample loss has occurred. This migration effect can be considerably decreased by shipping and storing the tubes at -20°C. (See Section 8.2.10.)

5.3 The precision of the method is limited by the reproducibility of the pressure drop and, therefore, the flow rates across the tubes. Because the pump is usually calibrated for one particular tube, differences in flow rates can occur when sampling through other tubes and can cause sample volumes to vary.

6. **Apparatus**

6.1 **Personal Sampling Pump.** The pump should be a properly calibrated personal sampling pump for personal and area samples. It should be calibrated with a representative sorbent tube in the sampling line. A dry or wet test meter or a glass rotameter that will determine the flow rate (50 mℓ/min) to within ± 5% may be used for the calibration.

6.2 **Sorbent Tubes.** The glass tubes have both ends flame sealed. Each is 7 cm long, 6-mm o.d., 4-mm i.d., and contains two sections of 20/40-mesh activated carbon separated by a 2-mm portion of urethane foam. The activated carbon is prepared from coconut shells and is fired at 600°C prior to packing to remove adsorbed materials. The primary adsorbing section contains 100 mg of sorbent, the backup section 50 mg. A 3-mm portion of urethane foam is placed between the outlet end of the tube and the backup section. A plug of silanized glass wool is placed in front of the adsorbing section. The pressure drop across the tube must be less than 2 in. of water at a flow rate of 0.2 ℓ/min.

6.3 Gas chromatograph equipped with a flame ionization detector.

6.4 Stainless steel column (20 ft x 0.125 in.) packed with 10% SE-30 on 80/100-mesh Chromosorb W (acid washed, silanized with dimethyldichlorosilane). Other columns capable of performing the required separations may be used.

6.5 A mechanical or electronic integrator or a recorder and some method for determining peak area.

6.6 Vials (2-mℓ) that can be sealed with caps containing Teflon-lined silicone rubber septa.

6.7 Microliter syringes (10-μℓ, and convenient sizes for making standards).

6.8 Gas-tight syringe (1-mℓ, with a gas-tight valve).

6.9 Pipettes (0.5-mℓ delivery pipettes or 1.0-mℓ type graduated in 0.1-mℓ increments).

6.10 **Volumetric Flasks (10-mℓ, or convenient sizes for making solutions).** It is preferable to have plastic stoppers for the volumetric flasks.

7. **Reagents**

7.1 Carbon disulfide, spectroquality or better grade.

7.2 Vinyl chloride, lecture bottle, 99.9% minimum purity.

7.3 Toluene, chromatographic quality.

7.4 Purified helium.

7.5 Prepurified hydrogen.

7.6 Filtered compressed air.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware used for the laboratory analysis should be washed with detergent and thoroughly rinsed with distilled water.

8.2 **Collection and Shipping of Samples**

8.2.1 Immediately before sampling, the ends of two tubes are broken to provide an opening at least one-half the internal diameter of the tube (2 mm).

8.2.2 The second sorbent tube is used as a backup and is positioned next to the sampling pump in tandem with the first tube.

8.2.3 The sorbent tubes are placed in a vertical position with the larger section of sorbent pointing up during sampling to minimize channelling of the vinyl chloride through the sorbent.

8.2.4 Air being sampled is not to be passed through any hose or tubing before entering the sorbent tubes.

8.2.5 The flow rate and time, or volume, must be measured as accurately as possible. The sample is taken at a flow rate of 50 mℓ/min. The maximum volume to be sampled should not exceed 5 liters. (See Section 2.2.)

8.2.6 Relatively large volumes (10 to 20 liters) of air also should be sampled through other sorbent tubes at the same time personal samples are taken. These bulk air samples will be used by the analyst to identify possible interferences before the personal samples are analyzed.

8.2.7 If the temperature and pressure of the atmosphere being sampled are significantly different from 25°C or 760 mmHg, they should be measured and recorded.

8.2.8 The sorbent tubes are capped with the supplied plastic caps immediately after sampling. Under no circumstances are rubber caps to be used.

8.2.9 One tube is handled in the same manner as a sample tube (break, seal, and transport), except that no air is sampled through this tube. This tube is labeled as a blank.

8.2.10 Capped tubes are packed tightly before they are shipped to minimize tube breakage during transport to the laboratory. The use of two tubes in series has eliminated the need for cooling during shipping. However, if *two* tubes are *not* used, *i.e.*, only one tube is used, and if the samples will spend a day or more in transit, then cooling (*e.g.*, with Dry Ice) is necessary to minimize migration of vinyl chloride to the backup section.

8.2.11 Samples received at the laboratory are logged in and immediately stored in a freezer (around - 20°C) until time for analysis. Samples may be stored in this manner for long periods of time with no appreciable loss of vinyl chloride (2 months). Even around -20°C, vinyl chloride will equilibrate between the two sections of activated carbon, *i.e.*, it will migrate to the backup section. This phenomenon is observable after 2 weeks and may be confused with sample loss after 1 to 2 months.

8.3 Analysis of Samples

8.3.1 **Preparation and Desorption of Samples.** The two tubes used in the collection of a single sample are analyzed separately. Each tube is scored with a file and broken open at each end. The glass wool is discarded. Both sections of each tube are transferred to a small vial containing 1 mℓ of carbon disulfide. It is important to add the sorbent to the carbon disulfide and not the carbon disulfide to the sorbent. The vial is topped with a septum cap. The separating section in each tube is discarded. Tests indicate that desorption is complete in 30 min if the sample is agitated occasionally during this period. The samples should be analyzed within 60 min after addition to carbon disulfide. If only one tube is used for sampling, then each section of activated carbon should be analyzed separately.

8.3.2 **Gas Chromatographic Conditions.** The typical operating conditions for the gas chromatograph are:

1. Helium carrier gas flow, 40 mℓ/min (80 psig).

2. Hydrogen gas flow to detector, 65 mℓ/min (20 psig).

3. Air flow to detector, 500 mℓ/min (50 psig).

4. Injector temperature, 230°C.

5. Detector temperature, 230°C.

6. Column temperature, 60°C.

8.3.3 **Injection.** The first step in the analysis is the injection of the sample into the gas chromatograph. To eliminate difficulties arising from blowback or distillation within the syringe needle, one should employ the solvent flush injection technique. The 10-$\mu\ell$ syringe is first flushed with solvent several times to wet the barrel and plunger. Two microliters of solvent is drawn into the syringe to increase the accuracy and reproducibility of the injected sample volume. The needle is removed from the solvent and the plunger is pulled back about 0.4 $\mu\ell$ to separate the solvent flush from the sample with a pocket of air to be used as a marker. The needle is then immersed in the sample, and a 5-$\mu\ell$ aliquot is withdrawn to the 7.4 $\mu\ell$ mark (2 $\mu\ell$ solvent + 0.4 $\mu\ell$ air + 5 $\mu\ell$ sample = 7.4 $\mu\ell$). After the needle is removed from the sample and prior to injection the plunger is pulled back a short distance to minimize evaporation of the sample from the tip of the needle. Duplicate injections of each sample and standard are made. No more than a 3% difference in area is to be expected. Automatic sampling devices may also be used.

8.3.4 **Measurement of Area.** The area under the sample peak is measured by an electronic integrator or some other suitable form of area measurement, and preliminary results are read from a standard curve prepared as discussed below.

8.4 Determination of Desorption Efficiency

8.4.1 **Importance of Determination.** The efficiency of desorption of a particular compound can vary from one laboratory to another and also from one batch of sorbent to another. Thus, it is necessary to determine at least once the percentage of vinyl chloride that is removed in the desorption process. Desorption efficiency should be determined on the same batch of sorbent tubes used in sampling. Results indicate that desorption efficiency varies with loading (total vinyl chloride on the tube), particularly at lower values, *e.g.*, 2.5 μg.

8.4.2 **Procedure for Determining Desorption Efficiency.** Sorbent tubes from the same batch as that used in obtaining samples are used in this determination. A measured volume of vinyl chloride gas is injected into a bag containing a measured volume of air. The bag is made of Tedlar (or a material that will retain the vinyl chloride and not absorb it) and should have a gas sampling valve and a septum injection port. The concentration in the bag may be calculated if room temperature and pressure are known. A measured volume is then sampled through a sorbent tube with a calibrated sampling pump. At least five tubes are prepared in this manner. These tubes are desorbed and analyzed in the same manner as the samples. (See Section 8.3.) Samples taken with a gas-tight syringe from the bag are also injected into the gas chromatograph. The concentration in the bag is compared to the concentration obtained from the tubes.

The desorption efficiency equals the amount of vinyl chloride desorbed from the sorbent divided by the quantity of vinyl chloride contained in the volume of synthetic atmosphere sampled, or

$$\frac{\text{(amount of vinyl chloride from sorbent)}}{\left(\begin{matrix}\text{concn of vinyl chloride}\\ \text{in bag}\end{matrix}\right) \times \left(\begin{matrix}\text{vol of atmosphere}\\ \text{sampled}\end{matrix}\right)}$$

9. Calibration and Standards

CAUTION: Laboratory Operations Involving Carcinogens

> Vinyl chloride has been identified as a human carcinogen and appropriate precautions must be taken in handling this gas. The Occupational Safety and Health Administration has promulgated regulations for the use and handling of vinyl chloride. They may be found in 29 CFR 1910.93q (Section 1910.93q in Title 29 of the Code of Federal Regulations available in the Federal Register, Vol. 39, No. 194, Friday, October 4, 1974, pp. 35890-35898).

A series of standards, varying in concentration over the range of interest, is prepared and analyzed under the same gas chromatographic conditions and during the same time period as the unknown samples. Curves are established by plotting concentration in μg/mℓ *versus* peak area. There are two methods of preparing standards and, as long as highly purified vinyl chloride is used, both are comparable.

If no internal standard is used in the method, standard solutions must be analyzed at the same time that the sample analysis is done. This will minimize the effect of day-to-day variations of the flame ionization response.

9.1 Standard Preparation

9.1.1 **Gravimetric Method.** Vinyl chloride is slowly bubbled into a weighed 10-mℓ volumetric flask containing approximately 5 mℓ of toluene. After 3 min, the flask is again weighed. A weight change of 100 to 300 mg is usually observed. The solution is diluted to exactly 10 mℓ with carbon disulfide and is used to prepare other standards by removal of aliquots with different sized syringes. Subsequent dilution of these aliquots with carbon disulfide results in a series of values that are linear from the range of 0.2 ng per injection, the minimum detectable amount of vinyl chloride, to 1.5 μg per injection.

322

9.1.2 **Volumetric Method.** A 1-mℓ gas sample of pure vinyl chloride is drawn into a gas-tight syringe and the valve is closed. The tip of the needle is inserted into a 10-mℓ volumetric flask containing approximately 5 mℓ of CS_2. The valve is opened and the plunger is withdrawn slightly to allow the CS_2 to enter the syringe. The action of the vinyl chloride dissolving in the CS_2 creates a vacuum and the syringe becomes filled with the solvent. An air bubble (2%) is present and has been found to be due to the void volume in the needle of the syringe. The solution is returned to the flask and the syringe is rinsed with clean CS_2 and the washings added to the volumetric flask. The volumetric flask is then filled to the mark with CS_2. Other standards are then prepared from this stock solution.

Standards are stored in a freezer at -20°C and have been found to be stable at this temperature for 3 days. Tight-fitting plastic tops on the volumetric flasks seem to retain the vinyl chloride better than ground-glass stoppers.

10. Calculations

10.1 The weight, in μg, corresponding to the area under each peak is read from the standard curve for vinyl chloride. No liquid volume corrections are needed because the standard curve is based on the number of micrograms in 1.0 mℓ of CS_2 and the volume of sample injected is identical to the volume of the standards injected.

10.2 Corrections for the blank are made for each sample.

$$\mu g = \mu g_S - \mu g_b$$

where: μg_S = μg found on sample tube.

μg_b = μg found on blank tube.

A similar procedure is followed for the backup sections.

10.3 The amounts present in the front and backup sections of the same sample tube are added to determine the total amount of vinyl chloride in the sample.

10.4 The total amount is corrected for the desorption efficiency at the level of vinyl chloride measured.

$$\text{Corrected amount (in } \mu g) = \frac{\text{amount (in } \mu g)}{\text{desorption efficiency}}$$

323

10.5 The concentration of vinyl chloride in air may be expressed in mg/m^3:

$$mg/m^3 = \frac{\text{corrected weight (in } \mu g)}{\text{volume of air sampled (in } \ell)}$$

10.6 The concentration may also be expressed in terms of parts per million (ppm) by volume:

$$ppm = mg/m^3 \times \frac{24.45}{M.W.} \times \frac{760}{P} \times \frac{T+273}{298}$$

where: 24.45 = molar volume (ℓ/mole) at 25°C and 760 mmHg.

M.W. = molecular weight.

P = pressure (mmHg) of air sampled.

T = temperature (°C) of air sampled.

11. **References**

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11.2 White, L.D., D.G. Taylor, P.A. Mauer, and R.E. Kupel, "A Convenient Optimized Method for the Analysis of Selected Solvent Vapors in the Industrial Atmosphere." Amer. Ind. Hyg. Ass. J.,31, 225 (1970).

CARBON DISULFIDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Carbon Disulfide	**Method No.:** P&CAM 179
Matrix: Air	**Range:** 5 to 100 mg/m³ in a 3 - liter sample
Procedure: Adsorption on activated carbon, atomic absorption spectrophotometry	**Precision (CV):** 0.04 (analytical) for 0.7 to 15 μg/mℓ in isoamyl acetate
Date Issued: 1/29/76	**Classification:** E (Proposed)
Date Revised:	

1. Principle of the Method

Carbon disulfide is adsorbed from a known volume of air in a small tube containing activated carbon as air is drawn through the sorbent. After desorption of the carbon disulfide from the sorbent with isoamyl acetate containing pyrrolidine, the resulting organic solution is shaken with an acid solution (pH 1.5 to 2.5) of cupric sulfate. The copper ions are extracted into the organic phase as they undergo chelation with pyrrolidine dithiocarbamate (PDTC) formed from carbon disulfide and pyrrolidine. The amount of copper in the organic phase is then determined by atomic absorption spectrophotometry at a wavelength of 324.7 nm. The number of moles of copper found is equal to half the number of moles of carbon disulfide collected from the air by the sorbent.

2. Range and Sensitivity

2.1 The detection limit of the method as described herein is 15 μg in a single sample. In a 3 - liter air sample, the smallest detectable concentration of carbon disulfide is therefore approximately 5 mg/m³. The sensitivity can be improved by reducing the volume of the extracting solvent or by increasing the volume of the air sampled. Also, higher sensitivities for copper are attainable with some atomic absorption spectrophotometers. (See Reference 11.1.)

2.2 The recommended range of carbon disulfide concentrations in air for which this method should be applied is determined by the region of linear response of the analytical method, which is 15 to 300 μg of carbon disulfide in 20 mℓ of extracting solvent. In 3 - liter air samples, this range corresponds to 5 to 100 mg/m³.

2.3 Amounts larger than 300 μg of carbon disulfide may be collected and measured by appropriate dilution of the solution resulting from the extraction of carbon disulfide from the sorbent tube. The maximum amount of carbon disulfide that may be collected in a sorbent tube without significant breakthrough is 1.7 mg. This amount in 20 mℓ of solvent should be diluted by a factor of 6 to fall within the linear range of the analytical procedure.

3. Interferences

3.1 High humidity decreases the capacity of the sorbent tube.

3.2 Hydrogen sulfide is not likely to interfere with this method since cupric sulfide is insoluble in isoamyl acetate. Its presence, however, could be eliminated by sampling the air through a cotton plug saturated with lead acetate, or cadmium chloride, prior to sampling the air through a sorbent tube. Other interferences have not been found.

4. Precision and Accuracy (See Reference 11.1)

4.1 For determinations of 7 to 150 μg of carbon disulfide in 10 mℓ of extracting solvent, the coefficient of variation was approximately 0.04.

4.2 Error in recoveries of 7 to 150 μg of carbon disulfide in 10 mℓ of isoamyl acetate ranged from 0.9 to 1.7%.

5. Advantages and Disadvantages of the Method

5.1 The sampling device is small and portable and involves no liquids.

5.2 Another advantage is that the analytical method involves a simple solvent extraction technique with a minimum of reagents and sample handling.

5.3 Also, the instrumental method, atomic absorption spectrophotometry, is a well-established technique that is simple to use.

5.4 One disadvantage of the method is the decrease in the loading capacity of the sorbent tube when sampling from humid air. This may lead to overloading of the front section of the sorbent tube. (See Section 6.2 for description of tubes.) If the amount of sample found in the backup section of the tube exceeds 25% of that found in the front section, the possibility of sample loss exists.

5.5 Another disadvantage is the limiting of the precision of the method by the irreproducibility of the pressure drop across the tubes. This drop will affect the flow rate and cause the sample volume to be imprecise since the pump is usually calibrated for a specific tube only.

6. Apparatus

6.1 **A Calibrated Personal Sampling Pump.** Each pump should be calibrated with a representative sorbent tube in the sampling line. A dry or wet test meter or a glass rotameter that will allow determination of the appropriate flow rate within an error of ±5% should be used in the calibration.

6.2 **Sorbent Tubes.** The tubes are made of glass, are 7 cm long with a 6-mm o.d. and a 4-mm i.d., and are flame sealed at both ends. Each tube contains two sections of 20/40-mesh activated carbon separated by a 2-mm portion of urethane foam. The activated carbon is prepared from coconut shells and is fired at 600°C prior to packing. The adsorbing section contains 100 mg of activated carbon, the backup section 50 mg. A 3-mm portion of urethane foam is placed between the outlet end of the tube and the backup section. A plug of silylated glass wool is placed in front of the adsorbing section. The pressure drop across the tube must be less than 1 in. of mercury at a flow rate of 1 ℓ/min.

6.3 An atomic absorption spectrophotometer with a copper hollow cathode lamp.

6.4 A mechanical shaker.

6.5 Glass vials (40 mℓ) with Teflon-lined caps.

6.6 Assorted laboratory glassware including pipettes and volumetric flasks of appropriate sizes.

7. Reagents

All reagents must be analytical reagent grade. Deionized distilled water should be used in the preparation of solutions.

7.1 **Isoamyl Acetate.** Distill weekly and store in a tightly capped bottle.

7.2 **Pyrrolidine.** Distill weekly and store under nitrogen.

7.3 **Pyrrolidine Solution (0.1 *M*).** Dilute 8.4 mℓ to 100 mℓ with water in a volumetric flask.

7.4 **Cupric Sulfate Solution (0.01 *M*).** Dissolve 0.2497 g in water and dilute the solution to 100 mℓ with water in a volumetric flask.

7.5 **Cupric Sulfate Solution (0.001 *M*).** Dilute 10 mℓ of 0.01 *M* $CuSO_4$ to 100 mℓ with water in a volumetric flask.

7.6 **Hydrochloric Acid (0.1 *M*).** Dilute 8.6 mℓ of concentrated hydrochloric acid to 1 liter with water.

7.7 **Carbon Disulfide, Standard Solution "A" (1.26 mg/mℓ).** Transfer 1 mℓ of carbon disulfide with a volumetric pipette into about 100 mℓ of isoamyl acetate in a liter volumetric flask and dilute to the mark with isoamyl acetate. Store in a tightly capped bottle.

7.8 **Carbon Disulfide, Standard Solution "B" (25.2 μg/mℓ).** Dilute 2 mℓ of carbon disulfide standard solution "A" to 100 mℓ in a volumetric flask with isoamyl acetate.

8. Procedure

8.1 **Cleaning of Equipment.** All glassware used for the laboratory analysis should be detergent washed and thoroughly rinsed with tap water and distilled water.

8.2 **Collection and Shipping of Samples**

8.2.1 Immediately before sampling, break the ends of the tube to provide an opening at least one-half the internal diameter of the tube (2mm).

8.2.2 The smaller section of sorbent is used as a backup and should be positioned nearest the sampling pump.

8.2.3 The sorbent tube should be placed in a vertical direction (with the larger section of sorbent pointing up) during sampling to minimize channeling through the sorbent.

8.2.4 Air being sampled should not be passed through any hose or tubing before entering the sorbent tube.

8.2.5 A maximum sample size of 3 liters is recommended for personal samples. Sample at a flow of 50 mℓ/min.

8.2.6 At the time personal samples are taken, relatively large volumes of air also should be sampled through other sorbent tubes. These bulk air samples will be used by the analyst to identify possible interferences before the personal samples are analyzed.

8.2.7 The temperature and pressure of the atmosphere being sampled should be recorded if they differ significantly from 25°C and 760 mmHg.

8.2.8 The sorbent tubes should be capped with the supplied plastic caps immediately after sampling. Under no circumstances should rubber caps be used.

8.2.9 One tube should be handled in the same manner as the sample tube (break, seal, and transport), except that no air is sampled through this tube. This tube should be labeled as a blank.

8.2.10 Capped tubes should be packed tightly and padded before they are shipped to minimize tube breakage during shipping. Transport and store the tubes in a cooled container (0°C).

8.3 Analysis of Samples

8.3.1 Preparation of Samples. Allow the tubes to warm to room temperature. In preparation for analysis, score each sorbent tube with a file in front of the larger sorbent section and break it open. Remove and discard the glass wool. Transfer the activated carbon in the larger sorbent section to a 40-mℓ vial and cap the vial. Remove and discard the separating section of foam. Transfer the backup sorbent section to another vial and cap the vial. Analyze the two sorbent sections separately.

8.3.2 Desorption and Analysis of Samples. Add 20 mℓ of isoamyl acetate and 1 mℓ of 0.01 *M* pyrrolidine to each vial. Cap each vial and shake it on a mechanical shaker for 30 min.Thenadd 10 mℓ of 0.001 *M* $CuSO_4$ and adjust the pH to 1.5 to 2.5 with 0.1 *M* HCl. Recap and shake the vial for 5 min on the mechanical shaker. Allow the phases to separate well and analyze the organic phase at 324.7 nm by atomic absorption spectrophotometry.

8.4 Determination of Desorption Efficiency

8.4.1 Importance of Determination. The desorption efficiency of a particular compound can vary from one laboratory to another and also from one batch of activated carbon to another. Thus, it is necessary to determine at least once the percentage of the specific compound that is removed in the desorption process, provided the same batch of activated carbon is used.

8.4.2 Procedure for Determining Desorption Efficiency. Activated carbon equivalent to the amount in the first section of the sampling tube (100 mg) is measured into a 7-cm long, 4-mm i.d. glass tube, flame sealed at one end. This activated carbon must be from the same batch as that used in obtaining the samples and can be obtained from unused sorbent tubes. The open end is capped with Parafilm. A known amount of the analyte in isoamyl acetate is injected directly into the activated carbon with a microliter syringe, and the tube is capped with more Parafilm.

The amount injected is equivalent to that present in a 3-liter air sample at the selected level. Since the injection of sufficiently small amounts of pure carbon disulfide is impractical, 5- to 10-μℓ injections of carbon disulfide solutions in isoamyl acetate will suffice.

Six tubes at each of three levels (0.5X, 1X and 2X the OSHA standard) are prepared in this manner and allowed to stand for at least overnight to assure complete adsorption of the analyte onto the sorbent. These tubes are referred to as the samples. A parallel blank tube should be treated in the same manner except that no sample is added to it. The sample and blank tubes are desorbed and analyzed in exactly the same manner as the sampling tube described in Section 8.3. The carbon disulfide solutions in isoamyl acetate are analyzed with the samples.

The desorption efficiency (D.E.) equals the average weight (in μg) recovered from the tube divided by the weight (in μg) added to the tube, or

$$\text{D.E.} = \frac{\text{average weight (in } \mu\text{g) recovered}}{\text{weight (in } \mu\text{g) added}}$$

The desorption efficiency may be dependent on the amount of analyte collected on the sorbent. Plot the desorption efficiency *versus* weight of analyte found. This curve is used in Section 10.4 to correct for adsorption losses.

9. Calibration and Standards

9.1 **Preparation of Standards.** A set of standards is prepared by diluting each of 0.5-,1-, 5-, 10- and 15-mℓ aliquots of carbon disulfide standard solution "B" (25.2 μg/mℓ) to 25 mℓ with isoamyl acetate in a volumetric flask.

9.2 **Measurement of Standards.** Transfer 20 mℓ of each standard into a 40-mℓ vial with a volumetric pipette. Add 1 mℓ of 0.01 *M* pyrrolidine to each vial, cap it, and shake it on a mechanical shaker for 30 min. Then add to each vial 10 mℓ of 0.001 *M* $CuSO_4$ and adjust the pH to 1.5 to 2.5 with 0.1 *M* HCl. Recap each vial and shake it for 5 min on the mechanical shaker. Allow the phases to separate well and measure the absorbance of the organic phase at 324.7 nm by atomic absorption spectrophotometry.

9.3 **Construction of the Calibration Curve.** To facilitate calculations involving sample unknowns, plot the absorbance of each standard solution as a function of the amount (in μg) of carbon disulfide in the 20-mℓ aliquot of each standard solution. The amount will range from 10 to 302 μg. The amount of carbon disulfide in a sample unknown can therefore be read directly from the calibration curve.

10. Calculations

10.1 Read the weight (in μg) of carbon disulfide in each sample unknown directly from the calibration curve.

10.2 Corrections for the blank must be made for each sample.

$$\mu g = \mu g \text{ sample} - \mu g \text{ blank}$$

where: μg sample = μg found in front section of sample tube.

μg blank = μg found in front section of blank tube.

A similar procedure is followed for the backup sections.

10.3 Add the weights found in the front and backup sections to get the total weight in the sample.

10.4 Read the desorption efficiency from the curve (see Section 8.4.2) for the amount found in the front section. Divide the total weight by this desorption efficiency to obtain the corrected weight in each sample.

$$\text{Corrected weight} = \frac{\text{total weight}}{\text{D.E.}}$$

10.5 The concentration of carbon disulfide in air may be expressed in mg/m^3:

$$mg/m^3 = \frac{\text{corrected weight (in } \mu g)}{\text{volume of air sampled (in } \ell)}$$

10.6 The concentration may also be expressed in terms of parts per million (ppm) by volume:

$$ppm = mg/m^3 \times \frac{24.45}{M.W.} \times \frac{760}{P} \times \frac{T+273}{298}$$

where: 24.45 = molar volume (ℓ/mole) at 25°C and 760 mmHg.

M.W. = molecular weight

P = pressure (mmHg) of air sampled.

T = temperature (°C) of air sampled.

11. References

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ARSENIC IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Arsenic	Method No.: P&CAM 180
Matrix: Air	Range: 0.1 to 1 mg/m^3 in 25 liters of air
Procedure: Collection on a membrane filter; reaction kinetic analysis	Precision (CV): 0.02 (analytical) at 0.4 mg/m^3
Date Issued: 1/29/76	Classification: E (Proposed)
Date Revised:	

1. Principle of the Method

1.1 Particulate material containing arsenic is collected from air on a membrane filter. The filter and trapped particulate material are wet-ashed with a mixture of nitric and sulfuric acids or dry-ashed in a low-temperature asher. The nitric acid is removed from the wet-ashed sample, or the dry-ashed sample is dissolved in sulfuric acid. The resulting As(V) is reduced to As(III) with an acidic solution of potassium iodide and sodium formate. The chemically reduced sample is neutralized, and As(III) is determined by the kinetics of a catalytic reaction.

1.2 In the catalytic reaction, As(III) promotes the osmium (Os(VIII)) catalysis of the oxidation of iodide to iodine by bromate. The rate of iodine production is proportional to the concentration of As(III) and is determined after a four-minute reaction time by measurement of the absorbance at 580 nm with starch as an indicator.

2. Range and Sensitivity

2.1 The detection limit of the analysis procedure described in Section 8.3 (excluding the digestion and chemical reduction steps) is estimated to be 2.5 μg. This corresponds to an arsenic concentration of 0.1 mg/m^3 in a 25-liter sample of air.

2.2 The rate of the catalyzed reaction is a linear function of amounts of arsenic in the range of 2.5 to 25 μg. In a 25-liter sample of air, this corresponds to 0.1 to 1 mg/m^3. The method can be extended to lower levels by sampling larger volumes of air or to higher levels by diluting the digested filter sample prior to the chemical reduction step. (See Section 8.3.3.)

3. Interferences

3.1 The effects of various ions on the determination of 10 μg of As(III) in the analysis procedure described in Section 8.3 (excluding the digestion and chemical reduction steps) are as follows:

Ion	Added as:	Amount added, μg	Recovery of As(III),%
As^{5+}	arsenate	10	112
Cu^{2+}	sulfate	100	121
Cu^{2+}	sulfate	10	100
Fe^{2+}	ammonium sulfate	10	217
Fe^{3+}	chloride	100	147
Fe^{3+}	chloride	10	116
Hg^{2+}	chloride	1000	103
Bi^{3+}	nitrate	1000	113
Bi^{3+}	nitrate	100	103
Co^{2+}	nitrate	1000	100
Ag^{+}	nitrate	1000	100
Ni^{2+}	nitrate	1000	101
Pb^{2+}	nitrate	1000	100
Ca^{2+}	nitrate	1000	100
Mg^{2+}	sulfate	1000	103
Mn^{2+}	sulfate	1000	103
PO_4^{3-}	sodium salt	1000	100
SiO_3^{2-}	sodium salt	1000	99
SO_3^{2-}	sodium salt	100	82

Of the ions tested, only As(V), Cu(II), Fe(II), Fe(III), and sulfite produce significant interferences.

3.2 As(III) cannot be reliably determined in the presence of As(V). If the determination of the total amount of arsenic in the sample is desired, as in this method, the reduction of As(V) to As(III) is necessary prior to the catalytic reaction. (If the determination of only As(III) is desired, As(III) can be readily separated from As(V) by solvent extraction. See References 11.1 and 11.2.)

3.3 The interference caused by a tenfold excess of Cu(II), Fe(II), or Fe(III) can be masked by EDTA (ethylenediaminetetraacetic acid), pyrophosphate, or potassium fluoride, respectively, as shown below:

Ion, 100 μg	Masking agent	Recovery of As(III), %
Cu^{2+}	EDTA, 2% (1 mℓ)	100
Fe^{2+}	Pyrophosphate, 0.001 *M* (1 mℓ)	108
Fe^{3+}	Potassium fluoride (500 mg)	100

Also, iron can be removed from the digested sample with a cation exchange resin. (See Reference 11.1.)

3.4 As(III) cannot be reliably determined in the presence of a tenfold excess of weight of sulfite, which may inhibit the participation of As(III) in the catalytic reaction. The ashing of samples should oxidize any sulfite collected on filters to sulfate.

4. Precision and Accuracy

4.1 The precision of the overall sampling and analytical method has not been evaluated. However, the coefficient of variation for the analysis procedure (excluding the digestion and chemical reduction steps) for the determination of 10 μg of As(III) has been estimated to be 0.02. In a 25-liter sample of air, 10 μg of arsenic corresponds to 0.4 mg/m^3. (See Reference 11.1.)

4.2 An estimate of the accuracy of the analysis procedure including the digestion and chemical reduction steps has been made from the analysis of samples of human lung tissues with an arsenic concentration of 13.93 μg/g. (See Reference 11.1.) Some samples were wet-ashed; others were dry-ashed with a low-temperature asher. Results from the wet-ashed samples indicated an arsenic concentration of 14.7 μg/g (106% of the specified value), while results from the dry-ashed samples indicated an arsenic concentration of 11.1 μg/g (79% of the specified value). It appears that some arsenic may be lost through volatilization when samples are dry-ashed in a low-temperature asher. Wet-ashing is therefore recommended.

5. Advantages and Disadvantages of the Method

5.1 One advantage is that the method does not require any complex equipment.

5.2 Another advantage is that the method is free from interference by phosphate and silicate. Also, interferences from Cu(II), Fe(II), and Fe(III) can be eliminated.(See Section 3.3.)

5.3 One disadvantage is that the reaction of bromate with iodide is strongly influenced by the pH of the sample solution since hydrogen ion is a reactant. The samples must therefore be strongly buffered.

6. Apparatus

6.1 **Air Sampling Equipment.** The sampling unit for the collection of particulate samples has the following components:

6.1.1 **Filter Unit.** The unit consists of the filter and an appropriate filter holder such as the Millipore Aerosol Analysis Monitor, Catalog No. MAWP 037 AO, or equivalent. This unit is a plastic disposable holder preassembled with a filter in place. The recommended filter is the Millipore Type AA, a mixed cellulose ester with a pore size of 0.8 μm and a diameter of 37 mm.

6.1.2 **Adaptor.** An adaptor such as the Millipore Aerosol Adaptor, Catalog No. XX62 000 04, is inserted into the filter unit to allow a hose to be attached.

6.1.3 **Personal Sampling Pump.** The pump should be calibrated with a representative filter unit in the sampling line. A wet or dry test meter or glass rotameter capable of measuring the appropriate flow rate (1.5 to 2 ℓ/min) to within ± 5% may be used in the calibration.

6.2 Constant temperature water bath (25 ± 1°C).

6.3 Recording spectrophotometer, with a jacketed cell compartment to maintain a constant temperature of 25°C.

6.4 pH Meter.

6.5 Timer capable of measuring minutes and seconds.

6.6 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric pipettes, and volumetric flasks of appropriate sizes.

7. Reagents

All reagents must be at least analytical reagent grade. Solutions should be prepared with distilled deionized water.

7.1 Sulfuric acid, concentrated.

7.2 Nitric acid, concentrated.

7.3 **Potassium Iodide Solution** (2%). Dissolve 2 g of potassium iodide in water and dilute to 100 mℓ.

7.4 **Potassium Iodide Solution** (0.25 *M*). Dissolve 4.15 g of potassium iodide in water in a 100-mℓ volumetric flask and dilute to the mark with water.

7.5 **Sodium Formate Solution** (10%). Dissolve 10 g of sodium formate in water and dilute to 100 mℓ.

7.6 **Acetate Buffer Solution (pH 4.8 to 5.0).** Mix sodium acetate solution (~2 *M*) and acetic acid solution (~2 *M*) in appropriate proportions to yield a pH of 4.8 to 5.0.

7.7 **Potassium Bromate Solution** (0.025 *M*). Dissolve 0.417 g of potassium bromate in water in a 100-mℓ volumetric flask and dilute to the mark with water.

7.8 **Starch Indicator Solution** (1%). Dissolve 1 g of soluble starch in boiling water and dilute to 100 mℓ with water. Prepare a fresh solution daily.

7.9 **Osmium (Os(VIII)), Standard Solution "A" (0.01 *M*).** Dissolve 0.636 g of osmium tetroxide (OsO_4) in water in a 250-mℓ volumetric flask and dilute to the mark with water.

7.10 **Osmium (Os(VIII)), Standard Solution "B" (0.0001 *M*).** Transfer 1 mℓ of osmium standard solution "A" to a 100-mℓ volumetric flask and dilute to the mark with water.

7.11 **Arsenic (As(III)), Standard Solution "C" (2500 μg/mℓ).** Dissolve 0.6600 g of arsenic trioxide (As_2O_3) in 10 mℓ of 1 *N* NaOH. Immediately after dissolution of the arsenic trioxide, neutralize the solution with dilute hydrochloric acid and cool it to room temperature. Transfer the solution quantitatively to a 200-mℓ volumetric flask and dilute to the mark with water.

7.12 **Arsenic (As(III)), Standard Solution "D" (5 μg/mℓ).** Transfer 1 mℓ of arsenic standard solution "C" into a 500-mℓ volumetric flask and dilute to the mark with water.

8. **Procedure**

8.1 **Cleaning of Equipment.** All equipment used for the laboratory analysis should be washed with detergent solution and rinsed with tap water and distilled deionized water.

8.2 **Collection and Shipping of Samples**

8.2.1 Connect the exit end of a filter unit to the adaptor. Connect the adaptor to the pump with a short piece of flexible tubing.

8.2.2 Sample the air at a flow rate of 1.5 to 2 ℓ/min. Measure the flow rate and time as accurately as possible. A minimum sample of 25 liters should be collected. If larger volumes are sampled, be sure that the filter does not become loaded with dust to the point that loose material could fall off or the filter become plugged.

8.2.3 The filter holders should be shipped in a suitable container to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample is dislodged from the filter nor that the sample surface is disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the holder on top of the sample filter.

8.2.4 Unexposed filters in filter holders should be labeled as blanks and shipped with the samples.

8.3 **Analysis of Samples**

8.3.1 Carefully remove the filter from the filter unit by opening the unit, gently pushing up the filter and its backing, and grasping the unexposed edge of the filter with stainless steel forceps.

8.3.2 Place the filter in a 250-mℓ Erlenmeyer flask. Add 2 mℓ of concentrated sulfuric acid, 20 mℓ of concentrated nitric acid, and a few glass beads to the flask. Heat gently to prevent excess frothing. Occasionally, add small amounts of distilled water to the flask to release trapped gas. Add additional amounts (5 mℓ) of concentrated nitric acid until the organic matter is destroyed. After the sample has been solubilized and the yellowish color has disappeared, heat the solution until white fumes appear.

8.3.3 If a preliminary estimate can be made of the arsenic content of the digested sample, dilute the sample solution to an appropriate volume with water so that a 10-mℓ aliquot contains less than 25 μg. Otherwise, dilute the sample solution to 10 mℓ. If iron is present, it can be removed by passing the solution through a small column (1 cm in diameter and 10 cm in length) filled with Dowex 50W-X8 (50/100 mesh) cation exchange resin.

8.3.4 To the diluted sample solution, or a 10-mℓ aliquot of the diluted sample solution, add 1 mℓ of concentrated sulfuric acid, 1 mℓ of 2% potassium iodide solution, and 1 mℓ of 10% sodium formate solution. Boil the solution for 5 min, cool, and neutralize with 3 *N* NaOH. Quantitatively transfer the neutral solution to a 25-mℓ volumetric flask and dilute to the mark with water.

8.3.5 Adjust the temperature of the sample solution and all of the reagent solutions for the kinetic reaction to 25 ± 1°C in the water bath. After mixing the solutions, as described below, maintain the temperature of the reaction mixture at 25 ± 1°C until the absorbance is measured.

8.3.6 To a 100-mℓ beaker, add the 25-mℓ sample solution, 5 mℓ of acetate buffer solution, 2 mℓ of potassium bromate solution, 2 mℓ of potassium iodide solution, and 1 mℓ of 1% starch solution. Quickly add 5 mℓ of osmium standard solution "B" and stir the mixture well. Begin timing the reaction when the addition of the osmium is initiated. (The reaction rate for the oxidation of As(III) prior to the addition of Os(VIII) is extremely slow. See References 11.4 and 11.5.)

8.3.7 Transfer the reaction mixture to an absorption cell and place the cell in the temperature controlled cell compartment of the spectrophotometer. After 4.0 ± 0.2 min have elapsed from the time of addition of the osmium, measure the absorbance of the solution at 580 nm with water in the reference cell.

9. Calibration and Standards

Two types of standards are prepared. Standard solutions of arsenic are made by diluting appropriate volumes of arsenic standard solution "D." These solutions are used for calibration. Filter standards are prepared as described in Section 9.3. They are used to determine the efficiency of recovery of known amounts of arsenic from filters.

9.1 **Standard Solutions.** Five or six standard solutions are necessary to establish the calibration curve. To prepare a standard solution add 0 to 5 mℓ of arsenic standard solution "D" to a 25-mℓ volumetric flask and dilute to the mark with water. Follow the instructions detailed in Section 8.3.5 through 8.3.7 for the catalytic reaction and measurement of the absorbance of each standard sample solution.

9.2 **Calibration Curve.** Plot the absorbance of each standard sample solution as a function of the amount (in μg) of arsenic in the solution. The amount of arsenic in an unknown sample can be read directly from the curve.

9.3 **Arsenic Filter Standards.** Prepackaged filters of the type used for sampling are removed from their plastic cases and separated from their support mats. The filters are placed on the center ring supports of the plastic cases so that only the edge of the filter is touching the rim of its holder.Arsenic standard solution "C" is appropriately diluted to provide 2.5- to 25-μg amounts of arsenic in 10-μℓ volumes. These solutions are placed on the centers of the filters and allowed to evaporate and air-dry for approximately 30 min. Each filter is then placed back in its holder. These filter standards are digested and analyzed along with the unknown samples and filter blanks according to the procedure described in Section 8.3. The results obtained from the filter standards are used to estimate the percent recovery of arsenic.

10. Calculations

10.1 Read from the calibration curve the amount (in μg) of arsenic in a sample.

10.2 Correction for the blank must be made for each sample.

$$\mu g = \mu g \text{ sample} - \mu g \text{ blank}$$

where: μg sample = μg found in sample filter.

μg blank = μg found in blank filter.

10.3 Divide the total weight by the recovery to obtain the corrected μg/sample.

$$\text{Corrected } \mu g/\text{sample} = \frac{\text{total weight}}{\text{recovery}}$$

10.4 The concentration of arsenic may be expressed in mg/m^3, which is numerically equal to $\mu g/\ell$:

$$mg/m^3 = \frac{\text{corrected weight (in } \mu g) \text{ of arsenic in sample}}{V_S\ (\ell)}$$

where: V_S = volume of air sampled.

11. References

11.1 Tarumoto, T., and H. Freiser, "Determination of Trace Level Quantities of Arsenic *via* a Novel Kinetic Method," Anal. Chem., 47, 180 (1975).

11.2 Sandell, E.B., *"Colorimetric Determination of Traces of Metals,"* Interscience, New York, 1959, pp. 280-282.

11.3 Naidu, P.P., and G.G. Rao, "Microdetermination of Arsenic(III) and Osmium(VIII) through Osmium-Thiourea Reaction," Microchem.J., 18, 422 (1973).

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11.5 Burgess, A.E., and J.M. Ottaway, "Catalysis of the Arsenic(III)-Potassium Bromate Reaction. II. Catalytic Determination of Osmium," Talanta, 22, 401 (1975).

SELENIUM IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Selenium	Method No.: P&CAM 181
Matrix: Air	Range: 0.0006 to 0.01 mg/m^3 in 90 liters of air
Procedure: Collection on a membrane filter; reaction kinetic analysis	Precision (CV): 0.03 (analytical) at 0.01 mg/m^3
Date Issued: 1/29/76	Classification: E (Proposed)
Date Revised:	

1. Principle of the Method

1.1 Particulate material containing selenium is collected from air on a membrane filter. The filter and trapped particulate material are wet-ashed with a mixture of nitric and sulfuric acids. The resulting solution is neutralized with sodium hydroxide, and selenium is determined by the kinetics of a catalytic reaction.

1.2 Selenite in the digested sample solution is reduced to elemental selenium by sulfite and sulfide. (See Reference 11.2.) The elemental selenium catalyzes the sulfide reduction of methylene blue to the colorless leuco form in alkaline solution. Fe(III) is used to promote the catalysis; ethylene diaminetetraacetic acid (EDTA) is used to mask the effect of other cations; and formaldehyde and ethanolamine are used to stabilize the blank. (See Reference 11.3.) The selenium concentration is proportional to the rate of decolorization of the methylene blue. The rate is determined spectrophotometrically by measuring the time required for the complete bleaching of the sample solution.

2. Range and Sensitivity

2.1 The detection limit of the method, excluding the sampling and wet-ashing steps, is estimated to be 0.05 μg. In a 90-liter sample of air, this corresponds to a selenium concentration of 0.0006 mg/m^3.

2.2 The rate of decolorization of methylene blue is proportional to amounts of selenium present in the range of 0.05 to 1 μg in the procedure described in Section 8.3. This corresponds to a selenium concentration range of 0.0006 to 0.01 mg/m^3 in a 90-liter sample of air. The method can be extended to lower levels by sampling greater volumes of air or to higher levels by diluting the digested filter sample prior to the catalytic reaction. (See Section 8.3.6.)

3. Interferences

The following ions have been investigated for interference in the analysis procedure described in Section 8.3 (excluding the digestion step):

Chemical group of ionized element	Ion
Ia	Li^+, Na^+, K^+
Ib	Cu^{2+}, Ag^+
IIa	Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}
IIb	Zn^{2+}, Cd^{2+}, Hg^{2+}
IIIb	$B_4O_7^{2-}$, Al^{3+}
IVb	HCO_3^-, CO_3^{2-}, SiO_3^{2-}, Sn^{2+}, Pb^{2+}
IVa	Zr^{4+}
Vb	NH_4^+, NO_3^-, PO_4^{3-}, $HAsO_4^{2-}$, Sb^{5+}, Bi^{3+}
Va	VO_3^-
VIb	SO_3^{2-}, SO_4^{2-}, TeO_3^{2-}
VIa	Cr^{3+}, MoO_4^{2-}, WO_4^{2-}
VIIb	F^-, Cl^-, Br^-, I^-
VIIa	Mn^{2+}
VIII	Fe^{3+}, Co^{2+}, Ni^{2+}
Lanthanides	Ce^{4+}

A visual method of detection was used in these experiments. (See Reference 11.2.) The amount of selenium was 1 μg and that of the interfering compound was 50 μg. With the exception of Cu^{2+}, Ag^+, Sb^{5+}, and Bi^{3+}, none of the ions interfered. Cu^{2+}, Ag^+, and Bi^{3+} caused positive interferences leading to unrealistically high recoveries of selenium. When the amounts of Ag^+ and Bi^{3+} were reduced to 25 μg and that of copper to 10 μg, the interferences were not observed. The interference due to Cu^{2+} could also be eliminated by separating copper from selenium with the use of a cation exchange resin. Sb^{5+} was found to inhibit the catalytic reaction slightly and led to a 25% loss in the recovery of selenium. However, when the amount of Sb^{5+} was reduced to 25 μg, no interference was observed.

4. Precision and Accuracy

The precision and accuracy of the method as applied to air samples have not been evaluated. However, the precision of the analysis procedure described in Section 8.3 (excluding the digestion step) has been estimated from the statistical analysis of calibration curves. (See Reference 11.1.) The coefficient of variation is 0.03 in the determination of 1 μg of selenium, which corresponds to a concentration of 0.01 mg/m^3 in a 90-liter sample of air.

5. Advantages and Disadvantages of the Method

5.1 The primary advantages of the method are its simplicity and high sensitivity. No complex equipment is needed. The rate of the catalytic reaction is very sensitive to selenium concentration.

5.2 Another advantage is that interference by ions of the other elements investigated is small.

5.3 A disadvantage is that the sulfide solution is unstable and will cause variations in results from day to day.

6. Apparatus

6.1 **Air Sampling Equipment.** The sampling unit for the collection of particulate samples has the following components:

6.1.1 **Filter Unit.** The unit consists of the filter and an appropriate filter holder such as the Millipore Aerosol Analysis Monitor, Catalog No. MAWP 037 AO, or equivalent. This unit is a plastic disposable holder preassembled with a filter in place. The recommended filter is the Millipore Type AA, a mixed cellulose ester with a pore size of 0.8 μm and a diameter of 37 mm.

6.1.2 **Adaptor.** An adaptor such as the Millipore Aerosol Adaptor, Catalog No. XX62 000 04, is inserted into the filter unit to allow a hose to be attached.

6.1.3 **Personal Sampling Pump.** The pump should be calibrated with a representative filter unit in the sampling line. A wet or dry test meter or glass rotameter capable of measuring a flow rate of 1.5 ℓ/min to within ± 5% may be used in the calibration.

6.2 Recording spectrophotometer, with a jacketed cell compartment to maintain a constant temperature of 25 ± 1°C.

6.3 Timer capable of measuring minutes and seconds.

6.4 Test tubes (or vials) with screw caps (40 mℓ).

6.5 Constant temperature water bath (25 ± 1°C).

6.6 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric pipettes, and volumetric flasks of appropriate sizes.

7. Reagents

All reagents must be at least analytical reagent grade. Solutions should be prepared with distilled deionized water.

7.1 Sulfuric acid, concentrated.

7.2 Nitric acid, concentrated.

7.3 **Sodium Hydroxide Solution** (3 *N*). Dissolve 120 g in water and dilute to 1 liter with water.

7.4 **Methyl Red Indicator Solution** (0.05 to 0.1%). Dissolve 0.05 to 0.1 g of the sodium salt of methyl red (2-[*p*-(dimethylamino)-phenylazo]-benzoic acid) in water and dilute to 100 mℓ with water.

7.5 **Conditioner Solution.** Dissolve 25 g of the disodium salt of EDTA, 0.4 g of ferric chloride ($FeCl_3 \cdot 6H_2O$), and 50 mℓ of triethanolamine in water. Dilute the solution to 1 liter with water.

7.6 Formaldehyde (37%).

7.7 **Alkaline Sodium Sulfide Solution** (0.1 *M*). Dissolve 2.40 g of sodium sulfide ($Na_2S \cdot 9H_2O$), 2.40 g of sodium sulfite (Na_2SO_3), and 4 g of sodium hydroxide in water in a 100-mℓ volumetric flask. Dilute to the mark with water. The solution is relatively stable for 2 days.

7.8 **Methylene Blue Solution** (0.05%). Dissolve 0.05 g of methylene blue (3.9-bisdimethylaminophenazothionium chloride) in water and dilute to 100 mℓ with water.

7.9 **Selenium (Se(IV)), Standard Solution "A"** (500 μg/mℓ). Dissolve 50 mg of pure selenium metal in a few drops of concentrated nitric acid. Boil the solution gently to expel brown fumes and dilute to 100 mℓ in a volumetric flask.

7.10 **Selenium (Se(IV)), Standard Solution "B"** (100 μg/mℓ). Transfer 20 mℓ of selenium standard solution "A" to a 100-mℓ volumetric flask and dilute to the mark with water.

7.11 **Selenium (Se(IV)), Standard Solution "C"** (0.1 μg/mℓ). Transfer 1 mℓ of selenium standard solution "B" to a 1-liter volumetric flask and dilute to the mark with water.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware should be washed with dilute hydrochloric acid and then rinsed well with distilled deionized water.

8.2 Collection and Shipping of Samples

8.2.1 Connect the exit end of a filter unit to the adaptor. Connect the adaptor to the pump with a short piece of flexible tubing.

8.2.2 Sample the air at a flow rate of 1.5 ℓ/min. Measure the flow rate and time as accurately as possible.

8.2.3 The filter holders should be shipped in a suitable container to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample is dislodged from the filter nor that the sample surface is disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the holder on top of the sample filter.

8.2.4 Unexposed filters in filter holders should be labeled as blanks and shipped with the samples.

8.3 Analysis of Samples

8.3.1 Carefully remove the filter from the filter unit by opening the unit, gently pushing up the filter and its backing, and grasping the unexposed edge of the filter with stainless steel forceps.

8.3.2 Place the filter in a 250-mℓ Erlenmeyer flask. Add 1 mℓ of concentrated sulfuric acid, 10 mℓ of concentrated nitric acid, and a few glass beads to the flask.

8.3.3 Reflux the solution at 120°C for several hours until the sample is solubilized and the solution has become colorless.

8.3.4 Remove the nitric acid from the sample solution by heating until white fumes of sulfur trioxide appear.

8.3.5 Add 5 mℓ of water and 1 drop of methyl red indicator solution. Add 3 *N* NaOH until the color of the indicator changes from red to yellow.

8.3.6 If a preliminary estimate can be made of the selenium content of the digested sample, dilute the sample solution to an appropriate volume with water so that a 10-mℓ aliquot contains less than 1 μg. To extend the range of the method to concentrations in air near the OSHA standard of 0.2 mg/m^3, dilute the sample solution by a factor of 20.

8.3.7 Adjust the temperature of the sample solution and all of the reagent solutions for the kinetic reaction to 25 ± 1°C in the water bath. After mixing the solutions, as described below, maintain the temperature of the reaction mixture at 25 ± 1°C until the absorbance is measured.

8.3.8 Transfer a 10-mℓ aliquot of the sample solution to a 40-mℓ screw-capped test tube. Add 5 mℓ of the conditioner solution, 5 mℓ of 37% formaldehyde, and 1 mℓ of the alkaline sodium sulfide solution. Mix the contents of the test tube well. Add 2 drops of the 0.05% methylene blue solution, cap the test tube, and shake it well. *Begin timing the reaction when the methylene blue is added.*

8.3.9 Immediately transfer a portion of the sample solution from the test tube to a 1-cm cuvette maintained at 25 ± 1°C and measure the absorbance at 634 nm as a function of time. Use a reagent blank solution in the reference cell. Continue timing the decolorization of the solution until the absorbance is zero. Alternatively, discontinue timing after an absorbance-time curve has been established and extrapolate the curve to zero absorbance to determine the decolorization time.

8.3.10 Analyze a filter blank in the same way the samples are analyzed.

9. Calibration and Standards

Two types of standards are prepared. Standard solutions of selenium to be used for calibration are made by dilution of appropriate volumes of selenium standard solution "C." Filter standards are prepared as described in Section 9.3. These filter standards are used to determine the efficiency of recovery of known amounts of selenium from filters.

9.1 **Standard Solutions.** Five or six standard solutions are necessary to establish the calibration curve. To prepare standard solutions, dilute selenium standard solution "C" appropriately with water to provide 10-mℓ solutions that contain 0 to 1 μg of Se(IV). Follow the instructions detailed in Section 8.3.7 through 8.3.9 for the analysis of each standard solution.

9.2 **Calibration Curve.** Plot the reciprocal of the time required for decolorization of each standard sample solution as a function of the amount (in μg) of selenium in the standard solution. The amount of selenium in an unknown sample can be read directly from the curve.

9.3 **Selenium Filter Standards.** Prepackaged filters of the type used for sampling are removed from their plastic cases and separated from their support mats. The filters are placed on the center ring supports of the plastic cases so that only the edge of the filter is touching the rim of its holder. Selenium standard solution "B" is appropriately diluted to provide 0.1 to 1 μg amounts of selenium in 10-$\mu\ell$ volumes. These solutions are placed on the centers of the filters and allowed to evaporate and air-dry for approximately 30 min. Each filter is then placed back in its holder. These filter standards are digested and analyzed along with the unknown samples and filter blanks according to the procedure described in Section 8.3. The results obtained with the filter standards are used to estimate the percent recovery of selenium.

10. **Calculations**

10.1 Read from the calibration curve the amount (in μg) of selenium in a sample.

10.2 Correction for the blank must be made for each sample .

$$\mu g = \mu g \text{ sample} - \mu g \text{ blank}$$

where: μg sample = μg found in sample filter.

μg blank = μg found in blank filter.

10.3 Divide the total weight by the recovery to obtain the corrected μg/sample.

$$\text{Corrected } \mu g/\text{sample} = \frac{\text{total weight}}{\text{recovery}}$$

10.4 The concentration of selenium may be expressed in mg/m^3, which is numerically equal to $\mu g/\ell$:

$$mg/m^3 = \frac{\text{corrected weight (in } \mu g) \text{ of selenium in sample}}{V_S \quad (\ell)}$$

where: V_S = volume of air sampled.

11. **References**

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11.2 Feigl, F., and P.W. West, "Test for Selenium Based on a Catalytic Effect," Anal Chem., 19, 351 (1947).

11.3 West, P.W., and T.V. Ramakrishna, "A Catalytic Method for Determining Traces of Selenium," Anal. Chem., 40, 966 (1968).

11.4 Mesman, B.B., and H.A. Doppelmayr, "Photometric Device for Determination of Trace Amounts of Selenium," Anal. Chem., 43, 1346 (1971).

CHROMIUM IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Chromium (VI)	**Method No.:** P&CAM 182
Matrix: Air	**Range:** 0.01 to 0.25 mg/m^3 in a 100-liter sample of air
Procedure: Collection on a PVC filter, extraction with water, reaction kinetic analysis	**Precision (CV):** 0.03 (analytical) at 0.05 mg/m^3
Date Issued: 1/29/76	**Classification:** E (Proposed)
Date Revised:	

1. **Principle of the Method**

   1.1 Particulate material containing chromium is collected from air on a polyvinyl chloride (PVC) filter. Cr(VI) is extracted from the filter into deionized distilled water by ultrasonic agitation, separated from interfering ions with a cation exchange resin, and finally determined by the kinetics of a catalytic reaction.

   1.2 In the catalytic reaction, Cr(VI) catalyzes the oxidation by hydrogen peroxide of *o*-dianisidine to its semiquinone in an aqueous-ethanolic solution near pH 4. The rate of production of the semi quinone is proportional to the concentration of Cr(VI) and is determined by the spectrophotometric measurement of absorbance at 450 nm as a function of time. (See References 11.1 and 11.2.)

2. **Range and Sensitivity**

   2.1 The detection limit of the method, excluding the collection step, is estimated to be 1.0 μg in a single sample, which corresponds to a Cr(VI) concentration of 0.01 mg/m^3 in a 100-liter sample of air.

   2.2 The rate of the catalyzed reaction is proportional to amounts of Cr(VI) in the range of 0.5 to 25 μg in the recommended analysis procedure. In a 100-liter sample of air, this corresponds to a range of 0.01 to 0.25 mg/m^3. The method can be extended to lower levels by sampling larger volumes of air or by decreasing the volume of water used to wash the filter so that the concentration of Cr(VI) in the resulting solution is no smaller than 0.001 $\mu g/m\ell$. The method can be extended to higher levels by diluting the wash solution. (See Section 8.3.2)

3. Interferences

The effects of various ions on the rate of the catalyzed reaction in the determination of 0.01 μg of Cr(VI) are as follows:

Ion	Ratio of amount of foreign ion to amount of Cr (VI)	Reaction rate (k), 10^{-3} min^{-1}	k/k_0
None	–	4.54 (k_0)	1.00
Na^+	100	4.45	0.98
Ca(II)	100	4.46	0.98
Pb(II)	100	0.35	0.08
Cu(II)	10	32.7	7.20
Ni(II)	100	5.01	1.10
Co(II)	100	4.84	1.07
Cd(II)	100	4.40	0.97
Mn(II)	100	4.75	1.05
Cr(III)	1	7.72	1.70
Fe(III)	100	8.84	1.95
Al(III)	1000	1.79	0.28
Al(III)	100	4.22	0.92
Sn(IV)	100	3.46	0.76
V(V)	100	8.35	1.84
Mo(VI)	100	5.04	1.11

The major interferences are caused by Pb(II), Cu(II), Cr(III), Fe(III), Sn(IV), and V(V). (See Reference 11.1.) The ion exchange treatment described in Section 8.3.3 has proved successful in eliminating these major interferences.

4. Precision and Accuracy

4.1 The precision of the overall sampling and analytical method has not been evaluated. However, the coefficient of variation for the analysis procedure described in Section 8.3 (excluding the extraction and ion exchange steps) is estimated to be 0.07 in the determination of 0.002 to 0.008 μg/mℓ of Cr(VI) and 0.03 in the determination of 0.008 to 0.05 μg/mℓ in the 10-mℓ reaction solution described in Section 8.3.4. The range of 0.002 to 0.05 μg/mℓ in the reaction solution corresponds to a range of 0.01 to 0.25 mg/m^3 in a 100-liter sample of air. The coefficient of variation is about 0.03 for the analytical procedure (excluding the extraction and ion exchange steps) in the analysis of a 100-liter air sample with a concentration of Cr(VI) near 0.05 mg/m^3

4.2 The accuracy of the overall sampling and analytical method has not been evaluated. However, the analysis of PVC filters that had been spiked with 0 to 28.5 μg of Cr(VI), transported by mail, and stored for 1.5 weeks prior to analysis indicated that the amounts recovered were within 6% of the amounts added.

5. Advantages and Disadvantages of the Method

5.1 The primary advantage is that the method is fast and simple. The extraction of Cr(VI) from a PVC filter by ultrasonic agitation can be done much faster than the digestion of filters by wet- or dry-ashing procedures. No complex equipment is required in the analysis procedure.

5.2 Another advantage is that the major interfering metal ions listed in Section 3 can easily be removed with an ion exchange resin.

5.3 A disadvantage is that the method is especially sensitive to contamination of glassware by Cr(VI) and to fluctuations of temperature in the cell compartment of the spectrophotometer.

6. Apparatus

6.1 **Air Sampling Equipment.** The sampling unit for the collection of particulate samples has the following components:

6.1.1 **Filter Unit.** The unit consists of a PVC filter with a diameter of 37 mm and a pore size of 0.6 μm and an appropriate filter holder, such as the Millipore two- or three- piece filter cassette, Millipore Corporation, Bedford, Mass.

6.1.2 **Adaptor.** An adaptor is connected to the filter unit to allow a hose to be attached.

6.1.3 **Personal Sampling Pump.** The pump should be calibrated with a representative filter unit in the sampling line. A wet or dry test meter or glass rotameter capable of measuring the appropriate flow rate (1.5 to 2 ℓ/min) to within ±5% may be used in the calibration.

6.2 Ultrasonic cleaner.

6.3 Ion Exchange Column. Prepare in the following manner:

6.3.1 With concentrated HCl and distilled deionized water, thoroughly clean a glass column (0.05 cm i.d. and 12 cm long) fitted with a Teflon stopcock. Insert a small Pyrex wool plug in the tip near the stopcock.

6.3.2 Wash about 0.5 g of the cation exchange resin, Dowex 50W-X8, with distilled deionized water. Decant the wash water and rinse the resin several times with more water.

6.3.3 Pour the washed resin into the column to a height of about 1.5 cm. Pass 5 mℓ of 1 *M* NaCl solution through the column, and then pass distilled deionized water until no chloride appears in the eluate, as indicated by no precipitation with silver ion.

6.3.4 Keep the resin wet when not in use by maintaining the water level above the top of the resin in the column.

6.4 Constant temperature water bath (25 ± 1°C).

6.5 Recording spectrophotometer with a jacketed cell compartment to maintain the temperature of the cell at 25 ±1°C. An automatic sample changer is recommended.

6.6 Assorted laboratory glassware, including beakers, graduated cylinders, Erlenmeyer flasks, volumetric pipettes, and volumetric flasks of appropriate sizes.

7. Reagents

All reagents must be analytical reagent grade. Solutions should be prepared with distilled deionized water.

7.1 Dowex 50W-X8 cation exchange resin, or equivalent.

7.2 **Sodium Chloride Solution** (1 *M*). Dissolve 58.5 g of NaCl in water and dilute to 1 liter with water.

7.3 Ethanol, absolute.

7.4 **Hydrogen Peroxide Solution** (5.0 *M*). Dilute 54.9 mℓ of hydrogen peroxide (30%) to 100 mℓ with water.

7.5 ***o*-Dianisidine Solution** (0.036 M). Dissolve 0.879 g of *o*-dianisidine (3,3'-dimethoxybenzidine), practical grade, in absolute ethanol in a 100-mℓ volumetric flask and dilute to the mark with ethanol. This solution may be stored in a brown bottle and is stable for 1 week when protected from light.

7.6 **Hydrochloric Acid Solution (0.001 *M*).** Dilute 8.3 mℓ of concentrated HCl to 1 liter with water to obtain 0.1 *M* HCl. Then dilute 1 mℓ of 0.1 *M* HCl to 100 mℓ with water.

7.7 **Chromium (Cr(VI)), Standard Solution "A" (2500 μg/mℓ).** Dissolve 0.7072 g of potassium dichromate in water in a 100-mℓ volumetric flask and dilute to the mark with water.

7.8 **Chromium (Cr(VI)), Standard Solution "B" (2.5 μg/mℓ).** Transfer 1 mℓ of chromium standard solution "A" to a 1-liter volumetric flask and dilute to the mark with water.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware used for the laboratory analysis should be washed with detergent and thoroughly rinsed with distilled water, 1 *M* HCl, and finally with deionized, doubly distilled water. *Chromic acid cleaning solution must not be used.*

8.2 **Collection and Shipping of Samples**

8.2.1 Connect the exit end of a filter unit to the adaptor. Connect the adaptor to the pump with a short piece of flexible tubing.

8.2.2 Sample the air at a flow rate of 1.5 to 2 ℓ/min. Measure the flow rate and time as accurately as possible. A minimum sample of 100 liters should be collected. Larger volumes are desirable, provided the filter does not become loaded with dust to the point that loose material could fall off or the filter become plugged.

8.2.3 The filter holders should be shipped in a suitable container to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample is dislodged from the filter nor that the sample surface is disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the holder on top of the sample filter.

8.2.4 Unexposed filters in filter holders should be labeled as blanks and shipped with the samples.

8.3 Analysis of Samples

8.3.1 Carefully remove the filter from the filter unit by opening the unit, gently pushing up the filter, and grasping the unexposed edge of the filter with stainless steel forceps. Transfer the filter to a small Erlenmeyer flask.

8.3.2 Extract Cr(VI) from the filter by adding 15 mℓ of distilled deionized water to the flask. Cover the flask tightly with paraffin film and place it in the ultrasonic bath for 20 min. Then quantitatively transfer the contents of the flask to a 50-mℓ volumetric flask and dilute to the mark with deionized distilled water. If it is anticipated that the sample solution will contain more than 25 μg of Cr(VI), dilute it appropriately with distilled deionized water.

8.3.3 Separate Cr(VI) from interfering ions in the sample solution in the following manner:

1. Pass 1 mℓ of the sample solution through the ion exchange column and discard the eluate.

2. Then pass 10 mℓ of the solution through the column and save the eluate for the kinetic procedure.

3. Wash the column with distilled deionized water to prepare it for the next sample.

8.3.4 Analyze the sample eluate by the following kinetic procedure:

1. Adjust the temperature of the sample solution and all of the reagent solutions for the kinetic reaction to 25 ± 1°C in the water bath. After mixing the solutions, as described below, maintain the temperature of the reaction mixture at 25 ± 1°C.

2. In a 10-mℓ volumetric flask, mix 4 mℓ of ethanol, 1 mℓ of 5 *M* H_2O_2 solution, 1 mℓ of the sample eluate, and 1 mℓ of 0.036 *M* *o*-dianisidine solution. Fill the flask to the mark with 0.001 *M* HCl and mix the resulting solution thoroughly.

3. Measure the absorbance of the reaction mixture at 450 nm as a function of time with ethanol in the reference cell. Record the absorbance for 15 to 20 min. Maintain the temperature of the cell at 25 ± 1°C during the absorbance measurements. The reaction rate is the slope of the absorbance *versus* time curve.

9. Calibration and Standards

Two types of standards are prepared. Standard solutions of Cr(VI) are made by dilution of appropriate volumes of chromium standard solution "B." They are used for calibration. Filter standards are prepared as described in Section 9.3. They are used to determine the efficiency of recovery of known amounts of Cr(VI) from filters.

9.1 **Standard Solutions.** Five or six standard solutions are necessary to establish the calibration curve. To prepare the standard solutions, add 0 to 10 mℓ of chromium standard solution "B" to 50-mℓ volumetric flasks and dilute to the mark with water. The resulting solutions will contain 0 to 25 μg of Cr(VI) (0 to 0.5 μg/mℓ). Follow the instructions detailed in Sections 8.3.3 and 8.3.4 for the determination of the reaction rate for each standard sample solution.

9.2 **Calibration Curve.** Plot the reaction rate for each standard sample solution as a function of the amount (in μg) of Cr(VI) in the standard solution. The amount of Cr(VI) in an unknown sample can be read directly from the curve.

9.3 **Chromium Filter Standards.** Filters of the type used for sampling are placed on the center ring supports of the filter holders so that only the edge of each filter is touching the rim of its holder. Chromium standard solution "A" is appropriately diluted to provide 5 to 25 μg amounts of Cr(VI) in 10-μℓ volumes. These solutions are placed on the centers of the filters and allowed to evaporate and air-dry for approximately 30 min. Each filter is then placed back in its holder. These filter standards are digested and analyzed along with the unknown samples and filter blanks according to the procedure described in Section 8.3. The results obtained with the filter standards are used to estimate the percent recovery of Cr(VI).

10. Calculations

10.1 Read from the calibration curve the amount (in μg) of Cr(VI) in a sample.

10.2 Correction for the blank must be made for each sample.

$$\mu g = \mu g \text{ sample} - \mu g \text{ blank}$$

where: μg sample = μg found in sample filter.

μg blank = μg found in blank filter.

10.3 Divide the total weight by the recovery to obtain the corrected μg/sample.

$$\text{Corrected } \mu\text{g/sample} = \frac{\text{total weight}}{\text{recovery}}$$

10.4 The concentration of Cr(VI) may be expressed in mg/m^3, which is numerically equal to $\mu g/\ell$:

$$mg/m^3 = \frac{\text{corrected weight (in } \mu g) \text{ of Cr(VI) in sample}}{V_s \quad (\ell)}$$

where: V_s = volume of air sampled.

11. References

11.1 Kneebone, B.M., and H. Freiser. "Determination of Chromium (VI) in Industrial Atmospheres by a Catalytic Method," Anal. Chem., 47, 595 (1975).

11.2 Dolmanova, I.F., G.A. Zolotova, L.V. Tarasova, and V.M. Peshkova. "Determination of Microamounts of Chromium(VI) by Means of the Catalytic Oxidation of *o*-Dianisidine by Hydrogen Peroxide," Zh. Anal. Khim., 24, 1035 (1969).

POLYNUCLEAR AROMATIC COMPOUNDS IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Polynuclear aromatic compounds (See table on page 183-16)	Method No.: P&CAM 183
Matrix: Air	Range: 2 to 1000 $\mu g/m^3$ of each compound in a 0.5 m^3 sample of air
Procedure: Collection on a membrane filter, extraction, GC separation, spectrophotometric determination	Precision: Not determined
Date Issued: 1/29/76	Classification: D (Operational)
Date Revised:	

1. Principle of the Method

Polynuclear aromatic compounds are collected from air on a filter and extracted with benzene. After the addition of an internal standard, the extract is concentrated by evaporation and a portion of the concentrate is injected into a gas chromatograph. The polynuclear aromatic compounds and the internal standard are trapped individually as they elute from the GC column. The amount of each compound in the collected fraction is determined by uv spectrophotometry. Appropriate corrections are applied as determined by the recovery of the internal standard.

2. Range and Sensitivity

The range of the method as outlined is approximately 1 to 500 μg of each polynuclear aromatic compound on a filter. For a sample volume of 0.5 m^3, this range corresponds to a concentration range of 2 to 1000 $\mu g/m^3$ for each compound. The range and sensitivity depend on the volume of air sampled, the amount of particulate matter present, and the fraction of organic material in the filter. The detection limits for the individual compounds lie in the range from 0.2 to 2.7 μg/sample.

3. Interferences

3.1 Any compound that has approximately the same gas chromatographic retention time and a similar ultraviolet absorption spectrum as one of the compounds being determined will interfere.

3.2 Any compound that has approximately the same retention time as one of the internal standards may interfere.

4. Precision and Accuracy

The precision and accuracy of this method have not been determined.

5. Advantages and Disadvantages

5.1 The method is sensitive and is capable of measuring several polynuclear aromatic compounds in a single filter sample. The method can be extended to include the determination of compounds other than those listed in the table on page 183-16.

5.2 The method requires a considerable amount of skill and experience for satisfactory performance.

5.3 Because of the sensitivity of the polynuclear aromatic hydrocarbons to photooxidation, it is necessary to conduct most of the operations under yellow light and to minimize exposure of the samples to atmospheric oxygen.

5.4 The method is sensitive to interference from unknown compounds in the samples and to contamination from extraneous sources.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Glass fiber filters, 37 mm, free of organic binder, with an efficiency of at least 99% as determined by the DOP penetration test.

6.1.2 Silver membrane filters, 37 mm, 0.8 μm pore size, Selas Corporation of America, No. FM-37-(0.8), or equivalent.

6.1.3 Disposable plastic filter holder (cassette) for 37-mm filters, Millipore [illegible]000 037 PO, or equivalent. In order to accommodate the two filters, the cassette must be held together with plastic tape or a shrinkable cellulose band.

6.1.4 Personal sampling pump capable of operating for 4 hr at 2.0 ℓ/min, or of sampling about 0.5 m^3 of air. The pump should be calibrated with a representative filter assembly in the line. A wet or dry test meter or a glass rotameter capable of measuring the flow rate to within ± 5% may be used for the calibration.

6.2 Ultrasonic Bath. Dynasonics Corporation Model NT-201, 90 kHz, 60 W, or equivalent. An ultrasonic bath operating at a lower frequency and having a higher power output may improve extraction efficiency. (See Reference 11.1.) A test tube support is needed for use in the ultrasonic bath.

6.3 Gas Chromatograph

6.3.1 A gas chromatograph equipped with a hydrogen flame ionization detector and a 10:1 effluent splitter is required. The smaller portion of the effluent flows to the flame ionization detector and the larger portion flows to the trapping assembly.

6.3.2 The GC column consists of a 10-ft by 0.125-in. (o.d.) stainless steel tube packed within 3% of Dexsil 300 GC (Analabs, Inc.) on 80/100-mesh acid-washed and silanized Chromosorb G (Johns-Manville), or the equivalent.

6.3.3 To prepare the column packing material, dissolve 3.0 g of Dexsil 300 GC in a mixture of 100 mℓ of chloroform and 100 mℓ of methylene chloride. Pour this solution over 100 g of Chromosorb G spread out in a glass evaporating tray. Allow the solvent to evaporate in a hood. Occasionally stir gently with a spatula. Place the tray on a hot plate and heat at about 40°C until the last traces of solvent have been removed. Pack a 10-ft section of the stainless steel tubing, using gentle vibration to obtain a uniform packing. Place silanized glass wool plugs in each end of the column. Condition the column at 350°C overnight with a helium flow rate of about 20 mℓ/min. After conditioning, connect the column to the splitter.

6.3.4 Strip chart recorder with 1 mV full-scale sensitivity for use with the gas chromatograph.

6.3.5 Electronic integrator, Hewlett Packard Model 3370A, or equivalent.

6.4 Traps for Collecting Effluent Fractions

6.4.1 Figure 1 shows the design of the trapping assembly. Ten of these traps are required for each analysis. The trapping assembly is connected to the high-flow outlet of the splitter. The effluent compounds leaving the GC column are condensed in the 3/16-in. (o.d.) by 12-in. stainless steel tubes and are subsequently washed out with cyclohexane.

6.4.2 It is convenient to provide a ten-place rack to hold the traps in a vertical position while they are being washed out with the solvent. The rack should have ten numbered positions, one for each trap. The base of the rack should accommodate ten 1-cm absorption cells, one for each trap. Cyclohexane is introduced in small increments into the top of each trap from the nozzle of a plastic wash bottle and is collected at the bottom in the absorption cell.

6.5 Recording Spectrophotometer. Cary Model 14, Varian Associates, or equivalent, with rectangular 1-cm silica cells with stoppers. The cells should be marked to contain 1.0 mℓ and 3.0 mℓ of cyclohexane.

6.6 **Glassware**

6.6.1 Test tubes, 100 mm by 16 mm, with Teflon-lined screw caps.

6.6.2 Small glass vials with Teflon-lined screw caps.

6.6.3 Allihn tubes, fine-porosity fritted glass, 30 mℓ.

6.6.4 Glass evaporating tray, 6 by 12 in.

6.6.5 Assorted other laboratory glassware of various sizes, including microsyringes, volumetric pipettes, graduated pipettes, graduated cylinders, volumetric flasks, and beakers.

6.7 **Miscellaneous Apparatus**

6.7.1 Hot plate, Corning Model No. PC-100, or equivalent.

6.7.2 Wood applicator sticks, 6 in. long by 1/16 in. diameter.

6.7.3 Weighing paper, 3.5 by 4.5 in.

6.7.4 Tweezers.

6.7.5 Plastic washing bottles, 500 mℓ.

7. **Reagents**

7.1 **Solvents.** Benzene, chloroform, methylene chloride, and toluene, ACS reagent grade; cyclohexane, Fisher Scientific Company, Spectranalyzed®, or equivalent.

7.2 **Polynuclear Aromatic Compounds.** Each of the compounds listed in the table should be checked for purity by gas chromatography and by comparison with infrared and ultraviolet reference spectra. Impure compounds should be purified by liquid chromatography. (See Reference 11.2.)

7.3 ***o*-Terphenyl Internal Standard "A," 1 μg/μℓ.** Weigh 0.1000 g of *o*-terphenyl in a tared 10-mℓ beaker. Transfer quantitatively to a 100-mℓ volumetric flask with toluene and fill to the mark with toluene and mix. **NOTE:** *o*-Terphenyl is a severe eye and respiratory irritant. Use with caution.

7.4 *o*-Terphenyl Internal Standard "B," 1.0 to 8.0 μg/mℓ. Weigh 3 to 5 mg of *o*-terphenyl to the nearest 0.01 mg and transfer it to a 25-mℓ volumetric flask. Dissolve in cyclohexane and dilute to the mark with cyclohexane. Transfer aliquots of appropriate size to each of four 50-mℓ volumetric flasks so that upon dilution to the mark with cyclohexane the concentrations of *o*-terphenyl in the flasks will be, respectively, 1.0, 2.0, 4.0, and 8.0 μg/mℓ.

7.5 Bibenzyl Internal Standard, 1 μg/μℓ. Weigh 0.1000 g of bibenzyl in a tared 10-mℓ beaker. Transfer quantitatively to a 100-mℓ volumetric flask with toluene. Dilute to the mark with toluene and mix.

7.6 Standard Solutions of Sample Compounds, 1.0 to 8.0 μg/mℓ. Prepare standard solutions in cyclohexane of each of the compounds listed in the table in exactly the manner described in Section 7.4 for the *o*-terphenyl internal standard "B."

8. Procedure

8.1 Cleaning of Equipment. Wash all glassware with detergent solution and rinse with tap water and distilled water. Then rinse with acetone and spectral grade cyclohexane and dry.

8.2 Collection and Shipping of Samples

8.2.1 Assemble the filters in the filter holder so that the air being sampled passes first through the glass fiber filter and then through the silver membrane filter. Remove the small plugs from the filter holder and connect the filter holder to the sampling pump by means of an adapter and a length of tubing. Sample at least 0.5 m^3 of air at 2.0 ℓ/min. The optimum sample volume will depend on the type of workroom environment being sampled. Since high concentrations of particulate material or certain types of mists may plug the filters, the flow rate should be checked about once every hour. On completion of sampling, reinsert the small plugs into the inlet and outlet of the filter holder.

8.2.2 With each group of samples prepare a blank consisting of a filter holder with representative filters that have been handled in the same manner as the sample filters, except that no air is drawn through them. The samples and the blank should be shipped promptly in a light-proof container to prevent photooxidation or damage during transit.

8.3 Analysis of Samples

8.3.1 Extraction

1. Remove the top portion of the filter holder. Hold the bottom portion containing the filters over a piece of weighing paper to catch any particulate material that may fall out. Remove the small plug from the bottom portion of the filter holder and insert an applicator stick through the hole. Gently raise the filters and grasp the unexposed edge with tweezers. Fold the filters in half and then into quarters lengthwise. Insert the folded filters into a test tube and push them gently to the bottom with the applicator stick. Add to the test tube any particulate material remaining in the filter holder or collected on the weighing paper.

2. Pipette 5.0 mℓ of benzene into the test tube. This amount of solvent should completely cover the filters.

3. Place the test tubes in the test tube support in the ultrasonic bath so that the level of water in the bath is above the level of the solvent. Turn on the ultrasonic bath and sonicate the sample for 5 min.

4. Decant the solvent into an Allihn tube and collect the filtrate in a small vial. Force the solvent through the Allihn filter under positive nitrogen pressure. This may be accomplished by connecting the nitrogen cylinder to a glass "T," leaving one arm of the "T" open and connecting the other arm to a No. 4 one-hole rubber stopper, which is pressed against the top of the Allihn tube; pressure is controlled with a finger placed against the open arm of the "T." When the filtration has been completed, close the vial tightly with a Teflon-lined screw cap.

5. Insofar as possible, all subsequent operations should be carried out under yellow light.

8.3.2 Preparation of Sample Solution

1. Transfer 3.0 mℓ of the benzene extract to a 30-mℓ beaker. Add exactly 40 μℓ of the *o*-terphenyl internal standard "A." Evaporate the contents of the beaker to dryness in a hood. Add about 0.5 mℓ of toluene to the beaker, swirl to dissolve the residue, and transfer quantitatively to a 1-dram vial. Rinse the beaker with several additional small volumes of toluene and transfer the washings to the vial. Evaporate the solution in the vial to approximately 30 μℓ. The solution should now appear pale yellow to light brown.

2. If the solution in the vial is dark brown or black, it is likely to contain relatively large amounts of polynuclear aromatic compounds that would interfere in the subsequent recording of the ultraviolet absorption peak of the *o*-terphenyl. Under these circumstances, the sample solution is prepared in a somewhat different manner. Add 80 μℓ of *o*-terphenyl internal standard "A" and 80 μℓ of bibenzyl internal standard solution to the benzene extract of the filter in the 30-mℓ beaker. Evaporate this solution to dryness in a hood and transfer to a 1-dram vial with 0.5 mℓ of toluene as described above. Evaporate the solution in the vial to approximately 70 μℓ (instead of 30 μℓ).

8.3.3 Gas Chromatographic Separation

1. Use the following GC conditions:

   Helium carrier gas flow rate, 40 mℓ/min.

   Hydrogen flow rate, 60 mℓ/min.

   Air flow rate, 400 mℓ/min.

   Injection port temperature, 340°C (do not exceed 350°C).

   Column temperature. Program at 6°C/min from 180 to 350°C; hold at 350°C for 12 min.

   Detector temperature, 350°C.

2. Inject approximately 8 μℓ of the sample solution prepared as described in Section 8.3.2. Since an internal standard is used, it is not necessary to measure this volume exactly or to record it. Approximately 41 min is required to elute all of the compounds.

3. For the sample solution containing *both* *o*-terphenyl and bibenzyl standards, record the peak areas (integrator counts) for these two compounds.

8.3.4 Collection of Effluent GC Fractions

1. Insert a stainless steel trap in the trapping assembly as each fraction elutes. Ten fractions are collected. (See table.) Some of these fractions will contain more than one compound. The approximate times for insertion and removal of the traps are ascertained by injecting a synthetic solution containing all of the polynuclear compounds and the internal standards. Watch the FID chromatogram in order to coordinate the insertion and removal of the traps at the precisely appropriate times. As each fraction is collected, place the trap in its proper place in the rack.

2. Insert the nozzle of a plastic wash bottle containing cyclohexane in the top of the stainless steel trap in the rack. Rinse the trap with small increments of cyclohexane. Catch the rinsings in the silica absorption cell. Dilute with cyclohexane to the 3.0-mℓ calibration mark on the cell. Mark each absorption cell so that it can be identified.

8.3.5 **Measurement of Ultraviolet Absorbance.** Set the scan speed of the Cary 14 spectrophotometer at 10 Å/sec and the slit control at 25. Record the absorption spectrum for each fraction. Some of the peaks are located in the ultraviolet region (200 to 350 nm) of the Cary 14 and some are located in the visible region (350 to 600 nm). (See the table for the peak wavelengths of the several compounds.) Determine the absorbance for each fraction at the specified wavelength. Use the baseline method described in Section 9.2.1 to determine the absorbance for each standard and each sample compound. If the observed absorbances are too high to measure accurately, dilute the solutions in the absorption cells as follows: With a syringe, remove 2.0 mℓ of solution from the cell and dilute the remaining 1.0 mℓ to 3.0 mℓ with cyclohexane; repeat this dilution step until an appropriate absorbance value is obtained; record the number (n) of dilution steps.

9. Calibration

CAUTION: Benzo(a)pyrene and other aromatic hydrocarbons commonly associated with it are known carcinogens. Avoid skin contact with these compounds and their solutions. Perform the evaporations and other manipulations in a hood.

9.1 **Identification of Gas Chromatographic Peaks.** With the GC conditions specified in Section 8.3.3 determine the retention time for each compound by injecting an aliquot of one of the standard solutions in cyclohexane prepared as described in Section 7.6. Also, prepare a synthetic mixture containing all of the compounds in appropriate concentrations and obtain the chromatogram of the mixture. Figure 2 is a chromatogram of such a synthetic mixture. These chromatograms will serve as a guide for trapping fractions eluted from samples. It will be observed that certain fractions contain more than one compound. The approximate retention times and the number of the trap containing each compound are shown in the table.

9.2 **Calibration for the Ultraviolet Analysis**

9.2.1 Record the ultraviolet absorption spectrum of each standard solution of the sample compounds and the internal standard *o*-terphenyl. These spectra are run with 1-cm cells at each of four concentrations, 1.0, 2.0, 4.0, and 8.0 μg/mℓ, using the standard solutions described in Sections 7.4 and 7.6. Use cyclohexane in the reference cell. Measure the absorbance by the baseline method at the wavelength of maximum absorbance specified in the table. If only one wavelength value is given in the fifth column of the table, draw a horizontal line on the absorption curve at the specified wavelength. If two wavelength values are given in the fifth column, draw a straight line between the two specified wavelengths on the absorption curve. The absorbance is measured at the wavelength of maximum absorbance by subtracting the absorbance value at the baseline from the absorbance value at the peak. This method of determining absorbance is illustrated in Figure 3.

9.2.2 Determine the average ultraviolet calibration factor for each sample compound and the internal standard by dividing concentration by absorbance. The method of calculation is illustrated below for a typical compound:

Concentration, μg/mℓ	Absorbance	Calibration factor, concentration/absorbance
8.0	0.840	9.52
4.0	0.416	9.63
2.0	0.211	9.48
1.0	0.105	9.52
	Average	9.54

9.3 **Determination of the Recovery Factors for the Internal Standards.** The recovery factors for the internal standards are determined either by ultraviolet spectrophotometry or by gas chromatography. The method chosen depends on the compounds that elute in Trap No. 1.

9.3.1 **Ultraviolet Method.** When the sample extract is relatively free of extraneous ultraviolet-absorbing compounds that elute in Trap No. 1 with *o*-terphenyl, the recovery factor R_{uv} is calculated from the absorbance of that fraction by the following equation:

$$R_{uv} = \frac{W_i}{A_i \times C_i}$$

where: W_i = µg of *o*-terphenyl added to the sample extract (Section 8.3.2).

A_i = Absorbance of the *o*-terphenyl collected in Trap No. 1 (Section 8.3.5).

C_i = Calibration factor for *o*-terphenyl (Section 9.2.2).

9.3.2 **Gas Chromatographic Method**

1. Some samples may contain compounds that are collected in Trap No. 1 and absorb ultraviolet radiation at the same wavelength as the internal standard, *o*-terphenyl, thus preventing the accurate measurement of the recovery of that internal standard. In such instances, the recovery of the internal standard is determined by comparing the peak area (integrator count) for the internal standard added to the sample (Section 8.3.2) with peak area for the same compound injected as a pure solution. Either *o*-terphenyl or bibenzyl is used for this purpose.

2. Inject exacly 15 µℓ of *o*-terphenyl internal standard "A" (Section 7.3) into the gas chromatograph and record the peak area (integrator counts) for the *o*-terphenyl peak. Similarly, inject 15 µℓ of bibenzyl internal standard (Section 7.5) and record its peak area. It is essential that the GC operating conditions be identical for these internal standard measurements and for sample measurements. Divide the peak area (integrator count) for each compound by the amount injected (15 µg) to obtain a GC calibration factor (O) in counts/µg.

3. Calculate a recovery factor R_{gc} for each internal standard by the following equation:

$$R_{gc} = \frac{W_i \times O \times 3.3}{D}$$

where: W_i = μg of internal standard added to the sample extract (Section 8.3.2).

O = GC calibration factor for the internal standard in counts/μg.

D = Integrator counts for the internal standard in the sample extract (Section 8.3.3).

3.3 = A constant taking into account the GC splitter ratio of 10:1 and the final volume (3.0 mℓ) of the solution of the trapped fraction.

4. The choice of whether to use the recovery factor for *o*-terphenyl or that for bibenzyl in the final calculation (Section 10.1) is based on the value of D. The response of the flame ionization detector is nearly the same for both compounds; therefore, the internal standard peak having the lower value for D will be more nearly free of interference from other compounds. In the subsequent final calculations in Section 10.1, use the recovery factor R for the internal standard having the lower value of D.

10. Calculations

10.1 Calculate the weight W_s (in μg) of each polynuclear aromatic compound on the filter by the following equation:

$$W_s = R \times A \times C \times 1.67$$

where: R = Recovery factor for the internal standard, as determined in Section 9.3.1 (uv) or 9.3.2 (GC). The analyst must choose the recovery factor that will give the best results.

A = Absorbance of the polynuclear aromatic compound as determined in Section 8.3.5.

C = Ultraviolet calibration factor for the polynuclear aromatic compound as determined in Section 9.2.2.

1.67 = A correction for taking a 3.0-mℓ aliquot of the 5.0-mℓ benzene extract of the filter (Section 8.3.2).

Another correction must be made if the cyclohexane washings from the traps were diluted before the measurement of absorbance (Section 8.3.5).

10.2 The concentration of the polynuclear aromatic compound in air is calculated by dividing the total weight of the compound on the filter (μg) by the volume of air sampled (m^3) to give the concentration in $\mu g/m^3$:

$$\text{Concentration } (\mu g/m^3) = \frac{\text{weight of compound } (\mu g)}{\text{volume of air sampled } (m^3)}$$

11. References

11.1 Alliger, H., Am. Lab., 7 (10), 75 (1975).

11.2 Schulte, K. A., D. J. Larsen, R. W. Hornung, and J. V. Crable, "Report on Analytical Methods Used in a Coke Oven Effluent Study", National Institute for Occupational Safety and Health, Cincinnati, Ohio, May 1974.

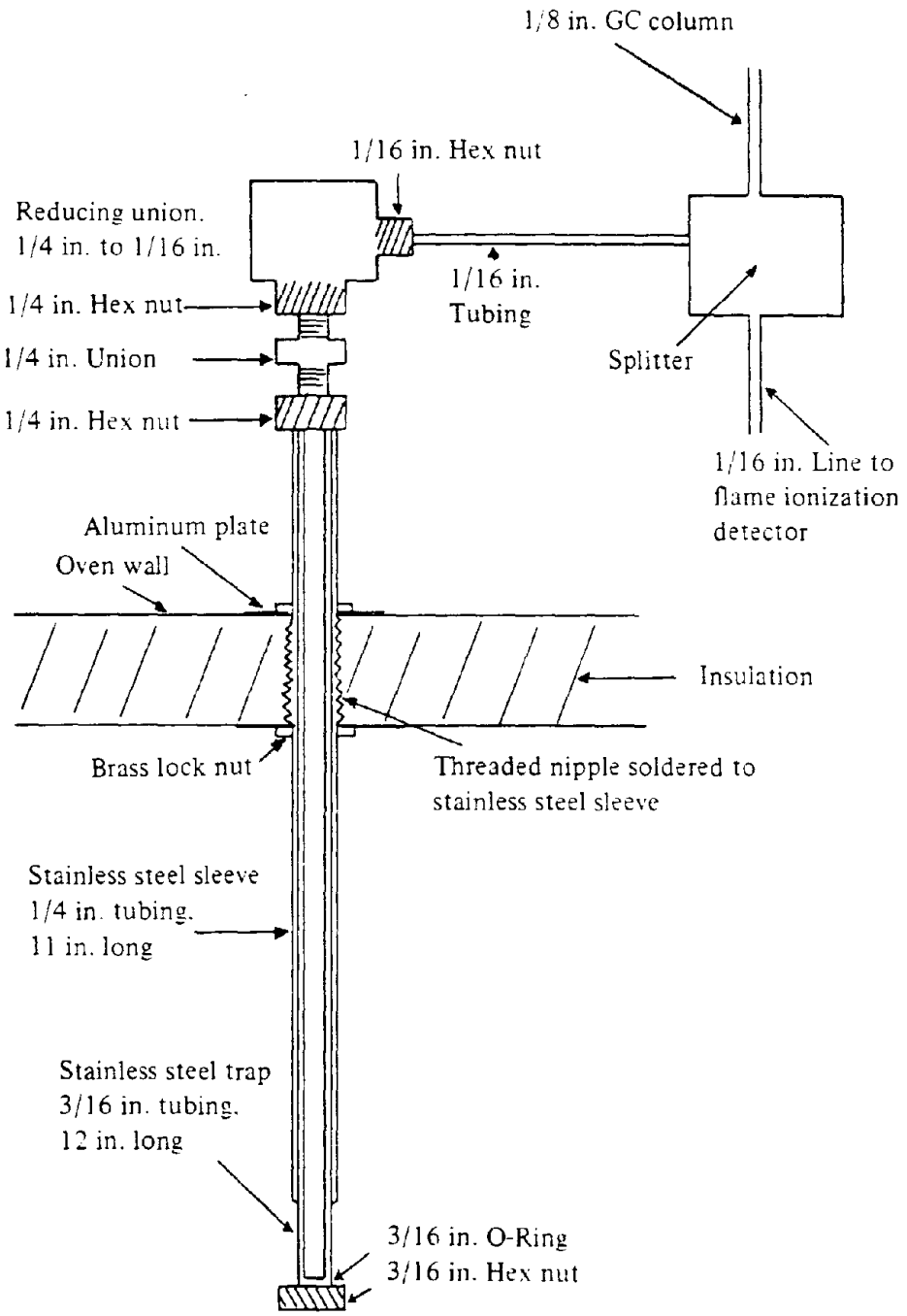


Figure 1. Trapping Assembly

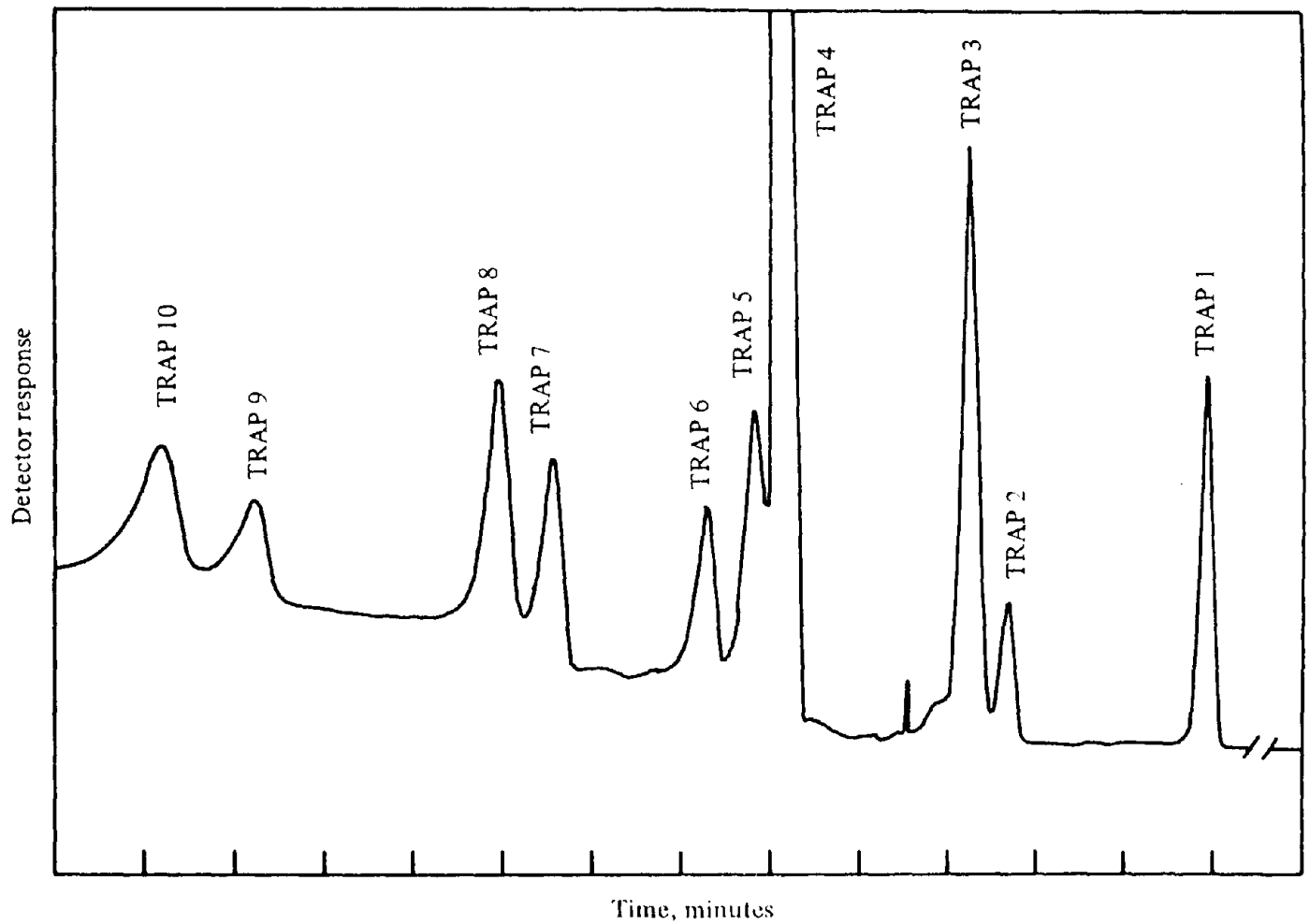


Figure 2. Chromatogram of Polynuclear Aromatic Synthetic Mixture. See Table for the Identity of the Compounds in the Various Traps.

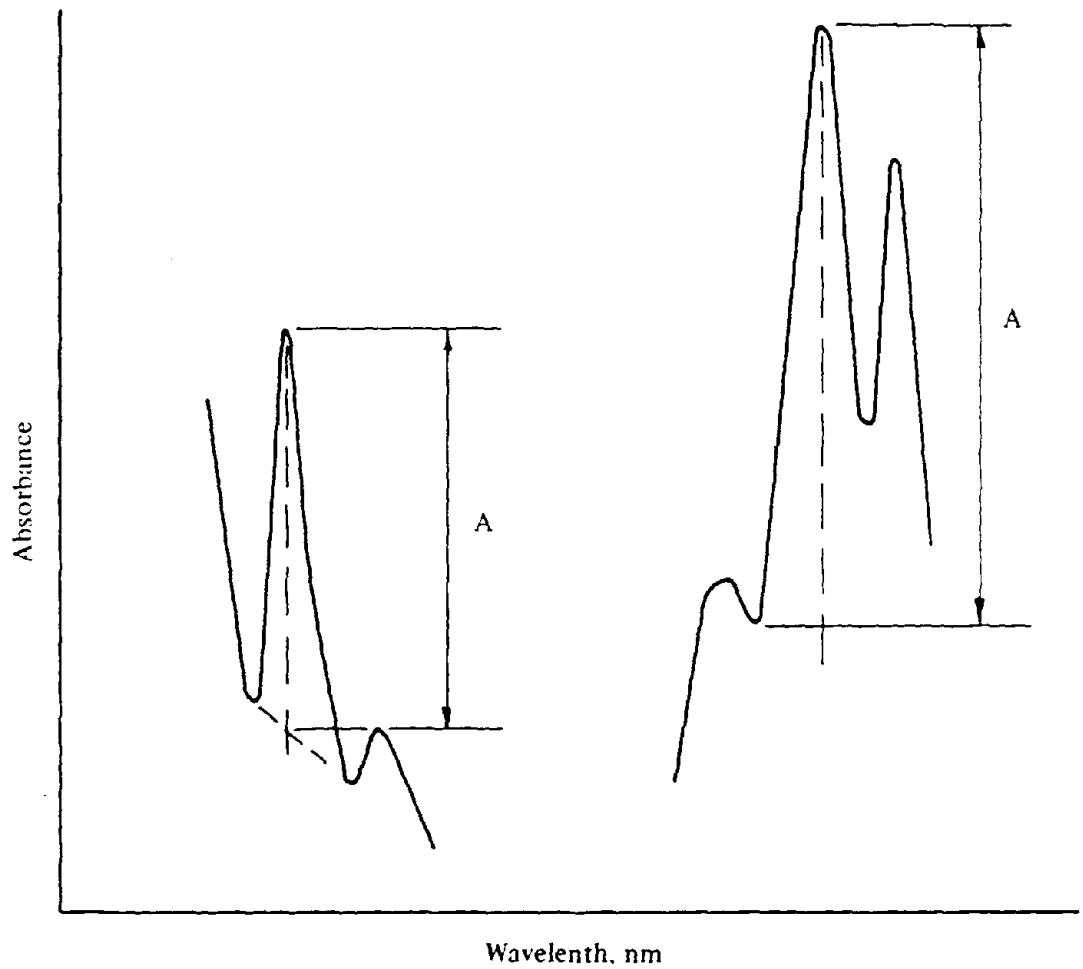


Figure 3. Measurement of Absorbance (A) by the Sloping Baseline and Horizontal Baseline Techniques

DATA FOR POLYNUCLEAR AROMATIC COMPOUNDS

Compound	Trap No.	Retention Time, Min.	Wavelength of Max. Absorb., nm	Baseline or Background Wavelength, nm	Detection Limit, μg/sample
Bibenzyl	-	5.0	-	-	-
o-Terphenyl	1	11.0	235	249	-
Fluoranthene	2	15.6	288	284, 292	0.7
Pyrene	3	17.1	336	327, 345	0.5
Benz(c)acridine	4	22.0	384	380, 392	2.3
Chrysene	5	23.0	267	260, 275	0.2
Benz(a)anthracene	5	23.0	289	297	0.3
Benz(a)anthrone	6	25.0	378	420	2.7
Benzo(b)fluoranthene	7	28.5	302	297	1.3
Benzo(j)fluoranthene	7	28.5	332	328, 337	1.0
Benzo(k)fluoranthene	7	28.5	402	397, 408	1.2
Benzo(a)pyrene	8	30.6	384	398	1.2
Benzo(e)pyrene	8	30.6	332	325, 338	0.8
Benz(a,h)anthracene	9	36.0	221	218, 228	1.3
Benzo(g,h,i)perylene	10	38.3	362	351, 372	2.7
Anthanthrene	10	38.3	430	442	0.5

POLYNUCLEAR AROMATIC HYDROCARBONS IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Polynuclear aromatic hydrocarbons (See Table on p. 184-13)	**Method No.:** P&CAM 184
Matrix: Air	**Range:** PAH content of 0 to 3 mg of benzene-soluble material
Procedure: Collection on a filter, extraction, column chromatography, spectrophotometric measurement	**Precision:** Not determined
Date Issued: 1/29/76	**Classification:** (D (Operational)
Date Revised:	

1. Principle of the Method

Polynuclear aromatic hydrocarbons are collected from air on a filter and extracted with benzene. The hydrocarbons are separated on an alumina column with *n*-pentane-diethyl ether mixtures as eluting solvents. The collected fractions are analyzed by a uv spectrophotometric method for nine hydrocarbons.

2. Range and Sensitivity

The range of the method depends upon the volume of air sampled, the amount of particulate matter collected, and the amount of organic material in the particulate matter. A normal sample size corresponds to 2 to 3 mg of benzene-soluble material. Larger amounts may be analyzed by diluting the sample solution and taking a small aliquot.

3. Interferences

Any substance that hinders the chromatographic separation or that absorbs radiation at the same wavelengths as the sample compounds may interfere.

4. Precision and Accuracy

The precision and accuracy of the method have not been determined. Precision for a similar procedure has been estimated to be ± 20% for samples taken at the same time and in the same vicinity. (See Reference 11.1.)

5. Advantages and Disadvantages of the Method

5.1 The method is sensitive and capable of measuring several polynuclear aromatic hydrocarbons in a single filter sample.

5.2 Careful attention to detail is required in performing the analytical procedure. Some practice and experience are necessary before an analyst can be expected to conduct a successful analysis with confidence.

5.3 The separation and quantification of the nine hydrocarbons is sensitive to the following factors: moisture content of the alumina; reproducibility of the packing of the columns; relative humidity of the laboratory air; quality of the solvents; interference from unknown compounds in the samples; photochemical effects; instrument performance; and contamination from extraneous sources.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Glass fiber filters, 37 mm, free of organic binder, with an efficiency of at least 99% as determined by the DOP penetration test.

6.1.2 Silver membrane filters, 37 mm, 0.8 μm pore size, Selas Corporation of America, No. FM- 37-(0.8), or equivalent.

6.1.3 Disposable plastic filter holder (cassette) for 37-mm filters, Millipore MOOO 037 PO, or equivalent. In order to accommodate the two filters, the cassette must be held together with plastic tape or a shrinkable cellulose band.

6.1.4 Personal sampling pump capable of operating for 4 hr at 2.0 ℓ/min or of sampling about 0.5 m^3 of air. The pump should be calibrated with a representative filter assembly in the line. A wet or dry test meter or a glass rotameter capable of measuring the flow rate to within ± 5% may be used for the calibration.

6.2 Ultrasonic Bath. Dynasonics Corporation Model NT-201, 90 kHz, 60 W, or equivalent. The use of an ultrasonic bath operating at a lower frequency and having a higher power output may improve extraction efficiency. (See Reference 11.2.) A test tube support is needed for use in the ultrasonic bath.

6.3 Recording Spectrophotometer. Cary Model 14, Varian Associates, or equivalent.

6.3.1 **Absorption Cells.** Suprasil, 10-mm pathlength, Catalog No. 14-385-904C, Fisher Scientific Company, or equivalent. Calibrate the cells – except for one reference cell – by adding 1.00 mℓ of water and marking the meniscus level. Make another calibration mark with 3.00 mℓ of water.

6.3.2 **Absorbance Screens.** Cary Part No. 1404109, Varian Associates, or equivalent. The set of screens has three absorbance values.

6.4 **Cuvette Washer.** Catalog No. 2735-304, Chemical Rubber Company, or equivalent.

6.5 **Column and Reservoir.** The chromatographic column is a 1.2- by 43-cm glass tube. A 500-mℓ round-bottom flask attached to the top of the column serves as a solvent reservoir. A stopcock with a 6-cm tip is attached to the bottom of the column. This assembly is illustrated in Figure 1.

6.6 Glassware

6.6.1 Beakers, graduated, 150 mℓ.

6.6.2 Pasteur pipettes, 5.75 and 9 in.

6.6.3 Cylinders, graduated to deliver, 10 mℓ.

6.6.4 Buchner funnels, 18.5 cm diameter, with a No. 9 rubber stopper.

6.6.5 Filtering flask, 200 mℓ.

6.6.6 Funnel, 60°, long stem.

6.6.7 Bottles, jug-form, with caps, 1 gal.

6.6.8 Bottles, wide mouth, 1 qt.

6.6.9 Bottles, 2 oz.

6.6.10 Test tubes, 100 by 16 mm, with Teflon-lined screw caps.

6.6.11 Allihn tubes, fine-porosity fritted glass, 30 mℓ.

6.6.12 Small glass vials with Teflon-lined screw caps.

6.6.13 Glass trays, 23 by 33cm.

6.6.14 Assorted other laboratory glassware of various sizes, including microsyringes, volumetric pipettes, graduated pipettes, graduated cylinders, volumetric flasks, and beakers.

6.7 Miscellaneous Items

6.7.1 Wood applicator sticks, 6 in. long and 1/16 in. diameter.

6.7.2 Weighing paper, 3.5 by 4.5 in.

6.7.3 Forceps

6.7.4 Glass wool (Pyrex or equivalent), washed with chloroform.

6.7.5 Freezer.

6.7.6 Pipette filler.

6.7.7 Plastic rod, Plexiglas or equivalent.

6.7.8 Rubber bulb, atomizer, attached by rubber tubing to a glass rod in a No. 6 rubber stopper.

6.7.9 Steel rod, 3 mm by 70 cm.

6.7.10 Rubber bulbs, 1- and 2-cm volume.

7. Reagents

7.1 Solvents. Chloroform and *n*-pentane, Spectranalyzed® grade, Fisher Scientific Company, or equivalent; benzene, chloroform, methanol, acetone, and diethyl ether (anhydrous), ACS reagent grade.

7.2 Hydrochloric acid, ACS reagent grade.

7.3 **Silica Gel.** The silica gel should be 100/200 mesh, Fisher Scientific Company, or equivalent. Prepare the silica gel by slurrying 200 to 500 g with anhydrous ether and filtering through filter paper in a Buchner funnel with suction. Repeat this washing four times. Allow the ether to evaporate overnight in a hood and then heat the silica gel for 2 hr at 130°C in an oven. Store in a tightly capped bottle.

7.4 **Alumina.** Any acid-washed 100/200-mesh alumina that yields satisfactory separations may be used.

7.5 **Elution Mixtures.** The elution mixtures are listed below in order of increasing polarity. The mixtures are prepared by mixing *n*-pentane and diethyl ether in the ratios indicated. To prepare the first mixture, transfer 970 mℓ of *n*-pentane to a 1000-mℓ graduated cylinder, add 30 mℓ of ether, and pour into a 1-gal. glass jug. Repeat to fill the jug. Label this mixture 3%. Prepare and label the other mixtures in like fashion.

n-Pentane, mℓ	Ether, mℓ	Label
1000	0	0%
940	60	6%
910	90	9%
880	120	12%
850	150	15%
820	180	18%
750	250	25%

7.6 **Polynuclear Aromatic Hydrocarbon Standards.** Each of the compounds listed in the table on page 184-13 should be checked for purity by gas chromatography and by comparison with infrared and ultraviolet reference spectra. Impure compounds should be purified by liquid chromatography. (See Reference 11.3.)

7.7 **Standard Solutions of Sample Compounds, 1 to 10 μg/ℓ.** Weigh exactly 10 mg of each of the nine compounds listed in the table into separate 30-mℓ beakers. Dissolve each compound in *n*-pentane and transfer quantitatively to separate 100-mℓ volumetric flasks. Fill to the mark with *n*-pentane. Transfer 1.0 mℓ of this solution to 100-, 50-, and 25-mℓ volumetric flasks, and 5.0 mℓ to a 50-mℓ flask. Dilute to volume with *n*-pentane. These solutions will contain, respectively, 1, 2, 4, and 10 μg/mℓ. Cover the flasks with aluminum foil.

7.8 **Mixed Standard, 50 μg/mℓ.** Weigh exactly 5 mg of each of the nine compounds into separate 30-mℓ beakers. Dissolve each compound in a small volume of chloroform (Spectranalyzed® grade) and transfer all of the solutions quantitatively to the same 100-mℓ volumetric flask. Dilute to volume with chloroform. Use this solution to determine the recovery factor for each compound as described in Section 9.3.

8. Procedure

8.1 **Cleaning of Equipment.** Clean all new glassware by rinsing first with dilute hydrochloric acid, then with distilled water, methanol, and *n*-pentane. Glassware that has been used in the analysis should be rinsed twice with chloroform, then with methanol and *n*-pentane.

8.2 Collection and Shipping of Samples

8.2.1 Assemble the filters in the filter holder so that the air being sampled passes first through a glass fiber filter and then through a silver membrane filter. Remove the small plugs from the filter holder and connect the filter holder to the sampling pump by means of an adapter and a length of tubing. Sample at least 0.5 m^3 of air at 2.0 ℓ/min. The optimum sample volume will depend on the type of workroom environment being sampled. Since high concentrations of particulate material or certain types of mists may plug the filters, the flow rate should be checked about once every hour. On completion of sampling, reinsert the small plugs into the inlet and outlet of the filter holder.

8.2.2 With each group of samples prepare a blank consisting of a filter holder with representative filters that have been handled in the same manner as the sample filters, except that no air is drawn through them. The samples and the blank should be shipped promptly in a light-proof container to prevent photooxidation or damage during transit.

8.3 Analysis of Samples

8.3.1 Extraction

1. Remove the top portion of the filter holder. Hold the bottom portion containing the filters over a piece of weighing paper to catch any particulate material that may fall out. Remove the small plug from the bottom portion of the filter holder and insert an applicator stick through the hole. Gently raise the filters and grasp the unexposed edge with tweezers. Fold the filters in half and then into quarters lengthwise. Insert the folded filters into a test tube and push them to the bottom with the applicator stick. Add to the test tube any particulate material remaining in the filter holder or collected on the weighing paper.

2. Pipette 5.0 mℓ of benzene into the test tube. This amount of solvent should completely cover the filters.

3. Place the test tubes in the test tube support in the ultrasonic bath so that the level of water in the bath is above the level of the solvent. Turn on the ultrasonic bath and sonicate the sample for 5 min.

4. Decant the solvent into an Allihn tube and collect the filtrate in a small vial. Force the solvent through the Allihn filter under positive nitrogen pressure. This may be accomplished by connecting the nitrogen cylinder to a glass "T," leaving one arm of the "T" open and connecting the other arm to a No. 4 one-hole rubber stopper, which is pressed against the top of the Allihn tube; pressure is controlled with a finger placed against the open arm of the "T." When the filtration has been completed, close the vial tightly with a Teflon-lined screw cap.

5. Insofar as possible, all subsequent operations should be carried out under yellow light.

8.3.2 Transfer a 3.0-mℓ aliquot of the benzene extract to a weighed 30-mℓ beaker and evaporate the solvent by gentle heating in a hood. Allow the beaker to cool to room temperature and record its weight to the nearest milligram. Calculate the number of milligrams of benzene-soluble material. Dissolve the residue in a known volume of chloroform (see note below) and transfer an appropriate aliquot to a 2-oz bottle containing 1.0 g of alumina (weighed under low humidity conditions and protected from atmospheric moisture). Allow the chloroform to evaporate in a hood. The alumina containing the adsorbed sample material is then ready for addition to the chromatography column.

Note: The volume of the chloroform in which the residue is dissolved and the size of the aliquot taken should be such that 2 to 3 mg of benzene-soluble material is transferred to the alumina. When the amount of benzene-soluble material is less than 3 mg, dissolve the residue in 5 mℓ of chloroform and transfer the entire sample to the alumina.

8.3.3 **Preparation of Columns**

1. Weigh into separate 2-oz bottles 9.0 g of alumina and 0.5 g of silica gel. The weighing should be done under low-humidity conditions, but not necessarily under dry-box conditions. Cover the bottles with aluminum foil and cap securely until the materials are used.

2. Attach the chromatography column securely to a ring stand at a convenient height to allow easy change of receiving bottles under the column. Tamp down a small amount of glass wool immediately above the stopcock. Pour the 9.0 g of alumina through a funnel into the column, keeping the stopcock open. Tap the funnel and column lightly so that all of the alumina falls into the lower portion of the column. Tamp down the alumina with the plastic rod.

3. Place a clean beaker under the column and add 130 mℓ of *n*-pentane to the column. Add the first 5 to 6 mℓ to the sides of the column with a dropper so that the alumina will not be disturbed. Using the atomizer bulb with stopper, apply pressure at the top of the column, forcing the solvent to flow through the alumina and driving out the entrapped air. Continue transferring *n*-pentane to the column and pressurizing until all air bubbles have been removed. Close the stopcock, leaving the *n*-pentane level 3 to 4 in. below the bottom of the reservoir.

4. With the funnel, add 0.5 g of silica gel slowly and evenly. Do not tap the column after the *n*-pentane has been added. With a medicine dropper filled with *n*-pentane, wash down any silica gel that has not fallen to the alumina. Carefully stir the silica gel (but not the alumina) with the steel rod until all air bubbles have risen.

5. Drain the *n*-pentane to a level about 1.5 in. above the silica gel. Adjust the volume of *n*-pentane in the beaker to 100 mℓ. Pour *n*-pentane from the beaker into the column until the solvent level is 3 to 4 in. below the reservoir.

6. With the aid of the funnel, add the 1.0 g of alumina containing the adsorbed sample. Add the alumina slowly and evenly so that no air is entrapped. Do not tap the column. Wash down any alumina adhering to the walls with *n*-pentane. Rinse the 2-oz sample bottle with *n*-pentane and transfer to the column. Rinse the funnel and remove it. Pour the remainder of the 100 mℓ of *n*-pentane from the beaker into the reservoir. Wrap the packed portion of the column with aluminum foil to protect the sample compounds from photooxidation.

8.3.4 **Elution of Sample Compounds.** As many as ten columns can be run at one time.

1. Separate the adsorbed compounds by eluting them from the column with successive additions of 100 to 150 mℓ of the elution mixtures (Section 7.5). Add the elution mixtures in order of increasing polarity (0 to 25% of ether). Add each elution mixture when the solvent level is about 2 in. above the packing. Add dropwise at first and then pour slowly so that the column packing is not disturbed.

2. Collect 15- to 20-mℓ fractions at the bottom of the column in numbered 2-oz bottles. About 45 to 50 fractions will be collected. Evaporate the solvent from the several collected fractions in a hood, cover the bottles, and store them in a freezer until ready for the next step.

3. The specific details of the elution process must be determined experimentally with a mixed standard prepared as described in Section 7.8. The details will depend upon the properties of the particular batch of alumina being used. The volumes of the elution mixtures and the number of elution mixtures needed to effect complete separation may vary from one batch of alumina to another. When the optimum conditions have been established for a given batch of alumina, they should be used with all columns packed with that alumina.

4. In a typical analysis, the nine compounds will be eluted in the order as listed in the table. Fluoranthene and benz(a)anthracene must be completely separated since their ultraviolet absorption peaks occur at the same wavelength, 287 nm.

8.3.5 Spectrophotometric Measurements

1. Remove a bottle containing a collected fraction from the freezer and allow it to come to room temperature. With a Pasteur pipette, a 1-mℓ rubber bulb, and *n*-pentane as solvent, quantitatively transfer the sample from the 2-oz bottle to a 1-cm absorption cell. Rinse the bottle several times with small amounts of *n*-pentane. Add *n*-pentane to the cell to bring the volume up to the 3-mℓ calibration mark and stir with the pipette.

2. Set the scan speed of the Cary 14 spectrophotometer at 10Å/sec and the slit control at 25. With *n*-pentane in the reference cell, record the spectrum of the sample solution over the wavelength range necessary to include the absorption peak for each compound present in the fraction. (See table). Using the baseline method as illustrated in Figure 2, measure the absorbances for each compound at the wavelengths indicated in the table.

3. If any absorption peak is off-scale, place one of the neutral density screens in the reference beam to lower both the baseline and the peak and record the spectrum again. If the peak is still off-scale, the solution must be diluted. Remove some of the solution from the cell with a pipette until the level is at the 1.0-mℓ mark. Add *n*-pentane to bring the level in the cell back to the 3.0-mℓ mark and mix. Record the spectrum of the diluted solution. If necessary, make additional dilutions and record the number (n) of dilution steps.

8.3.6 **Analysis of Combined Fractions.** After several column separations have been made and the absorption spectra have been recorded, various fractions found to contain the same compound may be combined in subsequent analyses. The number of spectra to be recorded will thereby be reduced and time will be saved. To combine fractions, dissolve the residues in the 2-oz bottles with *n*-pentane and transfer these solutions quantitatively to one bottle. Allow the solvent to evaporate and proceed with the analysis of the combined fractions as directed in Section 8.3.5.

9. **Calibration and Standards**

CAUTION: Benzo(a)pyrene and other aromatic hydrocarbons commonly associated with it are known carcinogens. Avoid skin contact with these compounds and their solutions. Perform the evaporations and other manipulations in a hood.

9.1 **Determination of Absorptivities of the Individual Compounds.** Transfer approximately 3 mℓ of the 1 μg/mℓ standard solution of pyrene (Section 7.7) to a 1-cm absorption cell. With *n*-pentane in the reference cell, record the spectrum as described in Section 8.3.5. Similarly, record the spectra of the other three pyrene standards and all of the standard solutions of the other eight compounds listed in the table. On each spectrum draw a baseline between the two wavelenths indicated in the table and subtract the absorbance value at the baseline from the absorbance value at the peak (see Figure 2).

Determine the absorptivity, a, at the wavelength of maximum absorbance for each compound by dividing absorbance by concentration for each standard solution and averaging the four values (the pathlength is 1 cm).

9.2 **Determination of Recovery Factors.** Transfer 0.5, 1.0, and 2.0 mℓ of the mixed standard solution (Section 7.8) to 2-oz bottles containing 1.0 g of alumina. Proceed with the analysis of these standards as directed in Section 8.3.2 and the subsequent sections. Analyze the standards on two or more columns. It is essential that all operating conditions be identical for the standards and for sample measurements. Calculate an average recovery factor, R_i, for each of the nine compounds by dividing the amount of the compound taken by the amount found.

10. **Calculations**

10.1 **Calculation When Separation is Complete.** When all of the compounds are completely separated on the column and all of each compound is completely contained in one fraction, calculate the amount of each compound in the sample by the following equation.

$$W = \left(\frac{A}{a} \times D\right) \times R \times \frac{V}{P} \times 1.67$$

where: W = amount (in μg) of compound on filter.

A = absorbance (Section 8.3.5).

a = absorptivity (Section 9.1).

D = volume (in mℓ) of *n*-pentane (normally 3.0 mℓ) in which the column fraction is dissolved for the absorbance measurement (Section 8.3.5). If this solution is diluted, $D = (3.0)^{n+1}$ where n = number of dilution steps.

R = recovery factor (Section 9.2).

V = total volume (in mℓ) of chloroform solution of sample (Section 8.3.2).

P = volume (in mℓ) of aliquot of chloroform solution added to alumina (Section 8.3.2).

1.67 = a correction for taking a 3.0-mℓ aliquot of the 5.0-mℓ benzene extract of the filter (Section 8.3.2).

If a compound is found in more than one fraction, the term $(\frac{A}{a} \times D)$ must be summed for all fractions in which that compound occurs.

10.2 **Calculation when Separation is Not Complete.** When the column separation is not complete and two or more compounds are found in the same fraction, the calculation becomes more complex. Under these circumstances, the absorptivity must be determined for each interfering compound at the wavelength of maximum absorbance of each other compound with which it interferes. If such interference is extensive, the result is a set of several simultaneous linear equations. These equations can best be solved by a suitably designed computer program.

11. References

11.1 "Tentative Method of Analysis for Polynuclear Aromatic Hydrocarbon Content of Atmospheric Particulate Matter," **Health Lab. Sci.**, 7 (No. 1), 31 (1970).

11.2 Alliger, H., **Am. Lab.** 7 (10), 75 (1975).

11.3 Schulte, K. A., D. J. Larsen, R. W. Hornung, and J. V. Crable, "Report on Analytical Methods Used in a Coke Oven Effluent Study," National Institute for Occupational Safety and Health, Cincinnati, Ohio, May 1974.

DATA FOR POLYNUCLEAR AROMATIC COMPOUNDS

Compound	Elution Order	Wavelength of Max. Absorb., nm	Baseline or Background Wavelength, nm
Pyrene	1	334	330, 340
Fluoranthene	2	287	284, 290
Benz(a)anthracene	3	287	282, 290
Chrysene	4	267	262, 270
Benzo(e)pyrene	5	331	328, 335
Benzo(a)pyrene	6	382	375, 390
Perylene	7	434	430, 440
Benzo(k)fluoranthene	8	401	395, 405
Anthanthrene	9	420	418, 423

BENZO (A) PYRENE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Benzo(a)pyrene	**Method No. :** P&CAM 186
Matrix: Air	**Range:** 0.02 to 0.8 $\mu g/m^3$ in a 0.5 m^3 sample of air
Procedure: Collection on a membrane filter; thin layer chromatographic separation; fluorometry	**Precision (CV):** 0.07 (analytical) over the above range
Date Issued: 1/29/76	**Classification:** D (Operational)
Date Revised:	

1. **Principle of the Method**

Benzo(a)pyrene (BaP), together with other aromatic hydrocarbons in the particulate material collected on a filter, is extracted with benzene. The BaP in the extract is separated from other components by thin layer chromatography (TLC). The portion of the TLC sheet containing the BaP is transferred to a fritted funnel, extracted with a solvent mixture, and the solvent is removed by evaporation. The residue is dissolved in sulfuric acid and the amount of BaP is measured fluorometrically (References 11.1 and 11.2)

2. **Range and Sensitivity**

The fluorometric response is linear over the range from 10 to 400 ng of BaP. The amount of BaP must be adjusted to this range by taking a sample or an aliquot of appropriate size. For a sample volume of 0.5 m^3, the linear range of response corresponds to a BaP concentration of 0.02 to 0.8 $\mu g/m^3$ in air. The limit of detection is about 4 ng of BaP. (Reference 11.1)

3. **Interferences**

The method is highly selective for BaP, but is sensitive to variations in technique. Interference is minimized by the use of highly purified reagents and by taking care to avoid contamination from extraneous sources during the analysis.

4. Precision and Accuracy

4.1 The coefficient of variation of the analytical procedure is estimated to be 0.07 in the range of 10 to 400 ng of BaP.

4.2 The accuracy of the method is affected by several variables such as the relative humidity of the laboratory, light-induced oxidation, uniformity of the coating on the TLC plates, the technique of spotting the plates, and other factors. Errors caused by these factors are minimized by carrying two known BaP standards through the complete procedure along with every group of six samples.

5. Advantages and Disadvantages

5.1 The TLC technique effectively separates BaP from other polynuclear aromatic compounds in complex particulate samples.

5.2 It is possible to make six BaP determinations in one workday, after the extractions have been completed.

5.3 Careful attention to detail is required in performing the analytical procedure. Some practice and experience are necessary before an analyst can be expected to conduct a successful analysis with confidence.

5.4 Because of the sensitivity of the polynuclear aromatic hydrocarbons to photooxidation, it is necessary to conduct most of the operations under yellow illumination and to minimize exposure of the samples to atmospheric oxygen.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Glass fiber filters, 37 mm, free of organic binder, with an efficiency of at least 99% as determined by the DOP penetration test.

6.1.2 Silver membrane filters, 37 mm, 0.8 μm pore size, Selas Corporation of America, No. FM-37-(0.8), or equivalent.

6.1.3 Disposable plastic filter holder (cassette) for 37-mm filters, Millipore MOOO 037 PO, or equivalent. In order to accommodate the two filters, the cassette must be held together with a plastic tape or a shrinkable cellulose band.

6.1.4 Personal sampling pump capable of operating for 4 hr at 2.0 ℓ/min, or of sampling about 0.5 m^3 of air. The pump should be calibrated with a representative filter assembly in the line. A wet or dry test meter or a glass rotameter capable of measuring the flow rate to within ± 5% may be used for the calibration.

6.2 **Ultrasonic Bath.** Dynasonics Corporation Model NT-201, 90 kHz, 60 W, or equivalent. An ultrasonic bath operating at a lower frequency and having a higher power output may improve extraction efficiency. (Reference 11.3.) A test tube support is needed for use in the ultrasonic bath.

6.3 **Thin Layer Chromatography Apparatus.** The items listed are supplied by Brinkmann Instruments, Inc. Equivalent apparatus may be used.

6.3.1 Coated aluminum foil sheets, 20 mm by 20 mm, coated with a 20-mm layer of alumina, without fluorescent indicator, Catalog No. ALUFOIL-FE22. Before use, the sheets are dried in a rack in an oven at 105°C for 30 min, cooled to room temperature, and stored in the humidity chamber. This treatment should be repeated if the sheets are not used within 10 days.

6.3.2 Drying rack, Catalog No. 0410100-6.

6.3.3 Drying oven, Catalog No. 6010010-1.

6.3.4 Marking template, horizontally graduated at 1-cm intervals, Catalog No. 2509200-7.

6.3.5 Humidity chamber, Catalog No. 0411500-7. The relative humidity is controlled at 45% at 25°C by placing in the bottom of the chamber a flat dish containing 100 mℓ of a saturated solution of $K_2CO_3 \cdot 2H_2O$.

6.3.6 Developing tank, Catalog No. 2510250-9. As an aid in attaining vapor phase equilibrium in the tank, a 20- by 20-cm sheet of stiff filter paper or a new ALUFOIL sheet should be placed inside the tank in the slot nearest the back wall.

6.3.7 Viewing cabinet, Catalog No. 0411100-1, with an ultraviolet lamp capable of locating a blue fluorescent spot containing 50 ng of BaP.

6.4 **Electric Hot Plate.** Fisher Scientific Company, Catalog No. 11-495-20, or equivalent. The hot plate should be a fast response type, with stepless control from 20 to 100°C, and should have a surface of about 1 ft^2. Asbestos sheets may be used on the surface of the hot plate to obtain an evaporation rate appropriate to the boiling range of the various solvents used in the procedure.

6.5 **Extraction Funnels.** Fisher Scientific Company, Catalog No. 10-358E, or equivalent. These funnels are 30-mm diameter Buchner type fritted glass funnels, medium porosity, with 75-mm stems. Nine are required. The funnels are supported on a 50- by 10- by 0.5-cm rack made from a strip of rigid plastic with nine 1.5-cm holes at convenient intervals. The plastic strip is supported at each end by clamps and ring stands. The funnel positions on the rack should be numbered 1 through 9.

6.6 **Glass Washing Bottles.** Fisher Scientific Company, Catalog No. 3-406A, or equivalent, 500 mℓ, modified as shown in Figure 1.

6.7 **Spectrophotofluorometer.** American Instrument Company, Model Ratio SPF, No. 4-8911, or equivalent, provided with matched 10-mm fluorescence cells.

6.8 **Glassware**

6.8.1 Test tubes, 100 mm by 16 mm, with Teflon-lined screw caps.

6.8.2 Allihn tubes, fine-porosity fritted glass, 30 mℓ.

6.8.3 Small glass-vials with Teflon-lined screw caps.

6.8.4 Lang Levy micropipettes, 5 and 10 µℓ, Fisher Scientific Company, Catalog No. 21-157-18, or equivalent.

6.8.5 Micropipettes, 5, 50, 100, and 200 µℓ.

6.8.6 Graduated Mohr pipettes, 2 and 5 mℓ.

6.8.7 Volumetric transfer pipettes, 1, 2, 5, 10, 25, and 30 mℓ.

6.8.8 Volumetric flasks, 100 mℓ.

6.8.9 Graduated cylinders, 10 and 100 mℓ.

6.8.10 Funnel, short stem, 50-mm diameter.

6.8.11 Beakers, 30 mℓ and 100 mℓ.

6.8.12 Bottle, glass-stoppered, 100 mℓ.

6.9 **Miscellaneous Apparatus**

6.9.1 Wood applicator sticks, 6 in. long and 1/16 in. diameter.

6.9.2 Weighing paper, 3.5 by 4.5 in.

6.9.3 Plastic washing bottles, 500 mℓ.

6.9.4 Metal shears, high quality, 6 in.

6.9.5 Forceps, 5 in.

6.9.6 Refrigerator.

6.9.7 Desk lamp, swing-arm type, 50 W, with yellow bulbs or a yellow light filter.

6.9.8 Analytical balance, single-pan type, 0.05 mg sensitivity.

7. Reagents

7.1 Solvents. Benzene, pentane, methanol, chloroform, methylene chloride, and cyclohexane, Fisher Scientific Company Spectranalyzed® grade, or equivalent.

7.2 Extraction Solvent Mixture.

7.2.1 Mix 300 mℓ of pentane, 30 mℓ of methanol, and 100 mℓ of methylene chloride in the glass washing bottle (Section 6.6) and use as directed in Section 8.3.11.

7.2.2 The quality of the extraction solvent mixture must be determined before it is used. Transfer 10 µℓ of BaP standard solution "B" (containing 200 ng of BaP) and 60 mℓ of the solvent mixture to a 100-mℓ beaker. Place the beaker on a hot plate set at 30°C and evaporate the solution to about 1 mℓ. Remove the beaker from the hot plate and allow the remaining solvent to evaporate while the beaker is cooling. Do not evaporate to dryness on the hot plate. Dissolve the residue in sulfuric acid and measure the fluorescence as described in Section 8.3.13. Compare with the fluorescence of 200 ng of BaP without the added extraction solvent mixture. The difference should not be greater than 10%. If the difference is greater than 10%, solvents of higher quality must be obtained.

7.3 Sulfuric Acid, ACS Reagent Grade.

7.3.1 Reserve a stock bottle of sulfuric acid exclusively for this analysis. Since oxygen dissolved in the sulfuric acid will retard the rate of formation of the fluorescent species derived from reaction with BaP, it is necessary to treat the acid before use in the analysis. Twenty minutes before use, remove the dissolved oxygen by passing nitrogen through 25 mℓ of acid in a 100-mℓ glass bottle.

7.3.2 To insure that oxygen is completely removed, measure the fluorescence intensity of a solution of 200 ng of BaP (10 µℓ of BaP standard solution "B") in 1.6 mℓ of acid as described in Section 8.3.13. Remove the cell from the fluorometer and place it in a dark location. After 20 min, again measure the fluorescence intensity. An increased value indicates that the oxygen has not been completely removed. The fluorescence should decrease slightly during the 20-min period. Once the conditions necessary for complete removal of oxygen have been established, this test need not be repeated.

7.4 **Nitric acid, ACS Reagent Grade.**

7.5 **Benzo(a)pyrene (BaP), 99% Purity.** If the quality of the BaP is unknown, it should be purified by column chromatography, or by thick layer chromatography on cellulose acetate with a mixture of ethanol, toluene, and water (18:4:4) as the developer. (See Reference 11.4)

7.6 **BaP Standard Solution "A", 200 ng/μℓ.** Dissolve 20 mg of BaP in 20 mℓ of benzene in a 100-mℓ volumetric flask, fill to the mark with benzene, and mix. Store in a cool dark place.

7.7 **BaP Standard Solution "B", 20 ng/μℓ** Transfer 10 mℓ of BaP standard solution "A" to a 100-mℓ volumetric flask, dilute to the mark with benzene, and mix. Store in a cool dark place.

7.8 **Nitrogen Gas, prepurified.**

8. **Procedure**

8.1 **Cleaning of Equipment.**

8.1.1 Reserve a complete set of glassware to be used only for this analysis. Work on a scrupulously clean surface in a laboratory that is free of dust, smoke, and acid fumes; such atmospheric contaminants may cause high or erratic results.

8.1.2 Clean all glassware with warm nitric acid. Rinse several times with distilled water and finally with methanol. Immediately after their initial use in the analysis procedure, rinse pipettes and other glassware with benzene and then with methanol; remove the solvent with clean dry air. Check all glassware for the presence of interfering fluorescent material with a uv lamp.

8.2 **Collection and Shipping of Samples**

8.2.1 Assemble the filters in the filter holder so that the air being sampled passes first through a glass fiber filter and then through a silver membrane filter. Remove the small plugs from the filter holder and connect the filter holder to the sampling pump by means of an adapter and a length of tubing. Sample at least 0.5 m^3 of air at 2.0 ℓ/min. The optimum sample volume will depend on the type of workroom environment being sampled. Since high concentrations of particulate material or certain types of mists may plug the filters, the flow rate should be checked about once every hour. On completion of sampling, reinsert the small plugs into the inlet and outlet of the filter holder.

8.2.2 With each group of samples prepare a blank consisting of a filter holder with representative filters that have been handled in the same manner as the sample filters, exept that no air is drawn through them. The samples and the blank should be shipped promptly in a light-proof container to prevent photooxidation or damage during transit.

8.3 Analysis of Samples

8.3.1 Extraction

1. Remove the top portion of the filter holder. Hold the bottom portion containing the filters over a piece of weighing paper to catch any particulate material that may fall out. Remove the small plug from the bottom portion of the filter holder and insert an applicator stick through the hole. Gently raise the filters and grasp the unexposed edge with tweezers. Fold the filters in half and then into quarters lengthwise. Insert the folded filters into a test tube and push them to the bottom with the applicatorstick.Add to the test tube any particulate material remaining in the filter holder or collected on the weighing paper.

2. Pipette 5.0 mℓ of benzene into the test tube. This amount of solvent should completely cover the filters.

3. Place the test tubes in the test tube support in in the ultrasonic bath so that the level of water in the bath is above the level of the solvent. Turn on the ultrasonic bath and sonicate the sample for 5 min.

4. Decant the solvent into an Allihn tube and collect the filtrate in a small vial. Force the solvent through the Allihn filter under positive nitrogen pressure. This may be accomplished by connecting the nitrogen cylinder to a glass "T," leaving one arm of the "T" open and connecting the other arm to a No. 4 one-hole rubber stopper, which is pressed against the top of the Allihn tube; pressure is controlled with a finger placed against the open arm of the "T." When the filtration has been completed, close the vial tightly with a Teflon-lined screw cap.

5. All subsequent operations up to the point of the fluorescence measurement should be conducted under yellow light.

8.3.2 **Preparation of Sample Solution.** Transfer 3.0 mℓ of the benzene extract quantitatively to a 30-mℓ beaker and evaporate to about 0.3 mℓ. Remove the beaker from the hot plate and allow the remaining solvent to evaporate while the beaker cools. Do not evapofate to dryness on the hot plate. After the beaker has cooled, add 50 μℓ of benzene with a micro-pipette and apply a gently rotary motion to the beaker to aid in dissolution of the residue. An aliquot of this solution is then applied immediately to the TLC sheet as described in Section 8.3.5.

8.3.3 **Preliminary Preparation of TLC Sheets.** Figure 2 is an illustration of a developed TLC sheet. Before spotting samples on the TLC sheet, mark the sheet with the marking template in the following manner. Place a pencil dot at 2-cm intervals on a line 1.5 cm above the bottom of the sheet. There will be nine dots; six for samples, two for BaP standards, and one for a blank. Place marks on the left and right sides of the sheet, 10 cm from the starting line, to indicate the maximum advance of the solvent front after development.

8.3.4 **Preparation of the Developing Tank**

1. At least 1 hr before spotting the TLC sheets, add 95 mℓ of pentane, 5.0 mℓ of chloroform, and 2.0 mℓ of methylene chloride to the developing tank. Quickly replace the cover, mix the solvents by swirling, and allow an hour for the vapor phase to reach equilibrium. The sheet of filter paper or alumina-coated foil in the back of the tank (Section 8.3.6) should be immersed in the solvent to a depth not greater than 1 cm. Empty the tank and add freshly prepared solvent mixture before each TLC separation.

2. It is essential that the relative humidity in the laboratory be in the range of 30 to 50%—ideally at 43%—for reproducible TLC separations. If the relative humidity is below 30%, the ratio of chloroform to pentane should be increased; if the relative humidity is above 50%, the ratio should be decreased. The appropriate ratio can be determined experimentally with standard BaP solution on a prepared TLC sheet.

8.3.5 **Spotting of the TLC Sheets.** With the aid of a small rubber bulb, fill a 10-μℓ Lang Levy pipette with a BaP standard solution "B" (20 ng/μℓ) to a level above the constriction, which is the index. Adjust the level of the solution to the index by touching the tip with lint-free tissue. Transfer the solution to Spot No. 1 on the TLC sheet by repeated, quick contact between the tip of the pipette and the sheet. Control the diameter of the wetted spot to 5 mm and avoid chipping the alumina coating. Similarly, apply another 10-μℓ aliquot of BaP standard solution "B" to Spot No. 9. Then, in immediate succession, apply 10-μℓ aliquots of sample solutions (include a filter blank) to Spots 2, 3, 4, 6, 7, and 8, using a fresh, clean Lang Levy pipette for each sample. To Spot No. 5 apply 10 μℓ of benzene; this spot represents the TLC blank.

8.3.6 **Development of the TLC Sheet.** Place the loaded TLC sheet vertically in a slot of the developing tank. The sheet should not be immersed in the solvent, to a depth greater than 1 cm. When the solvent front has migrated to the 10-cm mark (about 20 min), remove the developed sheet and place it in the viewing cabinet.

8.3.7 **Location of the BaP Spots on the TLC Sheet.** Use a clean 20- by 20-cm glass plate as a supporting platform for the developed sheet. Orient the TLC sheet on the viewer with the original location of the spots closest to the analyst. Find the standard BaP spots (No. 1 and No. 9) by their blue fluorescence. The fluorescense is more readily discernible if the room lights are off. As quickly as possible, place a pencil mark about 1 mm above the limit of each of the visible standard spots. Place another pencil mark about 2 mm below each of the standard spots; the lower mark will include the tail of the BaP spot. Immediately remove the sheet from the viewer to minimize exposure of the spots to the ultraviolet light.

8.3.8 **Evaluation of the Quality of the TLC Separation**

1. With the aid of a ruler gently draw two pencil lines horizontally, connecting the marks that locate the two BaP standards. The space between these lines will include the BaP in the samples as well as the standards. (See Figure 2.) If the distance between the parallel lines is more than 1.5 cm, or if the standards have not migrated at least 2 cm from the starting line, the quality of the TLC separation is not acceptable. Under these circumstances, discard the sheet and carry out another TLC separation; otherwise, proceed to Section 8.3.10.

2. An unsatisfactory TLC separation may be caused by a defective sheet, improper spotting technique, or a relative humidity lower than 30%. If the relative humidity is found to be less than 30%, it may be necessary to change the ratio of chloroform to pentane in the developing solvent mixture. (See Section 8.3.4.)

8.3.9 **Corrective Step.** If the first TLC separation is unsatisfactory, evaporate any benzene remaining in the 30-mℓ beaker (Section 8.3.2), but do not overheat. After the beaker has cooled, add another 50 μℓ of benzene, dissolve the residue, and repeat the TLC separation with greater care. If this corrective step is performed, it will be necessary in the subsequent calculations to take into account the difference in the amount of sample in the new aliquot applied to the TLC sheet.

8.3.10 Isolation of the Individual Spots on the TLC Sheet

1. Place the marking template above the upper horizontal pencil line that was drawn to locate the BaP spots (Section 8.3.8). Place the pencil mark 1 cm from the left edge of the sheet and additional marks at 2-cm intervals across the street. These marks will define the several BaP spots as 1.5- by 2-cm rectangles. (See Figure 2.) Place an "R" in the 1.5- by 1-cm space at the right edge of the sheet as a reference mark to identify the sequential location of the spots.

2. Cut along the upper horizontal pencil line with sharp metal shears while grasping the sheet near the lower edge. Avoid distortion or twisting of the sheet during the cutting; preliminary practice with a discarded sheet is recommended. Start again at the left edge of the sheet and cut along the lower horizontal line. As the cutting proceeds, allow the strip to come to rest on the glass plate. Do not touch the strip with the fingers. Grasp the strip at the point marked "R" with forceps. Cut off and discard the 1.5- by 1-cm piece at the other end of the strip. Make a second cut at the first 2-cm marked interval. This cut isolates Spot No. 1. Transfer this spot with forceps to the first extraction funnel (Section 6.5). Place the spot flat on the glass frit with the coating facing upward.

3. In sequence cut out the remaining eight spots and transfer them to the row of extraction funnels on the plastic rack, placing each spot in the correspondingly numbered position on the rack.

8.3.11 Extraction of BaP from the Spots

1. Locate the extraction funnel rack in an unlighted ventilated hood and place clean 100-mℓ beakers, marked 1 through 9, under the stems of the correspondingly numbered funnels. Add about 10 mℓ of extraction solvent mixture (Section 7.2.1) to each of the funnels from an all-glass washing bottle. Dispense the solvent mixture from the washing bottle by connecting the inlet tube to a low-pressure source of nitrogen and controlling the flow of nitrogen with a finger placed over the 2-mm hole in the inlet tube. (See Figure 1.) After 5 mℓ of solution has been collected in each beaker, continue adding the extraction solvent to the funnels in 5-mℓ increments until a total of 60 mℓ has been collected in each beaker.

2. Place the beakers on a hot plate set at 30°C and evaporate the solvent to a volume of about 1 mℓ. Remove the beakers and allow the remaining solvent to evaporate while the beakers are cooling. Cover the beakers with aluminum foil to reduce exposure to light and dust. Proceed with the fluorescence measurement as soon as possible. (Section 8.3.13).

8.3.12 **Adjustment of Instrument Parameters for Fluorescence Measurements**

1. First, determine the optimum excitation and emission wavelengths for BaP. Transfer 1.0 mℓ of BaP standard Solution "B" (20 ng/μℓ) to a 100-mℓ beaker. Allow the solvent to evaporate in a hood. Add 10 mℓ of sulfuric acid and stir. Fill A fluorescence cell at least half-full with this solution. With spectrophotofluorometer conditions established according to the manufacturer's instructions, open the shutter, turn the HV control to ratio, and set the excitation wavelength to approximately 520 nm and the emission wavelength to approximately 540 nm. Rotate the wavelength dials to obtain the maximum meter reading. Do not change the wavelength settings after the positions for maximum fluorescence have been established.

2. Discard the contents of the cell, rinse several times with water, and finally rinse with methanol from a washing bottle. Dry the cell with a jet of nitrogen while holding it firmly with lint-free tissue. Repeat this cell cleaning procedure each time a cell containing BaP is emptied.

3. Return the HV control to manual and close the shutter. Adjust the intensity meter to zero with the zero adjust vernier. Fill a clean cell with sulfuric acid, place it in the instrument, open the shutter, turn the HV control to ratio, set the sensitivity switch (meter multiplier) to 0.1, the sensitivity vernier to zero, and use the blank subtract vernier to adjust the intensity meter to zero.

8.3.13 **Measurement of Fluorescence of Samples.** With a 2-mℓ Mohr pipette, transfer 1.6 mℓ of oxygen-free sulfuric acid to Beaker No.1, which contains the extract from Spot No. 1 on the TLC sheet (Section 8.3.11). Carefully tilt and rotate the beaker until the acid has wetted the wall to within 1 cm of the rim. Hold the beaker upright, gently swirl the contents for 30 sec, and then pour into a clean 10-mm cell. With pure sulfuric acid in a reference cell, repeat the blank subtract adjustment described in Section 8.3.12. Remove the reference cell from the cell compartment and replace it with the cell containing the sample from Beaker No. 1. Open the shutter, set the mode switch at ratio, change the sensitivity switch to obtain a near mid-scale reading, and record the meter reading and the position of the sensitivity switch (meter multiplier). Return the mode switch to manual and close the shutter. Repeat these measurements with the remaining samples from Beakers 2 through 9.

9. **Calibration and Standards**

CAUTION: Benzo(a)pyrene and other aromatic hydrocarbons commonly associated with it are known carcinogens. Avoid skin contact with these compounds and their solutions. Perform the evaporations and other manipulations in a hood.

Calculate a calibration factor. F. for each set of samples and standards run as described in Section 8.3. The calibration factor is determined from the following equation:

$$F = \frac{A}{(B \times C) - (L \times M)}$$

where: F = calibration factor in ng of BaP per fluorescence intensity unit.

A = ng of BaP standard spotted on the TLC sheet (normally 200 ng).

B = fluorescence intensity (meter reading) obtained by averaging the values found for Spot No. 1 and Spot No. 9.

C = meter multiplier used to obtain the fluorescence intensity for Spot No. 1 and Spot No. 9.

L = fluorescence intensity (meter reading) for the TLC blank. Spot No. 5.

M = meter multiplier used to obtain the fluorescence intensity for Spot No. 5.

10. Calculations

10.1 **BaP Content of Filter Blank.** Calculate the BaP content of the unexposed filter by the following equation:

$$W_b = 1.67\left(\frac{50}{40}\right)^n (5F)\,[\,(D \times E) - (L \times M)\,]$$

where: W_b = ng of BaP in the blank obtained by extraction of an unexposed filter.

1.67 = a correction for taking a 3.0-mℓ aliquot of the 5.0-mℓ benzene extract of the filter

n = number of times the corrective step (Section 8.3.9) is applied. If the corrective step is omitted, n = 0 and the term $\left(\frac{50}{40}\right)^n = 1$.

F = calibration factor. The factor of 5 is the ratio between the volume (50 μℓ) of the concentrated extract (Section 8.3.2) and the volume of the aliquot (10 μℓ) applied to the TLC sheet (Section 8.3.5).

D = fluorescence intensity (meter reading) obtained for the filter blank.

E = meter multiplier used to obtain the fluorescence intensity for the filter blank.

L = fluorescence intensity (meter reading) for the TLC blank (Spot No. 5).

M = meter multiplier used to obtain the fluorescence intensity for Spot No. 5.

10.2 **BaP Content of Samples.** Calculate the BaP content of the samples by the following equation:

$$W_S = 1.67\left(\frac{50}{40}\right)^n (5F)\,[\,(G \times H) - (L \times M)\,] - W_b$$

where: W_S = ng of BaP in sample.

G = fluorescence intensity (meter reading) obtained for the sample.

H = meter multiplier used to obtain the fluorescence intensity for the sample.

The other terms are as defined in Section 10.1.

10.3 **Concentration of BaP in Air.** To obtain the concentration of BaP in air, divide the weight (W_S) of BaP found in the filter sample by the volume of air sampled. The concentration is usually expressed in $\mu g/m^3$.

11. **References**

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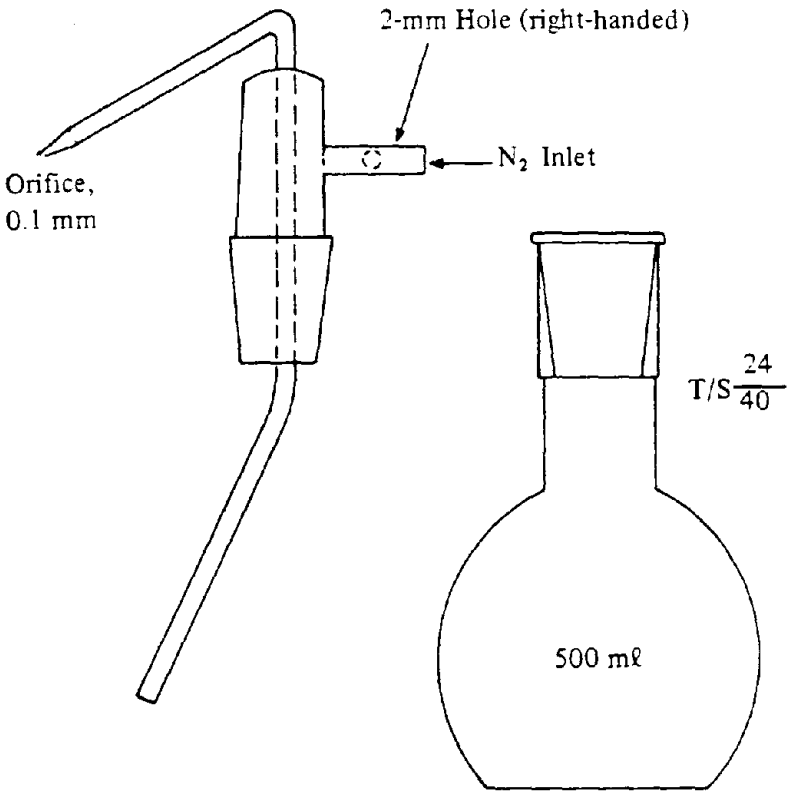


Figure 1. Glass Washing Bottle

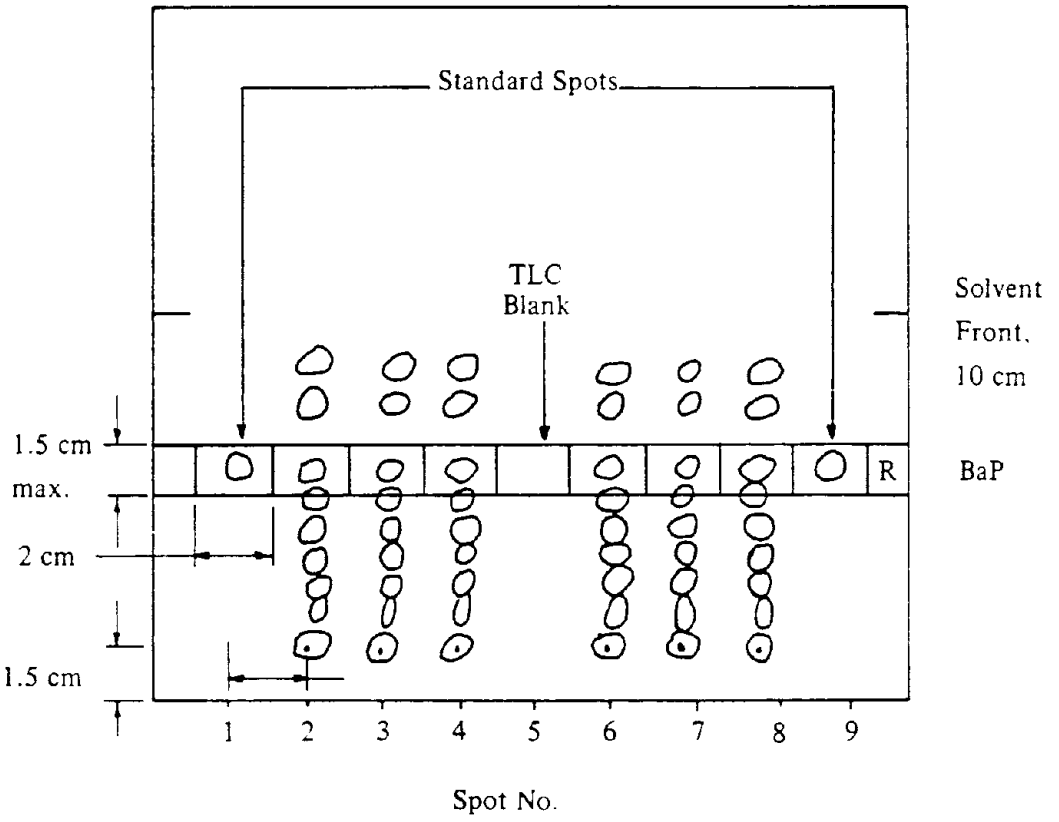


Figure 2. Developed TLC Sheet (20 cm by 20 cm)

SULFURIC ACID MIST IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Sulfuric acid	Method No.: P&CAM 187
Matrix: Air	Range: 0.1 to 4.0 mg/m^3 in a 100-liter sample of air
Procedure: Turbidimetry	Precision: (CV): 0.09 (analytical) at 2.6 mg/m^3
Date Issued: 3/1/74	
Date Revised:	Classification: E (Proposed)

1. Principle of the Method

Sulfuric acid mist in air is absorbed in 10 to 15 mℓ of water in a midget impinger. The sulfate in the sample solution is precipitated as barium sulfate and the turbidity of the suspension is measured at 420 nm with a spectrophotometer.

2. Range and Sensitivity

2.1 As little as 10 μg of sulfuric acid in 10 mℓ of impinger-bubbler sample solution can be determined. This lower limit of the range corresponds to a concentration of 0.1 mg/m^3 of sulfuric acid in a 100-liter sample of air.

2.2 When the amount of sulfuric acid in 10 mℓ of impinger-bubbler sample solution exceeds 0.4 mg, the suspension of barium sulfate may lose stability and the accuracy of the method may decrease. This upper limit of the range corresponds to a concentration of 4.0 mg/m^3 of sulfuric acid in a 100-liter sample of air. Larger concentrations of sulfuric acid may be determined by appropriate dilution of the impinger-bubbler sample solution.

3. Interferences

3.1 Colored or suspended matter in large concentrations will interfere. Filtration of the sample solution may remove some suspended matter. If the concentration of colored or suspended matter is small in comparison with that of sulfate, a correction may be made by subtracting the turbidity of the sample solution without added barium chloride from that of the barium sulfate suspension.

3.2 Silica in concentrations above 500 mg/ℓ in the sample solution will interfere.

3.3 Large concentrations of organic materials in the sample solution may prevent the satisfactory precipitation of barium sulfate.

3.4 Sulfate salts, if present as particulate material in the sample air, will be reported as sulfuric acid.

4. Precision and Accuracy

In the application of the turbidimetric method to water analysis, a coefficient of variation of 0.09 was found for a synthetic water sample containing a sulfate concentration of 0.26 mg/mℓ; when this synthetic water sample was appropriately diluted for analysis, the concentration of sulfate obtained corresponded approximately to that which would have been obtained from a 100-liter sample of air containing 2.6 mg/m^3 of H_2SO_4. The relative error was approximately 2%. (See Reference 11.2.) For the determination of sulfuric acid mist in air, where sampling errors may be significant, the accuracy and precision probably are poorer than for water analysis.

5. Advantages and Disadvantages

5.1 The method is simple to perform and relatively fast. It requires no elaborate preparation of reagents and no complex equipment other than a spectrophotometer.

5.2 The method is reasonably specific since no common anions other than sulfate form an insoluble barium salt in acid solution. The interferences discussed in Section 3 are not likely to be encountered in air in concentrations that would cause serious difficulty in the determination of sulfuric acid mist.

5.3 Conditions for the precipitation of barium sulfate must be carefully controlled to obtain good reproducibility.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 **Personal Sampling Pump.** A personal sampling pump capable of sampling accurately at 1.5 ℓ/min is required. The pump should be calibrated with a midget impinger containing 10 mℓ of water in the intake line. A wet or dry test meter or a glass rotameter capable of measuring the required flow rate to within ± 5% may be used in the calibration.

6.1.2 **Midget Impinger.** Glass midget impingers of a size to contain 25 mℓ of solution are required. Provision should be made for connection to the pump and for appropriate mounting during sampling.

6.2 **Sample Containers.** Bottles (25 mℓ) are needed for shipping samples to the laboratory. Alternatively, the samples may be shipped in the midget impingers, if suitable closures are available.

6.3 **Magnetic Stirrer.** The stirrer should preferably be equipped with a timing device. If it is not, a stopwatch may be used instead. The stirring speed should not vary appreciably.

6.4 Spectrophotometer with absorption cells, for use at 420 nm.

6.5 Volumetric flasks and pipettes.

7. Reagents

7.1 Distilled water for collection of samples.

7.2 SulfaVer IV powder, commercially available from Hach Chemical Company, or the equivalent. This reagent is supplied in a prepackaged "Powder Pillow" in an amount suitable for a single determination. (See Reference 11.1.)

7.3 **Standard Solution of Sulfate (0.1 mg/mℓ).** Dissolve 0.1479 g of ACS reagent grade anhydrous sodium sulfate in distilled water and dilute to 1 liter.

8. Procedure

8.1 **Cleaning of Equipment.** Wash all glassware with detergent solution and then rinse thoroughly with tap water and distilled water. After each use the absorption cells should be cleaned with detergent and a brush to prevent the accumulation of a deposit on the inside walls of the cell.

8.2 Collection and Shipping of Samples

8.2.1 Place approximately 10 mℓ of distilled water in a midget impinger and connect the impinger to the pump.

8.2.2 Pump air sample through the impinger at the rate of 1.5 ℓ/min for at least 60 min. Measure the flow rate and time as accurately as possible. If sulfuric acid concentrations greater that 4 mg/m^3 are expected, sample for a shorter length of time. Alternatively, the sample solution may be diluted in the laboratory.

8.2.3. One of every ten samples collected as directed above should be labeled as a blank. The absorbance of this sample will be measured without the addition of SulfaVer IV reagent to allow a correction to be made for color and suspended matter.

8.2.4 The samples should be packed for shipment to the laboratory in a manner that will prevent loss or damage in transit.

8.3 Analysis of Samples

8.3.1 Adjust the volume of the sample solution to 25 mℓ in a volumetric flask. Transfer the solution to a 100-mℓ Erlenmeyer flask and place the flask on the magnetic stirrer.

8.3.2 Add one package ("Powder Pillow") of SulfaVer IV reagent to the sample solution. The exact amount of SulfaVer IV powder added is not critical. However, the *same* amount must be added to all samples. The amount added must be in excess of the amount required to precipitate all of the sulfate as barium sulfate.

8.3.3 Start the magnetic stirrer immediately upon addition of the SulfaVer IV powder. Stir for exactly 1 min. The speed of stirring is not critical, but it should be constant for each set of samples and standards and should be adjusted to about the maximum rate at which no splashing occurs.

8.3.4 Immediately after the stirring has been stopped, fill an absorption cell and measure the turbidity at 420 nm at 30-sec intervals for 4 min. Record the maximum turbidity (or absorbance reading) observed during the 4-min period. The maximum turbidity usually occurs within 2 min and the readings remain constant thereafter for 3 to 10 min.

8.3.5 Dilute the "blank" sample to 25 mℓ and measure its turbidity (absorbance). Subtract this turbidity value from the sample values to correct for color and suspended matter.

9. Calibration and Standards

9.1 Pipette 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mℓ of the standard sulfate solution into 25-mℓ volumetric flasks and dilute to the mark.

9.2 Transfer the standards to 100-mℓ Erlenmeyer flasks and follow the procedure outlined in Section 8.3.

9.3 Check the calibration curve periodically by running a standard with every three or four samples.

10. Calculations

The concentration of sulfuric acid mist in air is calculated by the following equation:

$$mg/m^3 = \frac{mg\ H_2SO_4\ \text{in sample} \times 1000\ \ell/m^3}{\text{volume of sample in liters}}$$

78

11. References

11.1 *"Hach Water Analysis Handbook"*, Hach Chemical Company, Ames, Iowa, 1974.

11.2 *"Standard Methods for the Examination of Water and Wastewater"*, 13th Edition, American Public Health Association, Washington, D.C., 1971, pp. 334-335.

ARSENIC IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Arsenic	Method No: P&CAM 188
Matrix: Air	Range: 0.0005 to 0.005 mg/m^3 in 100 liters of air
Procedure: Collection on a membrane filter; anodic stripping voltammetry	Precision (CV_T): 0.08 over the above range
Date Issued: 10/5/73	Classification: E (Proposed)
Date Revised: 1/29/76	

1. Principle of the Method

Particulate material containing arsenic is collected from air on a membrane filter. The filter and trapped particulate material are wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The resulting As(V) is reduced to As(III) with an acidic solution of sodium bisulfite and ferrous perchlorate. An acid solution of hydrazine dihydrochloride is added to prevent air oxidation of Fe(II) ions. The chemically reduced sample is then analyzed by anodic stripping voltammetry (ASV) with a composite gold-graphite electrode (CGGE).

2. Range and Sensitivity

2.1 The sensitivity is limited by the reagent blank and the background characteristics of the electrode. A reasonable detection limit appears to be two times the standard deviation of the reagent blank. Statistically, the probability is 95% that arsenic is present in the sample if the signal is higher than this limit. The arsenic content of unused filters averages 44 ± 16 ng and the reagent blank averages 15 ± 8 ng. The detection limit is thus estimated to be 0.0005 mg/m^3 in a 100-liter sample of air.

2.2 The linear range of the electrode response extends from the detection limit to an upper limit of about 500 ng of arsenic, or 0.005 mg/m^3 in a 100-liter sample of air. This estimate allows for the amount of arsenic which may be found in an unused filter. The upper limit of the range of the analytical method may be extended to 0.250 mg/m^3 of arsenic in a 100-liter sample of air by analyzing a 100-μl aliquot of the digested filter solution.

3. Interferences

3.1 Antimony, bismuth, and tin will form resolvable shoulders on the cathodic edge of the arsenic peak. Mercury will form a shoulder on the anodic edge of the arsenic peak. Tin

has been found not to interfere at concentrations 100 times that of arsenic. Mercury, bismuth and antimony do not seriously affect the arsenic signal at concentrations up to 10 times that of arsenic. Higher concentration ratios of mercury, bismuth, and antimony have not been tested. However, mercury should present little, if any problem at higher concentrations since it is usually lost during digestion. If the concentrations of bismuth and antimony are suspected to be significant, as in samples taken in metallurgical industrial environments, the samples should also be analyzed with a composite mercury-graphite electrode (CMGE) at a plating potential of -350 mV to determine the amounts of bismuth and antimony present.

3.2 Copper and arsenic are not completely resolved at high levels of both metals. The available data suggest that when the absolute quantity of copper exceeds 1 μg, the linear range of electrode response to arsenic will decrease.

4. **Precision and Accuracy**

All of the factors that affect the accuracy and precision of the total method for the determination of arsenic in air have not been fully evaluated. An estimate of the coefficient of variation is 0.08; also, it is estimated that results of the analyses of sample unknowns should fall within ± 10% of the "true" values.

5. **Advantages and Disadvantages of the Method**

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 One disadvantage is that the current-voltage peaks for copper and arsenic are not completely resolved when their concentrations are comparable.

5.3 Another disadvantage is the occurrence of large variations in response from electrode to electrode.

6. **Apparatus**

6.1 **Air Sampling Equipment.** The sampling unit for the collection of particulate samples has the following components:

6.1.1 **Filter Unit.** The unit consists of the filter and an appropriate filter holder such as the Millipore Aerosol Analysis Monitor, Catalog No. MAWP 037 AO, or equivalent. This unit is a plastic disposable holder preassembled with a filter in place. The recommended filter is the Millipore Type AA, a mixed cellulose ester, with a pore size of 0.8 μm and a diameter of 37 mm.

6.1.2 **Adaptor.** An adaptor such as the Millipore Aerosol Adaptor, Catalog No. XX62 000 04, is inserted into the filter unit to allow a hose to be attached.

6.1.3 **Personal Sampling Pump.** The pump should be calibrated with a representative filter unit in the sampling line. A wet or dry test meter or glass rotameter capable of measuring the appropriate flow rate (1.5 to 2 ℓ/min) to within ± 5% may be used in the calibration.

6.2 Pyrex test tubes (20 x 100 mm) for digesting filter samples and subsequent use as analysis cells.

6.3 Digestion racks for analysis cells.

6.4 Micro pipettes, Eppendorf or equivalent (10, 100, 200, 300, and 500 μℓ, and 1 mℓ).

6.5 Disposable pipettes, graduated (1 and 5 mℓ).

6.6 Assorted laboratory glassware, including beakers, graduated cylinders, and volumetric flasks of appropriate sizes.

6.7 Potentiometric recorder (100-mV range).

6.8 **Anodic Stripping Voltammeter.** A suitable single-cell instrument with a composite mercury-graphite electrode (Model SA 2011) is available from Environmental Sciences Associates, Burlington, Mass. Equivalent instrumentation may be used. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations.

6.9 **Modification of the Model SA 2011.** The slight modification described below is suggested to improve the mixing of the high-salt-content solutions necessary for the determination of arsenic. This modification is best performed while the test electrode is out of the cell for routine maintenance. Throw the plate-strip switch to "strip" with the cell selector on "EXT." Disconnect and remove the test electrode. Place the test electrode in a beaker of water to keep it wet. With thumb and forefinger, grasp the bent end of the nitrogen bubbler and pull it down and away from the cell head until all of the platinum inside the tubing has been drawn past the retaining loop on the bottom of the reference electrode. Straighten the bend in the Teflon-covered platinum. With the platinum tip of the counter electrode, push the platinum wire in the nitrogen bubbler tube further up inside the bubbler tube so that the solution end of the tube is empty for the last 1 to 3 mm of its length. Rebend the platinum and tubing to the orginal configuration and return the bubbler tube to its original position and configuration. Replace the test and counter electrodes. Reconnect all wire junctions broken, and throw the plate-strip switch back to "plate." This procedure provides an unobstructed gas-solution interface so that micro precipitation due to evaporation of solution trapped inside of the bubbler will not lead to reduced stirring rates.

6.10 Composite Gold-Graphite Electrode (CGGE). A CGGE may be prepared from a new waxed graphite electrode or one which has been used as a composite mercury-graphite electrode.

6.10.1 If the waxed graphite electrode has previously been used as a CMGE, withdraw the electrode from the cell head and remove the mercury coat by wiping with a damp soft paper towel. Place the electrode back in the cell head and electrolytically remove the remaining mercury by holding the potential of the electrode at +1.0 V in 8 mℓ of a nitrogen-stirred solution of 1 *N* HNO_3 for 15 min. Remove the nitric acid solution and rinse the electrode thoroughly in a 250-mℓ beaker of distilled deionized water. Remove the electode from the cell head, wipe dry, and resurface the electrode. The resurfacing is accomplished by gently pinching the polished end of the electrode in 4/0 emery paper between thumb and forefinger of one hand. If the electrode is rotated a large number of times as it is withdrawn from the emery paper, a light gray silvery surface approximating that of spun aluminum will be obtained. Wipe the resurfaced area very gently with the dampened edge of a soft paper towel to remove any particles of graphite clinging to the refinished surface. Carefully place the electrode back into position in the cell head.

6.10.2 If the electrode is new, then resurface as described above; hold at +1.0 V in 8 mℓ of a nitrogen-stirred solution of approximately 1 *N* HNO_3, and rinse well with distilled deionized water.

6.10.3 Immerse the electrodes to be gold plated in analysis cells, each of which contains 5 mℓ of 5 *N* HCl and 100 μℓ of the gold plating solution (see Section 7.14). Stir for 60 sec with the nitrogen bubbler on and then turn off the nitrogen. Set the plating potential to -300 mV and throw the plate-strip switch to "plate". Plate the electrodes for 10 to 12 min from an unstirred solution and then for 60 sec from a stirred solution. Arrest plating by immersing the electrodes in the hydrazine dihydrochloride-acid solution. At the end of the plating step the electrodes may range in color from dull brown to bright yellow. Most of the electrodes will be a dull greenish yellow.

6.10.4 When they are not in use, store the electrodes at a potential of +300 mV in a solution consisting of 5 mℓ of 5 *N* HCl and 0.3 mℓ of the ferrous perchlorate reagent. Before use, soak each electrode in 5 mℓ of the ferrous perchlorate reagent for 5 min at -200 mV.

7. Reagents.

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in arsenic. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 **Acid Ashing Solution.** Prepare the acid ashing solution by adding 24 volumes of concentrated nitric acid to 24 volumes of perchloric acid (70%) and 1 volume of concentrated sulfuric acid.

7.5 **Sodium Bisulfite Solution (1 *N*).** Add 10.4 g of sodium bisulfite to a 100-mℓ volumetric flask, dissolve in water and dilute to the mark.

7.6 Hydrazine dihydrochloride.

7.7 **Ferrous Perchlorate Reagent.** There are two alternate methods of preparing this reagent.

7.7.1 Add 10 mg of iron (electrolytic grade) to a 50-mℓ beaker. Add 5 mℓ of distilled deionized water and 9 mℓ of 70% perchloric acid. Warm gently to aid in dissolution. After dissolution, transfer the contents of the beaker to a 100-mℓ volumetric flask containing 0.5 g of hydrazine dihydrochloride. Rinse the beaker several times with water and add the washings to the volumetric flask. Dilute to the mark with water.

7.7.2 Add 0.650 g of ferrous perchlorate, $Fe(ClO_4)_2 \cdot 6H_2O$, and 9 mℓ of 70% perchloric acid to a 100-mℓ volumetric flask containing 0.5 g of hydrazine dihydrochloride in approximately 30 mℓ of water. Dissolve and dilute to the mark with water.

7.8 Hydrochloric acid, concentrated.

7.9 **Hydrazine Dihydrochloride-Acid Solution (5 *N* HCl, 0.001 *N* $H_2NNH_2 \cdot (HCl)_2$).** Add 0.105 g of hydrazine dihydrochloride to a 1-liter volumetric flask. Add approximately 400 mℓ of water and swirl to dissolve the salt. Add 417 mℓ of concentrated hydrochloric acid, swirl, and dilute to the mark with water. This solution may be conveniently stored in a polyethylene bottle with a screw cap.

7.10 **Sodium Hydroxide Solution (1.5 *N*).** Add 6 g of sodium hydroxide to a 100-mℓ volumetric flask with a polyethylene stopper, dissolve in water, and dilute to the mark.

7.11 **Arsenic (As(III)), Standard Solution "A" (10 mg/mℓ).** Transfer 1.320 g of arsenic trioxide (As_2O_3) to a clean 100-mℓ volumetric flask. Add 35 mℓ of 1.5 *N* NaOH. Immediately after dissolution of the arsenic trioxide, acidify the solution with 25 mℓ of concentrated hydrochloric acid and cool to room temperature. Then dilute to the mark with water.

7.12 **Arsenic (As(III)), Standard Solution "B" (100 μg/mℓ).** Add approximately 80 mℓ of water and 2 mℓ of concentrated hydrochloric acid to a 100-mℓ volumetric flask. Swirl to achieve a homogeneous solution and then add 1 mℓ of standard solution "A". Dilute to the mark with water.

7.13 **Arsenic (As(III)), Standard Solution "C" (1 μg/mℓ).** Repeat the procedure for preparing standard solution "B", but add 1 mℓ of standard solution "B" instead of standard solution "A". During the first two months, prepare standard solution "C" daily and standard solution "B" weekly. Pour out the old solution and use the same flask for making up the new solution. After the two-month period for conditioning the flasks, prepare standard solution "C" weekly, standard solution "B" monthly, and solution "A" semiannually.

7.14 **Gold Plating Solution.** Place 1.00 g of high purity gold metal in a 100-mℓ beaker. In a hood, add 2.5 mℓ of concentrated nitric acid and 10 mℓ of concentrated hydrochloric acid to the beaker. Warm gently until all of the gold has dissolved. Add 5 mℓ more of concentrated hydrochloric acid and allow the beaker to cool. Quantitatively transfer the contents of the beaker to a clean 100-mℓ volumetric flask. Rinse the beaker several times with approximately 15-mℓ portions of water and add each rinse to the volumetric flask. Dilute to the mark with water. This solution now contains 10 mg/mℓ of gold in acid solution.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any arsenic from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot place with a surface temperature of 220 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove, and allow to cool. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom. Allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water.

8.2 Collection and Shipping of Samples

8.2.1 Connect the exit end of a filter unit to the adaptor. Connect the adaptor to the pump with a short piece of flexible tubing.

8.2.2 Sample the air at a flow rate of 1.5 to 2 ℓ/min. Measure the flow rate and time, or volume, as accurately as possible. A minimum sample of 100 liters should be collected. Larger volumes are desirable, provided the filter does not become loaded with dust to the point that loose material could fall off or the filter become plugged.

8.2.3 The filter holders should be shipped in a suitable container to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample is dislodged from the filter nor that the sample surface is disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the holder on top of the sample filter.

8.2.4 Unexposed filters in filter holders should be labeled as blanks and shipped with the samples.

8.3 Analysis of Samples

8.3.1 Carefully remove the filter from the filter unit by opening the unit, gently pushing up the filter and its backing, and grasping the unexposed edge of the filter with stainless steel forceps. Fold the filter in half, then into quarters, and subsequently into eighths without creasing or cracking the filter material. Insert the filter into an analysis cell and push it down into the cell with a glass rod.

8.3.2 Pipette 500 μℓ of the acid ashing solution into the analysis cell containing the filter. Wet the filter as thoroughly as possible by adding the acid slowly. Place the cell in a digestion rack on a hot plate with a surface temperature of 220 to 240°C. Carry out the digestion in a hood. The filter sample will clear in about 10 min, and a reflux line will be established about halfway up the side of the cell. At the end of 20 min, there will be approximately 100 to 200 μℓ of acid left in the cell, with most of the nitric acid having evaporated. The digestion should be continued for an additional 20 to 30 min until the acid is almost completely evaporated.

8.3.3 Add to the cell 1 mℓ of 1 *N* $NaHSO_3$, 200 μℓ of the ferrous perchlorate reagent, and 300 μℓ of concentrated hydrochloric acid. Heat the sample in a hood on a hot plate at 85 to 95°C for 15 min. Do not evaporate the solution to dryness. Allow the cell to cool room temperature and dilute the sample to 5 mℓ with the hydrazine dihydrochloride-acid solution.

8.3.4 If the sample is estimated to contain more than 500 ng of arsenic, an appropriate aliquot of the 5-mℓ digestate should be taken and diluted to 5 mℓ with the hydrazine dihydrochloride-acid solution.

8.3.5 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the solution under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -200 mV
- Auto-hold cutoff potential, 720 mV
- Plating time, 15 min
- Sweep rate, +60 mV/sec
- Current range, 0.5 to 1.0 mA (full scale)

The nitrogen stirring should be on during the plating step and should be turned off 15 sec prior to the stripping step. The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of arsenic plated on the electrode may occur. As the sample is stripped, the arsenic peak (+195 mV) occurs first, and the copper-iron peak occurs second. After the auto-hold cutoff potential is reached, remove the cell and rinse the electrode by swishing the cell-head assembly up and down while immersed in 230 mℓ of distilled deionized water containing 10 mℓ of concentrated hydrochloric acid. Place the next sample on the electrode cell head and reset the potential of the cell to the plating potential. Turn the nitrogen stirring on and reset the timer.

8.3.6 A baseline is drawn by connecting with a straight line the minimum point between the copper and arsenic peaks with the flat portion of the curve preceding the arsenic peak. The peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions: (1) filter and reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 9.3; (3) standard solutions of arsenic; (4) filter standards prepared as described below in Section 9.1. The sequence in which these four kinds of solutions are analyzed is as follows: (1) a set of standard solutions of arsenic; (2) sample solutions, with filter and reagent blanks and filter standards interspersed at the rate of one blank and one filter standard for every ten samples; and (3) a second set of standard solutions of arsenic. The standard solutions of arsenic are

used for calibration; the results for the samples are corrected for the amount of arsenic found in the reagent blank; and the filter standards are used to determine the efficiency of recovery of known amounts of arsenic from filters. The procedure described is for use with a four-cell ASV unit.

9.1 **Preparation of Arsenic Filter Standards.** Prepackaged filters of the type used for sampling are removed from their plastic cases and separated from their support mats. The filters are placed on the center ring supports of the plastic cases so that only the edge of the filter is touching the rim of its holder. Arsenic standard solution "B" is appropriately diluted to provide 10 to 500 ng amounts of arsenic in 10-μℓ volumes. These solutions are placed on the centers of the filters and allowed to evaporate and air-dry for approximately 30 min. Each filter is then placed back in its holder. These filter standards are analyzed along with the calibration standards as outlined in Section 9.3. The results obtained with the filter standards are used to estimate the percent recovery of arsenic.

9.2 **Initial Analysis of Four Standards.** Solutions consisting of 5 mℓ of the hydrazine dihydrochloride-acid reagent and 200 μℓ of ferrous perchlorate reagent are placed in each of four cells and analyzed by the procedure described in Section 8.3.5. The plating of each cell solution should be started sequentially at 1-min intervals. After stripping each cell, add 100 ng of arsenic (100 μℓ of standard arsenic solution "C") and plate and strip once again. A single point may be taken to describe the calibration curve, which has been determined to be linear over the range 25 to 600 ng of arsenic.

9.3 **Analysis of Samples and Controls.** The samples are then analyzed with blanks and filter standards (Section 9.1) interspersed at the rate of one blank and one filter standard per 12 samples. At least one arsenic filter standard and one unexposed filter (reagent blank) should be analyzed with each electrode that is used for the analysis of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 100 ng of arsenic will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA for each electrode since the peak heights may vary significantly from electrode to electrode. The response factor for a particular electrode is the amount of arsenic in the standard (100 ng) divided by the average of the two peak height values obtained for that electrode. For a 30-min plating time, typical response factors range from 0.75 to 1.4 ng/μA.

10. Calculations

10.1 The amount of arsenic in a sample is calculated from the peak height as measured according to Section 8.3.6, the response factor, and the correction factor, in the following manner:

$$\text{Amount (ng)} = \frac{\text{peak height } (\mu A)}{C} \times \text{response factor } (ng/\mu A)$$

where: C = a correction factor equal to the average fraction of arsenic recovered from the filter standards with the particular electrode used. If the correction factor is greater than 0.90, the value of C may be taken to be unity.

Any amount of arsenic found in the filter and reagent blank should be subtracted from the sample value to give the correct amount (in ng) of arsenic in the sample.

10.2 The concentration of arsenic may be expressed in mg/m^3, which is numerically equal to $\mu g/\ell$:

$$mg/m^3 = \frac{\text{corrected weight (in ng) of arsenic in sample}}{V_s\,(\ell) \times 1000\ (ng/\mu g)}$$

where: V_s = volume of air sampled.

11. References

11.1 Environmental Sciences Associates, Inc., Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Division of Laboratory and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

11.2 "General Method: Arsenic Analysis by Anodic Stripping Voltammetry (ASV)", ESA Methodology M-43, Environmental Sciences Associates, Inc., Burlington, Mass.

ANTIMONY IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Antimony	Method No.: P&CAM 189
Matrix: Air	Range: 0.00015 to 0.005 mg/m³ in 100 liters of air
Procedure: Collection on a membrane filter; anodic stripping voltammetry	Precision (CV): 0.10 (analytical) at 0.0005 mg/m³
Date Issued: 4/15/73	Classification: E (Proposed)
Date Revised: 1/29/76	

1. Principle of the Method

Particulate matter containing antimony is collected from air on a membrane filter. The filter and trapped particulate material are wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The resulting Sb(V) is reduced to Sb(III) with a solution of hydrochloric acid and hydrazine dihydrochloride. The chemically reduced sample is analysed by anodic stripping voltammetry (ASV) with a composite mercury-graphite electrode (CMGE). In principle, a hanging mercury drop electrode (HMDE) can also be used. However, optimum analysis parameters may vary significantly and must be established for each electrode system. The method as presented is designed for use with the CMGE.

2. Range and Sensitivity

2.1 The sensitivity of the method is limited by chemical interferences in the reduction of Sb(V) to Sb(III) and by interferences of copper and bismuth with the ASV determination of antimony. In the absence of copper and bismuth, as in reagent blanks, the detection limit is about 0.5 to 1 ng; however, in typical industrial atmospheres, which may contain high concentrations of copper and perhaps bismuth, the detection limit is 10 to 20 ng.

2.2 The linear range of the electrode response extends from the detection limit to 500 ng of antimony, which corresponds to 0.005 mg/m³ in a 100-liter sample of air. The upper limit of the analytical method may be extended by dilution of the sample prior to analysis.

3. Interferences

3.1 The presence of copper (II) ions in the sample digestate appears to accelerate the oxidation of antimony (III) to antimony (V). This interference is minimized by the presence of an amount of reducing agent in excess of that required for the reduction of antimony (V) to antimony (III) and copper (II) to copper (I).

3.2 Bismuth is stripped at the same potential as antimony. If this interference is suspected, the sample should be analyzed for bismuth after the addition of 20 $\mu\ell$ of 5% sodium hypochlorite solution to oxidize antimony (III) to antimony (V); a reduction of the peak height by 80% or more signifies that the peak was due to antimony only.

3.3 The formation of an interamalgam complex of copper and antimony during the plating step is minimized by the presence of a relatively large concentration of chloride ions.

4. Precision and Accuracy

4.1 Several processes determine the reproducibility of the overall analytical method: (1) sampling, (2) shipping, (3) preparation of samples, and (4) analysis. The variation introduced by sampling in industrial environments with personal sampling pumps has not been evaluated. The shipping of samples as described in Section 8.2.3 is expected to produce a negligible effect on precision. The coefficient of variation of the analysis step alone is 0.04 over the range of 10 to 500 ng. Measurements with two different electrodes with different histories may differ by a factor of two; however, the coefficient of variation remains 0.04 for measurements with each electrode. The coefficient of variation for the preparation and analysis steps combined is 0.10 over the range of 20 to 250 ng, which corresponds to 0.0002 to 0.0025 mg/m^3 in a 100-liter sample of air.

4.2 The estimated percent error in a measurement is less than about 10% of the "true" value in the concentration range of 0.0002 to 0.0025 mg/m^3.

5. Advantages and Disadvantages of the Method

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 The primary disadvantage is its sensitivity to interferences. (See Section 3.)

5.4 Another disadvantage is the occurrence of large variations in response from electrode to electrode.

6. Apparatus

6.1 Air Sampling Equipment. The sampling unit for the collection of particulate samples has the following components:

6.1.1 **Filter Unit.** The unit consists of the filter and an appropriate filter holder such as the Millipore Aerosol Analysis Monitor, Catalog No. MAWP 037AO, or equivalent. This unit is a plastic disposable holder preassembled with a filter in place. The recommended filter is the Millipore Type AA, a mixed cellulose ester, with a pore size of 0.8 μm and a diameter of 37 mm.

6.1.2 **Adaptor.** An adaptor such as the Millipore Aerosol Adaptor, Catalog No. XX62 000 04, is inserted into the filter unit to allow a hose to be attached.

6.1.3 **Personal Sampling Pump.** The pump should be calibrated with a representative filter unit in the sampling line. A wet or dry test meter or a glass rotameter capable of measuring the appropriate flow rate (1.5 to 2 ℓ/min) to within ± 5% may be used in the calibration.

6.2 Pyrex test tubes (20 x 100mm) for digesting filter samples and subsequent use as analysis cells.

6.3 Digestion racks for the analysis cells.

6.4 Micro pipettes, Eppendorf or equivalent (10, 50, 100, and 500 μℓ).

6.5 Model SA 2011 Single-Cell Anodic Stripping Voltammeter with composite-mercury graphite electrode, Environmental Sciences Associates, Inc., Burlington, Mass., or equivalent. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations. Instructions for the preparation and reconditioning of the CMGE are included in the instruction manual.

6.6 Potentiometric recorder (100-mV range).

6.7 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric pipettes, and volumetric flasks of appropriate sizes.

7. Reagents

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in antimony. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 **Acid Ashing Solution.** Prepare the acid ashing solution by adding 24 volumes of concentrated nitric acid to 24 volumes of perchloric acid (70%) and 1 volume of concentrated sulfuric acid.

7.5 **Hydrazine Dihydrochloride-Acid Solution** (5 *N* HCl, 0.001 *N* $H_2NNH_2 \cdot (HCl)_2$). Add 0.105 g of hydrazine dihydrochloride to a 1-liter volumetric flask. Add approximately 400 mℓ of water and swirl to dissolve the hydrazine salt. Add 417 mℓ of concentrated hydrochloric acid, swirl, and dilute to the mark with water. This solution may be conveniently stored in a polyethylene bottle with a screw cap. The high concentration of chloride ions is necessary to minimize interference by copper.

7.6 Antimony Reference Standard Solution (1000 μg/mℓ). Fisher Scientific Co., or equivalent.

7.7 **Antimony (Sb(III)), Standard Solution "B" (100 μg/mℓ).** Transfer 10 mℓ of the antimony reference standard solution (1 mg/mℓ) with a volumetric pipette to a 100-mℓ volumetric flask and dilute to the mark with water. Prepare fresh daily.

7.8 **Antimony (Sb(III)), Standard Solution "C" (1 μg/mℓ).** Transfer 1 mℓ of standard solution "B" with a volumetric pipette to a 100-mℓ volumetric flask and dilute to the mark with water. Prepare fresh daily.

7.9 Sodium Hypochlorite Solution (4 to 6%, w/w).

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any antimony from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 220 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove, and allow them to cool. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom. Allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water.

8.2 Collection and Shipping of Samples

8.2.1 Connect the exit end of a filter unit to the adaptor. Connect the adaptor to the pump with a short piece of flexible tubing.

8.2.2 Sample the air at a flow rate of 1.5 to 2 ℓ/min. Measure the flow rate and time, or volume, as accurately as possible. A minimum sample of 100 liters should be collected. Larger volumes are desirable, provided the filter does not become loaded with dust to the point that loose material could fall off or the filter become plugged.

8.2.3 The filter holders should be shipped in a suitable container to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample surface is disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the holder on top of the sample filter.

8.2.4 Unexposed filters in filter holders should be labeled as blanks and shipped with the samples.

8.3 Analysis of Samples.

8.3.1 Carefully remove the filter from the filter unit by opening the unit, gently pushing up the filter and its backing, and grasping the unexposed edge of the filter with stainless steel forceps. Fold the filter in half, then into quarters, and subsequently into eighths without creasing or cracking the filter material. Insert the filter into an analysis cell and push it down into the cell with a glass rod.

8.3.2 Pipette 500 μℓ of the acid ashing solution into the analysis cell containing the filter. Wet the filter as thoroughly as possible by adding the acid slowly. Place the cell in a digestion rack on a hot plate with a surface temperature of 220 to 240°C. Carry out the digestion in a hood. The filter sample will clear in about 10 min, and a reflux line will be established about halfway up the cell. At the end of 20 min, there will be approximately 100 to 200 μℓ of acid left in the cell with most of the nitric acid having evaporated. The digestion should be continued for an additional 20 to 30 min until the acid is almost completely evaporated.

8.3.3 Remove the sample from the hot plate, allow it to cool, and add 5 mℓ of the hydrazine dihydrochloride-acid solution. Allow the solution to run down the sides of the cell to insure that any spattered material is washed down. Then heat the cell to about 90°C for 15 min, remove it from the hot plate, and allow it to cool to room temperature. Readjust the volume of the sample solution to 5 mℓ with the hydrazine dihydrochloride-acid solution if necessary. If the digested and reduced sample is estimated to contain more than 500 ng of antimony, dilute a 100-μℓ aliquot (or an aliquot of appropriate volume) of the sample to 5 mℓ with the hydrazine dihydrochloride-acid solution.

8.3.4 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the solution under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -400 mV
- Auto-hold cutoff potential, 200 mV
- Plating time, 15 to 30 min. (The plating time for a set of samples and standards must be the same.)
- Sweep rate, +30 mV/sec
- Current range, 0.1 to 2 mA (full scale)

The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of antimony plated on the electrode may occur. A baseline is drawn by connecting with a straight line the flat portions of the curve before and after the antimony peak, which occurs at -180 mV. Peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions: (1) filter and reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solutions of antimony; (4) filter standards prepared as described below in Section 9.1. The sequence in which these four kinds of solutions are analyzed is as follows: (1) a set of standard solutions of antimony; (2) sample solutions, with filter and reagent blanks and filter standards interspersed at the rate of one blank for every 30 samples and one filter standard for every 10 samples; and (3) a second set of standard solutions of antimony. The standard solutions of antimony are used for calibration; the results for the samples are corrected for the amount of antimony found in the reagent blank; and the filter standards are used to determine the efficiency of recovery of known amounts of antimony from filters. The procedure described is for use with a four-cell ASV unit.

9.1 **Preparation of Antimony Filter Standards.** Prepackaged filters of the type used for sampling are removed from their plastic cases and separated from their support mats. The filters are placed on the center ring supports of the plastic cases so that only the edge of the filter is touching the rim of its holder. Antimony standard solution "B" is appropriately diluted to provide 50-, 100-, and 500-ng amounts in 10-μℓ volumes. These solutions are placed on the centers of the filters and allowed to evaporate and air-dry for approximately 30 min. Each filter is then placed back in its holder. These filter standards are analyzed along with the calibration standards as outlined in Section 9.3. The results obtained with the filter standards are used to estimate the percent recovery of antimony.

9.2 **Initial Analysis of Four Standards.** Standards of 50 ng of antimony, prepared by adding 50 $\mu\ell$ of standard solution "C" to 5 mℓ of the hydrazine dihydrochloride-acid solution, should be analyzed first. Each of the four standards should be analyzed with a different electrode. A single point may be taken to describe the calibration curve, which has been determined to be linear up to 500 ng of antimony. However, the calibration curves will vary from electrode to electrode, and a curve must be established for each electrode.

9.3 **Analysis of Samples and Controls.** The samples are then analyzed with procedure blanks and filter standards (Section 9.1) interspersed at the rate of one blank for every 30 samples and one filter standard for every 10 samples. One reagent blank and one filter standard should be analyzed with each electrode during the analysis of a set of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 50 ng of antimony will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA for each electrode since the peak heights may vary significantly from electrode to electrode. The response factor for a particular electrode is the amount of antimony in the standard (50 ng) divided by the average of the two peak height values obtained for that electrode. For a 30-min plating time, a typical response factor is 0.75 ng/μA.

10. **Calculations**

10.1 The amount of antimony in a sample is calculated from the antimony peak height (in μA), as measured according to Section 8.3.4, and the response factor in the following manner:

$$\text{Amount (ng)} = \frac{\text{peak height } (\mu\text{A})}{C} \times \text{response factor } (\text{ng}/\mu\text{A})$$

where: C = a correction factor equal to the average fraction of antimony recovered from the filter standards with the particular electrode used.

Any amount of antimony found in the reagent and filter blank should be subtracted from the sample value to give the correct amount (in ng) of antimony in the sample.

10.2 The concentration of antimony may be expressed in mg/m^3, which is numerically equal to μg/ℓ:

$$\text{mg/m}^3 = \frac{\text{corrected weight (in ng) in sample}}{V_s\ (\ell) \times 1000\ (\text{ng}/\mu\text{g})}$$

where: V_s = volume of air sampled.

11. Reference

Environmental Sciences Associates, Inc., Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Division of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

INDIUM IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Indium	Method No: P&CAM 190
Matrix: Air	Range: 0.00002 to 0.03 mg/m^3 in 100 liters of air
Procedure: Collection on a membrane filter; anodic stripping voltammetry	Precision (CV): 0.10 (analytical) at 0.001 mg/m^3
Date Issued: 4/15/73	Classification: E (Proposed)
Date Revised: 1/29/76	

1. Principle of the Method

Particulate matter containing indium is collected from air on a membrane filter. The filter and trapped particulate material are wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The residue is dissolved in an acetate buffer solution, and the buffered sample solution is analyzed by anodic stripping voltammetry (ASV) with a composite mercury-graphite electrode (CMGE). In principle, a hanging mercury drop electrode (HMDE) can also be used. However, optimum analysis parameters may vary significantly and must be established for each electrode system. The method as presented is designed for use with the CMGE.

2. Range and Sensitivity

2.1 Indium is not normally present in the reagents and filters in detectable amounts. Therefore, the sensitivity is limited only by the baseline current in the ASV analysis. The detection limit is estimated to be 2 ng, which corresponds to 0.00002 mg/m^3 in a 100-liter sample of air.

2.2 The linear range of the electrode response is estimated to extend from the detection limit to 3 μg of indium, which corresponds to 0.03 mg/m^3 in a 100-liter sample of air. The range may be extended by diluting the sample digestate with acetate buffer and taking an aliquot of the diluted solution for the ASV determination.

3. Interferences

In the ASV analysis, the indium peak occurs between lead and cadmium peaks. Consequently, microgram amounts of lead or cadmium interfere with the determination of indium. At lead levels greater than 2 μg, the indium peak occurs as a shoulder on the lead peak and measurements of indium peak heights become inaccurate. Interferences by cadmium can be reduced with no loss of response to indium by changing the plating potential from -1000 mV to -900 mV and changing the auto-hold cutoff potential from 980 mV to 780 mV.

4. Precision and Accuracy

4.1 Several processes determine the reproducibility of the overall analytical method: (1) sampling, (2) shipping, (3) preparation of samples, and (4) analysis. The variations introduced by sampling from workroom atmospheres and shipping the collected samples to a distant laboratory have not been evaluated. The coefficient of variation for the preparation and analysis steps has been empirically determined to be 0.10 over the range of 20 to 3000 ng, which corresponds to 0.0002 to 0.03 mg/m^3 in a 100-liter sample of air, and 0.20 over the range of 2 to 20 ng, which corresponds to 0.00002 to 0.0002 mg/m^3 in a 100-liter sample of air.

4.2 The estimated percent error in a measurement, neglecting any contribution from the sampling and shipping, is less than about 10% of the "true" value in the range of 20 ng to 3 μg, which corresponds to 0.00002 to 0.03 mg/m^3 in a 100-liter sample of air.

5. Advantages and Disadvantages of the Method

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 Also, the matrix in which the digested samples are analyzed, the acetate buffer solution, allows the accurate determination of lead, cadmium, and copper, as well as indium.

5.4 One disadvantage is that relatively high lead and cadmium concentrations interfere with the determination of indium. (See Section 3.)

6. Apparatus

6.1 Air Sampling Equipment. The sampling unit for the collection of particulate samples has the following components:

6.1.1 **Filter Unit.** The unit consists of the filter and an appropriate filter holder such as the Millipore Aerosol Analysis Monitor, Catalog No. MAWP 037 AO, or equivalent. This unit is a plastic disposable holder preassembled with a filter in place. The recommended filter is the Millipore Type AA, a mixed cellulose ester, with a pore size of 0.8 μm and a diameter of 37 mm.

6.1.2 **Adaptor.** An adaptor such as the Millipore Aerosol Adaptor, Catalog No. XX62 000 04, is inserted into the filter unit to allow a hose to be attached.

6.1.3 **Personal Sampling Pump.** The pump should be calibrated with a representative filter unit in the sampling line. A wet or dry test meter or a glass rotameter capable of measuring the appropriate flow rate (1.5 to 2 ℓ/min) to within ± 5% may be used in the calibration.

6.2 Pyrex test tubes (20 x 100 mm) for digesting filter samples and subsequent use as analysis cells.

6.3 Digestion racks for the analysis cells.

6.4 Micro pipettes, Eppendorf or equivalent (10, 50, 100, and 300 μl).

6.5 Model SA 2011 Single-Cell Anodic Stripping Voltammeter with composite mercury-graphite electrode, Environmental Sciences Associates, Inc., Burlington, Mass., or equivalent. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations. Instructions for the preparation and reconditioning of the CMGE are included in the instruction manual.

6.6 Potentiometric recorder (100-mV range).

6.7 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric pipettes, and volumetric flasks of appropriate sizes.

7. Reagents

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in indium. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 **Acid Ashing Solution.** Prepare the acid ashing solution by adding 24 volumes of concentrated nitric acid to 24 volumes of perchloric acid (70%) and 1 volume of concentrated sulfuric acid.

7.5 **Acetate Buffer Solution (1 *M* NaAc, 4 *M* LiCl, pH 5.2).** To prepare 1 liter of this reagent, dissolve 82 g of sodium acetate, 170 g of lithium chloride, and 22 mℓ of 70% $HClO_4$ in water in a 1-liter volumetric flask and fill to the mark with water. Remove trace metals from the resulting solution electrolytically with a system such as the Model 2014 PM Reagent Cleaning System, Environmental Sciences Associates, Inc., or equivalent. The acetate buffer maintains the pH of the resulting sample solution near 5.2, a pH favorable for the determination of indium. The relatively high concentration of chloride ion minimizes interference from lead and cadmium.

7.6 **Indium (In(III)), Standard Solution "A" (10 mg/mℓ).** Weigh accurately an amount of pure indium shot (99.99% pure) near 1 g and transfer the weighed indium to a 100-mℓ volumetric flask. Dissolve the metal in 30 mℓ of concentrated nitric acid and dilute to the mark with water.

7.7 **Indium (In(III)), Standard Solution "B" (100 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "A" to a 100-mℓ volumetric flask containing 2 to 3 mℓ of concentrated nitric acid and dilute to the mark with water. Prepare fresh daily to prevent loss of indium or contamination.

7.8 **Indium (In(III)), Standard Solution "C" (1 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "B" to a 100-mℓ volumetric flask containing 2 to 3 mℓ of concentrated nitric acid and dilute to the mark with water. Prepare fresh daily to prevent loss of indium or contamination.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any indium from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 220 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove, and allow to cool. Complete decontamination requires four repetitions of the procedure. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom. Allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water.

8.2 Collection and Shipping of Samples

8.2.1 Connect the exit end of a filter unit to the adaptor. Connect the adaptor to the pump with a short piece of flexible tubing.

8.2.2 Sample the air at a flow rate of 1.5 to 2 ℓ/min. Measure the flow rate and time, or volume, as accurately as possible. A minimum sample of 100 liters should be collected. Larger volumes are desirable, provided the filter does not become loaded with dust to the point that loose material could fall off or the filter become plugged.

8.2.3 The filter holders should be shipped in a suitable container to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample is dislodged from the filter nor that the sample surface is disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the holder on top of the sample filter.

8.2.4 Unexposed filters in filter holders should be labeled as blanks and shipped with the samples.

8.3 Analysis of Samples

8.3.1 Carefully remove the filter from the filter unit by opening the unit, gently pushing up the filter and its backing, and grasping the unexposed edge of the filter with stainless steel forceps. Fold the filter in half, then into quarters, and subsequently into eighths without creasing or cracking the filter material. Insert the filter into an analysis cell and push it down into the cell with a glass rod.

8.3.2 Pipette 300 μℓ of the acid ashing solution into the analysis cell containing the filter. Wet the filter as thoroughly as possible by adding the acid slowly. Place the cell in a digestion rack on a hot plate operating at 220 to 240°C. Carry out the digestion in a hood. The filter sample will clear in about 10 min, and a reflux line will be established about halfway up the cell. At the end of 20 min, there will be approximately 100 to 200 μℓ of acid left in the cell, with most of the nitric acid having evaporated. The digestion should be continued an additional 20 to 30 min until the acid is almost completely evaporated.

8.3.3 Remove the sample from the hot plate, allow it to cool, and add to it 4.5 mℓ of the acetate buffer solution.

8.3.4 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the buffered solution under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -1100 mV
- Auto-hold cutoff potential, 980 mV
- Plating time, 30 min
- Sweep rate, +60 mV/sec
- Current range, 0.05 to 2 mA (full scale)

The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of indium plated on the electrode may occur. As the sample is stripped, the cadmium peak (-675 mV) will occur first, followed by the indium peak (-650 mV), and then the lead peak (-510 mV). A baseline is drawn by connecting with a straight line the flat portion of the curve after the cadmium peak with the flat portion before the lead peak. At high concentrations of lead, a baseline is drawn by extending the shoulder of the lead peak from the end of the indium peak to its beginning. The peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions; (1) filter and reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solutions of indium in acetate buffer; (4) filter standards prepared as described below in Section 9.1. The sequence in which these four kinds of solutions are analyzed is as follows: (1) a set of standard solutions of indium in acetate buffer; (2) sample solutions, with filter and reagent blanks and filter standards interspersed at the rate of one blank and one filter standard for every ten samples; and (3) a second set of standard solutions of indium in acetate buffer. The standard solutions of indium in acetate buffer are used for calibration; the results for the samples are corrected for the amount of indium found in the reagent blank; and the filter standards are used to determine the efficiency of recovery of known amounts of indium from filters. The procedure described is for use with a four-cell ASV unit.

9.1 **Preparation of Indium Filter Standards.** Prepackaged filters of the type used for sampling are removed from their plastic cases and separated from their support mats. The filters are placed on the center ring supports of the plastic cases so that only the edge of the filter is touching the rim of its holder. Indium standard solution "B" is

appropriately diluted to provide 50-, 100-, 200-, and 500-ng amounts in 10-μℓ volumes. These solutions are placed on the centers of the filters and allowed to evaporate and air-dry for approximately 30 min. Each filter is then placed back in its holder. These filter standards are analyzed along with the calibration standards as outlined in Section 9.3. The results obtained with the filter standards are used to estimate the percent recovery of indium.

9.2 **Initial Analysis of Four Standards.** Four standards, each containing 100 ng of indium, are prepared by adding 100 μℓ of indium standard solution "C" to 4.5 mℓ of the acetate buffer solution. Each of the four standards should be analyzed with a different electrode by the procedure of Section 8.3. At the level of 100 ng of indium, the contributions of the reagent blank should be negligible and a single point for the indium calibration curve usually is sufficient.

9.3 **Analysis of Samples and Controls.** With every ten samples, a filter standard (Section 9.1) and a reagent blank are analyzed by the ASV procedure given in Section 8.3. At least one reagent blank and one filter standard should be analyzed with each electrode that is used for analysis of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 100 ng of indium will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA. The overall response factor is the amount of indium in the standard (100 ng) divided by the average of the eight peak heights (in μA). However, it will be necessary to calculate an individual response factor for an electrode if the average of the two peak height values for an individual electrode differs from the overall average of the eight peak heights by more than 10%. For example, if the average for the eight peak heights is 210 μA, and the average of the two values for one of the electrodes is 185 μA, then the response factor to be used for that electrode is 100 ng/185 μA, or 0.54 ng/μA. The overall response factor in this example is 100 ng/210 μA, or 0.48 ng/μA.

10. **Calculations**

10.1 The amount of indium in a sample is calculated from indium peak height (in μA), as measured according to Section 8.3.4, and the response factor in the following manner:

Amount (in ng) = peak height (in μA) x response factor (in ng/μA)

Any amount of indium found in the filter blank should be subtracted from the sample value to give the correct amount (in ng) of indium in the sample. The average recovery of indium from filter standards is usually greater than 90%. However, it may be necessary to include a factor in the above equation to correct for incomplete recovery.

435

10.2 The concentration of indium may be expressed in mg/m^3, which is numerically equal to $\mu g/\ell$:

$$mg/m^3 = \frac{\text{corrected weight (in ng) of indium in sample}}{V_s\ (\ell) \times 1000\ (ng/\mu g)}$$

where: V_s = volume of air sampled.

11. **Reference**

Environmental Sciences Associates, Inc., Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Division of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

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PART 2 OF 2

NIOSH MANUAL OF ANALYTICAL METHODS

SECOND EDITION

Part I

NIOSH Monitoring Methods

David G. Taylor, Ph.D.
Manual Coordinator

VOLUME 1

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Center for Disease Control
National Institute for Occupational Safety and Health
Cincinnati, Ohio
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LEAD AND CADMIUM IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analytes: Lead and Cadmium	**Method No.:** P&CAM 191
Matrix: Air	**Range:** In a 100-liter air sample, Pb: 0.00016 to 0.01 mg/m^3 Cd: 0.00008 to 0.01 mg/m^3
Procedure: Collection on a membrane filter; anodic stripping voltammetry	**Precision** (CV_T) : Pb: 0.09 at 0.002 mg/m^3 Cd: 0.13 at 0.0005 mg/m^3
Date Issued: 3/15/73	**Classification:** E (Proposed)
Date Revised: 1/29/76	

1. **Principle of the Method**

Particulate matter containing lead and cadmium is collected from air on a membrane filter. The filter and trapped particulate material are wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The residue is dissolved in an acetate buffer solution and the buffered sample solution is analyzed by anodic stripping voltammetry (ASV) with a composite mercury-graphite electrode (CMGE). In principle, a hanging mercury drop electrode (HMDE) can also be used. However, optimum analysis parameters may vary significantly and must be established for each electrode system. The method as presented is designed for use with the CMGE.

2. **Range and Sensitivity**

2.1 The sensitivity is limited by the reagent blank (filter plus reagents). A reasonable detection limit appears to be two times the standard deviation of the reagent blank. Statistically, the probability is 95% that lead or cadmium is present in the sample if the signal is higher than this limit. With this criterion, the detection limits are estimated to be 16 ng for lead and 8 ng for cadmium, which correspond to 0.00016 mg/m^3 of lead and 0.00008 mg/m^3 of cadmium in a 100-liter air sample.

2.2 The linear range of the electrode response extends from each of the above detection limits to about 1000 ng of lead or cadmium, which corresponds to 0.01 mg/m^3 of either lead or cadmium in a 100-liter air sample. The upper limit of the analytical method may be extended to 1 mg/m^3 of lead or cadmium in a 100-liter air sample by sample dilution prior to analysis. (See Section 8.3.3.)

3. Interferences

3.1 The thallium peak occurs between the lead and cadmium peaks. However, the thallium peak does not affect the height of either the lead or cadmium peak until thallium is present in roughly the same concentration as lead or cadmium. If the presence of a significant concentration of thallium is suspected, as in a sample from the workroom atmospheres of certain ore smelting or roasting industries, the sample should be analyzed for thallium. Thallium is determined by making the digested sample solution 0.01 *M* in EDTA (ethylenediaminetetraacetic acid) and analyzing the resulting solution by ASV at the same instrument settings as those recommended for the lead and cadmium analysis. A typical response factor for thallium is 4 ng/μA when the sample plating time is 30 min. Since the presence of EDTA prevents the ASV response to lead and cadmium but does not affect the response to thallium, the peak height obtained for thallium alone may be used to estimate the response due to lead or cadmium alone.

3.2 Incomplete digestion of a sample, indicated by a yellowish coloration, may lead to peak broadening and anomalous results.

4. Precision and Accuracy

4.1 Several processes determine the reproducibility of the overall analytical method: (1) sampling, (2) shipping, (3) preparation of samples, and (4) analysis. The precision of sampling has been evaluated for ambient air sampling with flow control by a critical orifice. When samples were collected at the rate of 2 ℓ/min through each of six filters arranged in the same relative geometry, no significant variation in the deposition of particles was noted. The precision of sampling with personal sampling pumps has not been evaluated; however, it is estimated that variations observed with personal sampling pumps will be greater than those with flow control by a critical orifice.

4.2 Shipping samples collected from ambient air according to the procedure described in Section 8.2 has no significant effect on precision. Since workroom atmospheres may contain more particulate material encompassing a wider size range than ambient air, precision may be affected by the loss of particles during shipping of filters loaded in such atmospheres. However, analyses of washings from filter holders loaded in workroom atmospheres have indicated that no lead-containing particulate is lost during transport.

4.3 The variations introduced by the preparation and analysis steps have been empirically derived and may be estimated for the entire analytical range from the following relations:

- lead,

$$CV = \frac{0.08}{[Pb]} + 0.05$$

- cadmium,

$$CV = \frac{0.04}{[Cd]} + 0.05$$

where: CV = the estimated coefficient of variation of a measurement.
[Pb], [Cd] = the measured concentration of lead or cadmium (in mg/m^3) in a 100-liter air sample.

The factor 0.08 or 0.04 in the numerator of the first term of the right-hand side of each expression reflects the scatter in the filter blank, which corresponds to standard deviations of ± 8 ng of lead and ± 4 ng of cadmium, or 0.00008 mg/m^3 of lead and 0.00004 mg/m^3 of cadmium in a 100-liter air sample. The second term represents the normal statistical variations in the preparation and analysis steps. (See References 11.1 and 11.2) The estimated coefficients of variation for the measurement of 0.002 mg/m^3 of lead and 0.0005 mg/m^3 of cadmium in air are, respectively, 0.09 and 0.13.

4.4 The accuracy of the overall sampling and analytical method has not been evaluated. However, it is estimated that the percent error in a measurement, neglecting any contribution from sampling and shipping, is less than about 10% in the determinations of levels near 200 ng of lead or 50 ng of cadmium in 100-liter air samples, which corresponds to concentrations near 0.002 or 0.0005 mg/m^3, respectively.

5. Advantages and Disadvantages of the Method

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 One disadvantage is that thallium, when present in comparable amounts, will interfere with the determinations of lead and cadmium.

6. Apparatus

6.1 **Air Sampling Equipment.** The sampling unit for the collection of particulate samples has the following components:

6.1.1 **Filter Unit.** The unit consists of the filter in an appropriate holder such as the Millipore Aerosol Analysis Monitor, Catalog No. MAWP 037 AO, or equivalent. This unit is a plastic disposable holder preassembled with a filter in place. The recommended filter is the Millipore Type AA, a mixed cellulose ester, with a pore size of 0.8 μm and a diameter of 37 mm.

6.1.2 **Adaptor.** An adaptor such as the Millipore Aerosol Adaptor, Catalog No. XX62 000 04, is inserted into the filter unit to allow a hose to be attached.

6.1.3 **Personal Sampling Pump.** The pump should be calibrated with a representative filter unit in the sampling line. A wet or dry test meter or a glass rotameter capable of measuring the appropriate flow rate (1.5 to 2 ℓ/min) to within ± 5% may be used in the calibration.

6.2 Pyrex test tubes (20 x 100 mm) for digesting filter samples and subsequent use as analysis cells.

6.3 Digestion racks for the analysis cells.

6.4 Micro pipettes, Eppendorf or equivalent (10, 100, and 300 $\mu\ell$).

6.5 Model SA 2011 Single-Cell Anodic Stripping Voltammeter with composite mercury-graphite electrode, Environmental Sciences Associates, Inc., Burlington, Mass., or equivalent. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations. Instructions for the preparation and reconditioning of the CMGE are included in the instruction manual.

6.6 Potentiometric recorder (100-mV range).

6.7 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric pipettes, and volumetric flasks of appropriate sizes.

7. Reagents

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in lead and cadmium. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 **Acid Ashing Solution.** Prepare the acid ashing solution by adding 24 volumes of concentrated nitric acid to 24 volumes of perchloric acid (70%) and 1 volume of concentrated sulfuric acid.

7.5 **Acetate Buffer Solution (1 *M* NaAc, 0.2 *M* NaCl, pH 5).** To prepare 1 liter of this reagent, dissolve 82 g of sodium acetate, 58.5 g of sodium chloride, and 30 mℓ of 70% $HClO_4$ in water in a 1-liter volumetric flask and fill to the mark with water. Remove trace metals from the resulting solution electrolytically with a system such as the Model 2014PM Reagent Cleaning System, Environmental Sciences Associates, Inc., or equivalent. The acetate buffer maintains the pH of the resulting sample solution near 5, a pH favorable for lead and cadmium determination. The chloride improves the resolution of the lead and cadmium peaks and reduces the effect of pH variations on the determination of lead and cadmium.

7.6 **Lead (Pb(II)), Standard Solution "A" (10 mg/mℓ).** Transfer 1.5987 g of lead nitrate ($Pb(NO_3)_2$) to a clean 100-mℓ volumetric flask. Dissolve the salt in approximately 30 mℓ of water, add 3 mℓ of concentrated nitric acid, and dilute to the mark with water.

7.7 **Lead (Pb(II)), Standard Solution "B" (100 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "A" to a 100-mℓ volumetric flask containing 1 mℓ of concentrated nitric acid and dilute to the mark with water.

7.8 **Lead (Pb(II)), Standard Solution "C" (1 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "B" to a 100-mℓ volumetric flask containing 1mℓ of concentrated nitric acid and dilute to the mark with water.

7.9 **Cadmium (Cd(II)), Standard Solution "D" (10 mg/mℓ).** Transfer 2.0315 g of cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) to a clean 100-mℓ volumetric flask. Dissolve the salt in approximately 50 mℓ of water, add 2 mℓ of concentrated nitric acid, and dilute to the mark with water.

7.10 **Cadmium (Cd(II)), Standard Solution "E" (100 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "D" to a 100-mℓ volumetric flask containing 1 mℓ of concentrated nitric acid and dilute to the mark with water.

7.11 **Cadmium (Cd(II)), Standard Solution "F" (1 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "E" to a 100-mℓ volumetric flask containing 1 mℓ of concentrated nitric acid and dilute to the mark with water.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any lead or cadmium from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 200 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove and allow to cool. Complete decontamination requires four repetitions of this procedure. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom. Allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water.

8.2 Collection and Shipping of Samples

8.2.1 Connect the exit end of a filter unit to the adaptor. Connect the adaptor to the pump with a short piece of flexible tubing.

8.2.2 Sample the air at a flow rate of 1.5 to 2 ℓ/min. Measure the flow rate and time, or volume, as accurately as possible. A minimum sample of 100 liters should be collected. Larger volumes are desirable, provided the filter does not become loaded with dust to the point that loose material could fall off or the filter become plugged.

8.2.3 The filter holders should be shipped in a suitable container to minimize contamination and to prevent damage in transit. Care must be taken during storage and shipping that no part of the sample is dislodged from the filter nor that the sample surface is disturbed in any way. Loss of sample from heavy deposits on the filter may be prevented by mounting a clean filter in the holder on top of the sample filter.

8.2.4 Unexposed filters in filter holders should be labeled as blanks and shipped with the samples.

8.3 Analysis of Samples

8.3.1 Carefully remove the filter from the filter unit by opening the unit, gently pushing up the filter and its backing, and grasping the unexposed edge of the filter with stainless steel forceps. Fold the filter in half, then into quarters, and subsequently into eighths without creasing or cracking the filter material. Insert the filter into an analysis cell and push it down into the cell with a glass rod.

8.3.2 Pipette 300 μℓ of the acid ashing solution into the analysis cell containing the filter. Wet the filter as thoroughly as possible by adding the acid slowly. Place the cell in a digestion rack on a hot plate operating at 220 to 240°C. Carry out the digestion in a hood. The filter sample will clear in about 10 min, and a reflux line will be established about halfway up the cell. At the end of 20 min, there will be approximately 100 to 200 μℓ of acid left in the cell, with most of the nitric acid having evaporated. The digestion should be continued an additional 20 to 30 min until the acid is almost completely evaporated.

8.3.3 Remove the sample from the hot plate and allow it to cool. If the sample is estimated to contain less than 1 μg of lead or cadmium, add 4.5 mℓ of the acetate buffer solution.

8.3.4 If the sample is estimated to contain more than 1 μg of lead or cadmium, add 10 mℓ of 1 N $HClO_4$. Heat the solution to about 90°C for 30 min, cool it, and readjust its volume to 10 mℓ with 1 N $HClO_4$ if necessary. Take an aliquot of appropriate volume (10 to 100 μℓ) from the sample solution for analysis. (The error resulting from the presence of lead or cadmium in a 100-μℓ aliquot of the dilute perchloric acid will not be greater than 0.5 to 1.0%.) Place the aliquot into another empty analysis cell and add 4.5 mℓ of the acetate buffer solution.

8.3.5 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the buffered solution under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -1000 mV
- Auto-hold cutoff potential, 960 mV
- Plating time, 15 to 30 min. (The plating time for a set of samples and standards must be the same.)
- Sweep rate, +60 mV/sec
- Current range, 0.1 to 2 mA (full scale)

The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of lead or cadmium plated on the electrode may occur. As the sample is stripped, the cadmium peak (-675 mV) will occur first, followed by the lead peak (-510 mV). A baseline is drawn by connecting with a straight line the flat portion of the curve before the cadmium peak with the flat portion after the lead peak. Each peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions: (1) filter and reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solutions of lead and cadmium in acetate buffer; (4) filter standards prepared as described below in Section 9.1. The sequence in which these four kinds of solutions are analyzed is as follows: (1) a set of standard solutions of lead and cadmium in acetate buffer; (2) sample solutions, with filter and reagent blanks and filter standards interspersed at the rate of one blank and one filter standard for every ten samples; and (3) a second set of standard solutions of lead and cadmium in acetate buffer. The standard solutions of lead and cadmium in acetate buffer are used for calibration; the results for the samples are corrected for the amounts of lead and cadmium found in the reagent blanks; and the filter standards are used to determine the efficiency of recovery of known amounts of lead and cadmium. The procedure described is for use with a four-cell ASV unit.

9.1 **Preparation of Lead and Cadmium Filter Standards.** Prepackaged filters of the type used for sampling are removed from their plastic cases and separated from their support mats. The filters are placed on the center ring supports of the plastic cases so that only the edge of the filter is touching the rim of its holder. Lead standard solution "B" and cadmium standard solution "E" are appropriately diluted to provide 50-, 100-, 200-, and 500-ng amounts in 10-$\mu\ell$ volumes. These solutions are placed on the centers of the filters and allowed to evaporate and air-dry for approximately 30 min. Each filter is then placed back in its holder. These filter standards are analyzed along with the calibration standards as outlined in Section 9.3. The results obtained with the filter standards are used to estimate the percent recovery of lead and cadmium.

9.2 **Initial Analysis of Four Standards.** Four standards, each containing 200 ng of lead and 50 ng of cadmium, are prepared by adding 200 $\mu\ell$ of lead standard solution "C" and 50 $\mu\ell$ of cadmium standard solution "F" to 4.5 mℓ of the acetate buffer solution. Each of the four standards should be analyzed with a different electrode by the procedure of Section 8.3. At the levels of 200 ng of lead and 50 ng of cadmium, the contributions of the reagent blank should be neglible and a single point for the lead or cadmium calibration curve usually is sufficient.

9.3 **Analysis of Samples and Controls.** With every ten samples, a filter standard (Section 9.1) and a reagent blank are analyzed by the ASV procedure given in Section 8.3. At least one reagent blank and one filter standard should be analyzed with each electrode that is used for analysis of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 200 ng of lead (or 50 ng of cadmium) will be obtained for each of the four electrodes. These values are used to calculate a response factor for lead (or cadmium) in ng/μA. The overall response factor is the amount of lead (or cadmium) in each standard (100 or 50 ng, respectively) divided by the average of the eight peak heights (in μA). However, it will be necessary to calculate an individual response factor for an electrode if the average of the two peak height values for lead (or cadmium) for an individual electrode differs from the overall average of the eight peak heights by more than 10%. For example, if the average for the eight peak heights for lead is 210 μA, and the average of the two values for one of the electrodes is 185 μA, then the response factor for lead to be used for that electrode is 200 ng/185 μA, or 1.08 ng/μA. The overall response factor for lead in this example is 200 ng/210 μA, or 0.95 ng/μA. A typical response factor for cadmium is 0.58 ng/μA.

10. Calculations

10.1 The amount of lead or cadmium in a sample is calculated from the lead or cadmium peak height (in μA), as measured according to Section 8.3.5, and the response factor in the following manner:

$$\text{Amount (in ng)} = \text{peak height (in } \mu\text{A)} \times \text{response factor (in ng/}\mu\text{A)}$$

The average amount of lead or cadmium found in unexposed filters and reagent blanks should be subtracted from the sample values to give the correct weight (in ng) of lead or cadmium in the sample. The average recovery of lead and cadmium from filter standards is usually greater than 90%. However, it may be necessary to include a factor in the above equation to correct for incomplete recovery.

10.2 The concentration of lead or cadmium may be expressed in mg/m^3, which is numerically equal to $\mu g/\ell$:

$$mg/m^3 = \frac{\text{corrected weight (in ng) in sample}}{V_s\ (\ell) \times 1000\ (ng/\mu g)}$$

where: V_s = volume of air sampled.

11. References

11.1 Environmental Sciences Associates, Inc., Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Divison of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

11.2 Matson, W.R., R.M. Griffin, and T.J. Sapienza, "A Total System and Quality Control Procedure for Micro Blood Lead Analysis for Anodic Stripping Voltammetry (ASV)," presented at the Laboratory Director's Conference, Department of Health, Education, and Welfare, Center for Disease Control, August 23, 1974.

ARSENIC IN BLOOD

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Arsenic	**Method No.:** P&CAM 192
Matrix: Blood	**Range:** 0.016 to 0.6 μg/mℓ in 1 mℓ of blood
Procedure: Anodic stripping voltammetry	**Precision (CV_T):** 0.08 over the above range
Date Issued: 10/5/73	**Classification:** E (Proposed)
Date Revised: 1/29/76	

1. **Principle of the Method**

A sample of whole blood is wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The resulting As(V) is reduced to As (III) with an acidic solution of sodium bisulfite and ferrous perchlorate. An acid solution of hydrazine dihydrochloride is added to prevent air oxidation of Fe(II) ions. The chemically reduced sample is then analyzed by anodic stripping voltammetry (ASV) with a composite gold-graphite electrode (CGGE).

2. **Range and Sensitivity**

2.1 The sensitivity is limited by the reagent blank and the background characteristics of the electrode. A reasonable detection limit appears to be two times the standard deviation of the reagent blank. Statistically, the probability is 95% that arsenic is present in the sample if the signal is higher than this limit. Blanks for arsenic are usually 15 ± 8 ng. The detection limit of the method is thus estimated to be about 16 ng/mℓ in a 1-mℓ sample of blood.

2.2 The linear range of the electrode response is estimated to extend from the detection limit to 600 ng of arsenic. This upper limit corresponds to 0.6 μg/mℓ of arsenic in a 1-mℓ sample of blood. The range may be extended by analyzing a smaller blood sample or by diluting the sample digestate with the acid solution of hydrazine dihydrochloride (Section 7.9) and taking an aliquot of the diluted solution for the ASV determination.

3. **Interferences**

3.1 Antimony, bismuth, and tin will form resolvable shoulders on the cathodic edge of the arsenic peak. Mercury will form a shoulder on the anodic edge of the arsenic peak. Tin has been found not to interfere at concentrations 100 times that of arsenic. Mercury, bismuth, and antimony do not seriously affect the arsenic signal at concentrations up

to 10 times that of arsenic. Higher concentration ratios of mercury, bismuth, and antimony have not been tested. However, mercury should present little, if any, problem at higher concentrations since it is usually lost during digestion. If the concentrations of bismuth and antimony are suspected to be significant, as in samples taken in metallurgical industrial environments, the samples should also be analyzed with a composite mercury-graphite electrode (CMGE) at a plating potential of -350 mV to determine the amounts of bismuth and antimony present.

3.2 Copper and arsenic are not completely resolved at high levels of both metals. The available data suggest that when the absolute quantity of copper exceeds 1 μg, the linear range of electrode response to arsenic will decrease.

3.3 Incomplete digestion of a sample, indicated by a yellowish coloration, may lead to peak broadening and anomalous results.

4. Precision and Accuracy

All of the factors that affect the accuracy and precision of the total method for the determination of arsenic in blood have not been fully evaluated. An estimate of the coefficient of variation is 0.08; also, it is estimated that results from the analysis of sample unknowns should fall within 10% of the "true" values.

5. Advantages and Disadvantages of the Method

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 One disadvantage is that the current-voltage peaks for copper and arsenic are not completely resolved when their concentrations are comparable.

5.4 Another disadvantage is the occurrence of large variations in response from electrode to electrode.

6. Apparatus

6.1 Vacutainer tubes (5 mℓ), heparinized; Becton, Dickinson, and Company, or equivalent.

6.2 Model W-104-E Sonifier, Heat Systems-Ultrasonics, Inc., or an equivalent ultrasonic mixer with a microprobe tip having a diameter no larger than 0.2 in.

6.3 Pyrex test tubes (20 x 100 mm) for digesting blood samples and subsequent use as analysis cells.

6.4 Digestion racks for analysis cells.

6.5 Micro pipettes (10, 50, 100, 200, 300, and 500 $\mu\ell$, and 1 mℓ, Eppendorf or equivalent.

6.6 Disposable pipettes, graduated (5 and 10 mℓ).

6.7 Potentiometric recorder (100-mV range).

6.8 Assorted laboratory glassware, including beakers, graduated cylinders, and volumetric flasks of appropriate sizes.

6.9 **Anodic Stripping Voltammeter.** A suitable single-cell instrument with a composite mercury-graphite electrode (Model SA 2011) is available from Environmental Sciences Associates, Burlington, Mass. Equivalent instrumentation may be used. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations.

6.10 **Modification of the Model SA 2011.** The slight modification described below is suggested to improve the mixing of the high-salt-content solutions necessary for the determination of arsenic. This modification is best performed while the test electrode is out of the cell for routine maintenance. Throw the plate-strip switch to "strip" with the cell selector on "EXT." Disconnect and remove the test electrode. Place the test electrode in a beaker of water to keep it wet. With thumb and forefinger, grasp the bent end of the nitrogen bubbler and pull it down and away from the cell head until all of the platinum inside the tubing has been drawn past the retaining loop on the bottom of the reference electrode. Straighten the bend in the Teflon-covered platinum. With the platinum tip of the counter electrode, push the platinum wire in the nitrogen bubbler tube further up inside the bubbler tube so that the solution end of the tube is empty for the last 1 to 3 mm of its length. Rebend the platinum and tubing to the original configuration and return the bubbler tube to its original position and configuration. Replace the test and counter electrodes, reconnect all wire junctions broken, and throw the plate-strip switch back to "plate". This procedure provides an unobstructed gas-solution interface so that micro precipitation due to evaporation of solution trapped inside of the bubbler will not lead to reduced stirring rates.

6.11 **Composite Gold-Graphite Electrode (CGGE).** A CGGE may be prepared from a new waxed graphite electrode or one which has been used as a composite mercury-graphite electrode.

6.11.1 If the waxed graphite electrode has previously been used as a CMGE, withdraw the electrode from the cell head and remove the mercury coat by wiping with a damp soft paper towel. Place the electrode back in the cell head and electrolytically remove the remaining mercury by holding the

potential of the electrode at +1.0 V in 8 mℓ of a nitrogen-stirred solution of 1 *N* HNO_3 for 15 min. Remove the nitric acid solution and rinse the electrode throughly in a 250-mℓ beaker of distilled deionized water. Remove the electrode from cell head, wipe dry, and resurface the electrode. The resurfacing step is accomplished by gently pinching the polished end of the electrode in 4/0 emery paper between thumb and forefinger of one hand while rotating the electrode with the other hand. If the electrode is rotated a large number of times as it is withdrawn from the emery paper, a light gray silvery suface approximating that of spun aluminum will be obtained. Wipe the resurfaced area very gently with the dampened edge of a soft paper towel to remove any particles of graphite clinging to the refinished surface. Carefully place the electrode back into position in the cell head.

6.11.2 If the electrode is new, then resurface as described above; hold at +1.0 V in 8 mℓ of a nitrogen-stirred solution of approximately \l *N* HNO_3, and rinse well with distilled water.

6.11.3 Immerse the electrodes to be gold plated in analysis cells, each of which contains 5 mℓ of 5 *N* HCl and 100 μℓ of the gold plating solution. (See Section 7.14.) Stir for 60 sec with the nitrogen bubbler on and then turn off the nitrogen. Set the plating potential to -300 mV and throw the plate-strip switch to "plate". Plate the electrodes for 10 to 12 min from an unstirred solution and then for 60 sec from a stirred solution. Arrest plating by immersing the electrodes in the hydrazine dihydrochloride-acid solution. At the end of the plating step the electrodes may range in color from dull brown to bright yellow. Most of the electrodes will be a dull greenish yellow.

6.11.4 When they are not in use, store the electrodes at a potential of +300 mV in a solution consisting of 5 mℓ of 5 *N* HCl and 0.3 mℓ of the ferrous perchlorate reagent. Before use, soak each electrode in 5 mℓ of the ferrous perchlorate reagent for 5 min at -200 mV.

7. Reagents

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in arsenic. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly vacuum distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 **Acid Ashing Solution.** Prepare the acid ashing solution by adding 24 volumes of concentrated nitric acid to 24 volumes of perchloric acid (70%) and 1 volume of concentrated sulfuric acid.

7.5 **Sodium Bisulfite Solution (1 *N*).** Add 10.4 g of sodium bisulfite to a 100-mℓ volumetric flask, dissolve in water, and dilute to the mark.

7.6 Hydrazine dihydrochloride.

7.7 **Ferrous Perchlorate Reagent.** There are two alternate methods of preparing this reagent.

7.7.1 Add 100 mg of iron (electrolytic grade) to a 50-mℓ beaker. Add 5 mℓ of distilled deionized water and 9 mℓ of 70% perchloric acid. Warm gently to aid in dissolution. After dissolution, transfer the contents of the beaker to a 100-mℓ volumetric flask containing 0.5 g of hydrazine dihydrochloride. Rinse the beaker several times with water and add the washings to the volumetric flask. Dilute to the mark with water.

7.7.2 Add 0.650 g of ferrous perchlorate, $Fe(ClO_4)_2 \cdot 6H_2O$, and 9 mℓ of 70% perchloric acid to a 100-mℓ volumetric flask containing 0.5 g of hydrazine dihydrochloride in approximately 30 mℓ of water. Dissolve and dilute to the mark with water.

7.8 Hydrochloric acid, concentrated.

7.9 **Hydrazine Dihydrochloride-Acid Solution (5 *N* HCl, 0.001 *N* $H_2NNH_2 \cdot (HCl)_2$).** Add 0.105 g of hydrazine dihydrochloride to a 1-liter volumetric flask. Add approximately 400 mℓ of water and swirl to dissolve the salt. Add 417 mℓ of concentrated hydrochloric acid, swirl, and dilute to the mark with water. This solution may be conveniently stored in a polyethylene bottle with a screw cap.

7.10 **Sodium Hydroxide Solution (1.5 *N*).** Add 6 g of sodium hydroxide to a 100-mℓ volumetric flask with a polyethylene stopper, dissolve in water, and dilute to the mark.

7.11 **Arsenic (As(III)), Standard Solution "A" (10 mg/mℓ).** Transfer 1.320 g of arsenic trioxide (As_2O_3) to a clean 100-mℓ volumetric flask. Add 35 mℓ of 1.5 *N* NaOH. Immediately after dissolution of the arsenic trioxide, acidify the solution with 25 mℓ of concentrated hydrochloric acid and cool to room temperature. Then dilute to the mark with water.

7.12 **Arsenic (As(III)), Standard Solution "B" (100 μg/mℓ).** Add approximately 80 mℓ of water and 2 mℓ of concentrated hydrochloric acid to a 100-mℓ volumetric flask. Swirl to achieve a homogeneous solution and then add 1 mℓ of standard solution "A." Dilute to the mark with water.

7.13 **Arsenic (As(III)), Standard Solution "C" (1 μg/mℓ).** Repeat the procedure for preparing standard solution "B", but add 1 mℓ of standard solution "B" instead of standard solution "A." During the first two months, prepare standard solution "C" daily and standard solution "B" weekly. Pour out the old solution and use the same flask for making up the new solution. After the two-month period for conditioning the flasks, prepare standard solution "C" weekly, standard solution "B" monthly, and standard solution "A" semiannually.

7.14 **Gold Plating Solution.** Place 1.00 g of high purity gold metal in a 100-mℓ beaker. In a hood, add 2.5 mℓ of concentrated nitric acid and 10 mℓ of concentrated hydrochloric acid to the beaker. Warm gently until all of the gold has dissolved. Add 5 mℓ more of concentrated hydrochloric acid and allow the beaker to cool. Quantitatively transfer the contents of the beaker to a clean 100-mℓ volumetric flask. Rinse the beaker several times with 15-mℓ portions of water and add each rinse to the volumetric flask. Dilute to the mark with water. This solution now contains 10 mg/mℓ of gold in acid solution.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any arsenic from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 220 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove, and allow them to cool. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom. Allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water. Polyethylene bottles used in sample collection are cleaned by soaking in 2.5% perchloric acid for 24 hr. The bottles should not be rinsed with concentrated acid as this may cause sample loss.

8.2 **Collection and Shipping of Samples.** The samples are collected in Vacutainer tubes according to established procedures. The samples should be shipped to the laboratory in the sampling containers in a box with a minimum of 0.5 in. of foam packing to prevent breakage.

8.3 Analysis of Samples

8.3.1 Rinse the ultrasonic mixer tip thoroughly in distilled deionized water. Place the tip into a blood sample within 2 cm of the bottom of the Vacutainer tube, and mix at the minimum permissible power for 20 to 30 sec. If a sample in a Vacutainer tube sits for more than 10 min after it has been ultrasonically mixed, then remix it before taking a portion for analysis. Pipette 1 mℓ of the blood into an analysis cell. Rinse the pipette once with distilled deionized water and add the rinse to the analysis cell. Duplicate 1-mℓ aliquots of the blood sample should be taken for analysis.

8.3.2 Pipette 1.5 mℓ of the acid ashing solution into the analysis cell containing 1 mℓ of blood. Swirl the cell slightly to mix the acid with the blood to prevent localized clotting.

8.3.3 Place the cell in a digestion rack on a hot plate with a surface temperature of approximately 130°C. Carry out the digestion in a hood. Initially, the samples will tend to foam. Watch closely during this phase of the digestion (approximately 10 to 15 min) to insure that the foam does not climb out of the cell. If loss by foaming seems inevitable, remove the cell from the rack and gently tap its base against the hood floor. Replace the cell in the rack. After foaming has ceased and the sample has evaporated to approximately half its original volume, raise the hot plate surface temperature to 220 to 240°C. At the end of 40 min, there will be approximately 300 to 500 μℓ of acid left in the bottom of the cell, with most of the nitric acid having evaporated. The digestion should be continued for an additional 40 to 60 min until the acid is almost completely evaporated.

8.3.4 Add 1 mℓ of 1 *N* $NaHSO_3$ 200 μℓ of the ferrous perchlorate reagent, and 300 μℓ of concentrated hydrochloric acid to the cell. Heat the cell on a hot plate to 85 to 95°C for 15 min. Do not evaporate the solution to dryness. Allow it to cool to room temperature and dilute it to 5-mℓ with the hydrazine dihydrochloride-acid solution.

8.3.5 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the sample solution with the CGGE under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -200 mV
- Auto-hold cutoff potential, 720 mV
- Plating time, 15 min
- Sweep rate, +60 mV/sec
- Current range, 0.5 to 1.0 mA (full scale)

The nitrogen stirring should be on during the plating step and should be turned off 15 sec prior to the stripping step. The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of arsenic plated on the electrode may occur. As the sample is stripped, the arsenic peak (+195 mV) occurs first, and the copper-iron peak occurs second. After the auto-hold cutoff potential is reached, remove the cell and rinse the electrode by swishing the cell-head assembly up and down while immersed in 230 mℓ of distilled deionized water containing 10 mℓ of concentrated hydrochloric acid. Place the next sample on the electrode cell head and reset the potential of the cell to the plating potential. Turn the nitrogen stirring on and reset the timer. A baseline is drawn by connecting with a straight line the minimum point between the copper and arsenic peaks with the flat portion of the curve preceding the arsenic peak. The peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions: (1) reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solutions of arsenic; (4) blood standards prepared as described below in Section 9.1. The sequence in which these four kinds of solutions are analyzed is as follows: (1) a set of standard solutions of arsenic; (2) sample solutions, with reagent blanks and blood standards interspersed at the rate of one blank and one blood standard for every ten samples; and (3) a second set of standard solutions of arsenic. The standard solutions of arsenic are used for calibration; the results for the samples are corrected for the amount of arsenic found in the reagent blank; and the blood standards are used to determine the efficiency of recovery of known amounts of arsenic from blood. The procedure described is for use with a four-cell ASV unit.

9.1 Preparation of Blood Standards

9.1.1 A "normal" blood sample from an individual not suspected of exposure to arsenic or a sample from a blood bank is analyzed by the method of standard additions. One-milliliter samples of the blood are placed in each of five Vacutainer tubes. Four of these samples are spiked with known amounts of arsenic (50-, 100-, 200-, and 300-μℓ aliquots of standard solution "C," containing 50, 100, 200, and 300 ng of arsenic, respectively). These samples are ultrasonically mixed, transferred to analysis cells, digested. and analyzed by ASV according to the procedure given in Section 8.3. The concentration of arsenic in the unspiked "normal" blood is then estimated.

9.1.2 Finally, an amount of standard arsenic solution is added to 1.0 mℓ of the unspiked "normal" blood to make the total amount of arsenic equal to 100 ng. This standard blood solution is subsequently analyzed along with calibration standards and blanks (Section 9.3) to determine the percent recovery of arsenic.

9.2 **Initial Analysis of Four Standards.** Solutions consisting of 5 mℓ of the hydrazine dihydrochloride-acid solution and 200 μℓ of the ferrous perchlorate reagent are placed in each of four cells and analyzed as indicated in Section 8.3.5. The plating of each cell solution should be started sequentially at 1-min intervals. After stripping is completed, add 100 ng of arsenic (100 μℓ of standard arsenic solution "C") and plate and strip once again. A single point may be taken to describe the calibration curve, which has been determined to be linear over the range 25 to 600 ng of arsenic.

9.3 **Analysis of Samples and Controls.** With every ten samples, a blood standard (Section 9.1.2) and a reagent blank are analyzed by the ASV procedure given in Section 8.3. At least one reagent blank and one blood standard should be analyzed with each electrode that is used for analysis of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 100 ng of arsenic will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA for each electrode since the peak heights may vary significantly from electrode to electrode. The response factor for a particular electrode is the amount of arsenic in the standard (100 ng) divided by the average of the two peak height values obtained for that electrode. For a 15-min plating time, typical response factors range from 0.75 to 1.4 ng/μA.

10. Calculations

10.1 The amount of arsenic in a sample is calculated from the peak height as measured according to Section 8.3.5, the response factor, and the correction factor in the following manner:

$$\text{Amount (ng)} = \frac{\text{peak height } (\mu A)}{C} \times \text{response factor } (ng/\mu A)$$

where: C = a correction factor equal to the average fraction of arsenic recovered from the blood standards with the particular electrode used. If the correction factor is greater than 0.90, the value of C may be taken to be unity.

Any amount of arsenic found in the reagent blank should be subtracted from the sample value to give the correct amount (in ng) of arsenic in the sample.

10.2 The concentration of arsenic in a blood sample may be expressed as $\mu g/m\ell$:

$$\mu g/m\ell = \frac{\text{corrected weight (in ng) of arsenic in sample}}{\text{volume (in m}\ell\text{) of sample x 1000 (ng/}\mu g\text{).}}$$

11. References

11.1 Environmental Sciences Associates, Inc., Burlington, Mass.. Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Division of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

11.2 "General Method: Arsenic Analysis by Anodic Stripping Voltammetry (ASV)", ESA Methodology M-43, Environmental Sciences Associates, Inc., Burlington, Mass.

ANTIMONY IN BLOOD

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Antimony	**Method No.:** P&CAM 193
Matrix: Blood	**Range:** 0.003 to 0.5 μg/mℓ in 1 mℓ of blood
Procedure: Anodic stripping voltammetry	**Precision** (CV): 0.10 (analytical) at 0.02 μg/mℓ
Date Issued: 4/10/73	**Classification:** E (Proposed)
Date Revised: 1/29/76	

1. Principle of the Method

A sample of whole blood is wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The resulting Sb (V) is reduced to Sb (III) with a solution of hydrochloric acid and hydrazine dihydrochloride. The chemically reduced sample is analyzed by anodic stripping voltammetry (ASV) with a composite mercury-graphite electrode (CMGE). In principle, a hanging mercury drop electrode (HMDE) can also be used. However, optimum analysis parameters may vary significantly and must be established for each electrode system. The method as presented is designed for use with the CMGE.

2. Range and Sensitivity

2.1 The sensitivity of the procedure for antimony is limited by chemical interferences in the reduction of Sb(V) to Sb(III) and by interferences of copper and bismuth with the ASV determination of antimony. In the absence of copper and bismuth, as in reagent blanks, the detection limit is about 0.5 to 1 ng; however, in typical industrial blood samples, which may contain high concentrations of copper and perhaps bismuth, the detection limit is 2 to 4 ng, or about 0.003 μg/mℓ in a 1.0-mℓ sample of blood.

2.2 The linear range of the electrode response extends from the detection limit to 500 ng of antimony, which corresponds to 0.5 μg/mℓ in a 1-mℓ sample of blood. The upper limit of the range can be extended to 2.5 μg/mℓ by taking a 200-μℓ blood sample and digesting it in 500 μℓ of the acid ashing solution instead of 1.5 mℓ. (See Sections 8.3.1 and 8.3.2.)

3. Interferences

3.1 The presence of copper (II) ions in the sample digestate appears to accelerate the oxidation of antimony (III) to antimony (V). This interference is minimized by the presence of an amount of reducing agent in excess of that required for the reduction of antimony (V) to antimony (III) and copper (II) to copper (I).

3.2 Bismuth is stripped at the same potential as antimony. If this interference is suspected, the sample should be analyzed for bismuth after the addition of 20 μℓ of 5% sodium hypochlorite solution to oxidize antimony (III) to antimony (V). A reduction of the peak height by 80% or more signifies that the peak was due to antimony only.

3.3 The formation of an interamalgam complex of copper and antimony during the plating step is minimized by the presence of a relatively large concentration of chloride ions.

3.4 Incomplete digestion of a sample, indicated by a yellowish coloration, may lead to peak broadening and anomalous results.

4. Precision and Accuracy

4.1 Several processes determine the reproducibility of the overall analytical method: (1) sampling, (2) shipping, (3) preparation of samples, and (4) analysis. The variation introduced by sampling blood in industrial plants has not been evaluated. The shipping of samples in Vacutainer tubes, as described in Section 8.2, and storage for 1 week prior to analysis produce a negligible effect on the precision. The coefficient of variation for the analysis step alone is 0.04 over the range of 10 to 500 ng. Measurements with two different electrodes with different histories may differ by a factor of two; however, the coefficient of variation remains 0.04 for measurements with each electrode. The coefficient of variation for the preparation and analysis steps combined is 0.10 over the range of 20 to 250 ng, which corresponds to 0.02 to 0.25 μg/mℓ in a 1.0-mℓ sample of blood.

4.2 The estimated percent error in a measurement, neglecting any contribution from sampling, is less than about 10% of the "true" value in the range of 20 to 250 ng.

5. Advantages and Disadvantages of the Method

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 The primary disadvantage is its sensitivity to interferences. (See Section 3.)

5.4 Another disadvantage is the occurrence of large variations in response from electrode to electrode.

6. Apparatus

6.1 Vacutainer tubes (5 mℓ), heparinized, Becton, Dickinson and Co., or equivalent.

6.2 Model W-140-E Sonifier, Heat Systems-Ultrasonics, Inc., or an equivalent ultrasonic mixer with a microprobe tip having a diameter no larger than 0.2 in.

6.3 Pyrex test tubes (20 x 100 mm) for digesting blood samples and subsequent use as analysis cells.

6.4 Digestion racks for the analysis cells.

6.5 Micro pipettes (5, 10, 20, 30, 50, and 500 μℓ, and 1 mℓ), Eppendorf or equivalent.

6.6 Model SA 2011 Single-Cell Anodic Stripping Voltammeter with composite mercury-graphite electrode, Environmental Sciences Associates, Inc., Burlington, Mass., or equivalent. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations. Instructions for the preparation and reconditioning of the CMGE are included in the instruction manual.

6.7 Potentiometric recorder (100-mV range).

6.8 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric flasks, volumetric pipettes, and graduated pipettes of appropriate sizes.

7. Reagents

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in antimony. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 Acid Ashing Solution. Prepare the acid ashing solution by adding 24 volumes of concentrated nitric acid to 24 volumes of perchloric acid (70%) and 1 volume of concentrated sulfuric acid.

7.5 **Hydrazine Dihydrochloride-Acid Solution** (5 *N* HCl, 0.001 *N* $H_2NNH_2 \cdot (HCl)_2$). Add 0.105 g of hydrazine dihydrochloride to a 1-liter volumetric flask. Add approximately 400 mℓ of water and swirl to dissolve the salt. Add 417 mℓ of concentrated hydrochloric acid, swirl, and dilute to the mark with water. This solution may be conveniently stored in a polyethylene bottle with a screw cap.

7.6 Antimony Reference Standard Solution (1000 μg/mℓ), Fisher Scientific Co., or equivalent.

7.7 **Antimony (Sb(III)), Standard Solution "B"** (100 μg/mℓ). Transfer 10 mℓ of the antimony reference standard solution (1000 μg/mℓ) with a volumetric pipette to a 100-mℓ volumetric flask and dilute to the mark with water. Prepare fresh daily.

7.8 **Antimony (Sb(III)), Standard Solution "C"** (1 μg/mℓ). Transfer 1 mℓ of standard solution "C" with a volumetric pipette to a 100-mℓ volumetric flask and dilute to the mark with water. Prepare fresh daily.

7.9 Sodium Hypochlorite Solution (4 to 6%, w/w).

8. **Procedure**

8.1 **Cleaning of Equipment**

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any antimony from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 220 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove, and allow them to cool. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom and allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water.

8.2 **Collection and Shipping of Samples.** The samples are collected in Vacutainer tubes according to established procedures. The samples should be shipped to the laboratory in the sampling containers in a box with a minimum of 0.25 in. of foam packing to prevent breakage.

8.3 Analysis of Samples

8.3.1 Rinse the ultrasonic mixer tip thoroughly in distilled deionized water. Place the tip into a blood sample within 2 cm of the bottom of the Vacutainer tube, and mix at the maximum permissible power for 20 to 30 sec. If a sample in a Vacutainer tube sits for more than 10 min after it has been ultrasonically mixed, remix it before taking an aliquot for analysis. Pipette 1 mℓ of the blood into an analysis cell. Rinse the pipette once with distilled deionized water and add the rinse to the analysis cell. Duplicate 1-mℓ aliquots of the blood sample should be taken for analysis.

8.3.2 Into the analysis cell containing 1 mℓ of blood, pipette 1.5 mℓ of the acid ashing solution.

8.3.3 Place the cell in a digestion rack on a hot plate operating at a surface temperature of about 150°C. Carry out the digestion in a hood. The sample will initially turn yellow and foam somewhat. Until the foaming stops, remove the cell from the hot plate when the foam nears the top. Gently tap the bottom of the cell against the hood base until the foam drops to the bottom of the cell and then replace it in the rack. The foaming will stop after about 10 min of heating at 150°C. When foaming ceases and the solution clears, adjust the temperature of the surface of the hot plate to 240°C. At the end of 1 hr, there will be approximately 100 to 200 μℓ of acid left in the bottom of the cell, with most of the nitric acid having evaporated. The digestion should be continued for an additional 1 to 1.4 hr until the acid is almost completely evaporated.

8.3.4 Remove the cell from the hot plate, cool it, and add 5 mℓ of the hydrazine dihydrochloride-acid solution. Allow the solution to run down the sides of the cell to insure that any spattered material is washed down. Heat the cell to about 90°C for 15 min, remove it from the hot plate, and allow it to cool to room temperature. Readjust the volume of the sample solution to 5 mℓ with the hydrazine dihydrochloride-acid solution if necessary.

8.3.5 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the sample solutions under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -400 mV
- Auto-hold cutoff potential, 200 mV
- Plating time, 15 to 30 min. (The plating time for a set of samples and standards must be the same.)
- Sweep rate, +30 mV/sec
- Current range, 0.1 to 2 mA (full scale)

The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of antimony plated on the electrode may occur. A baseline is drawn by connecting with a straight line the flat portions of the curve before and after the antimony peak, which occurs at -180 mV. The peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions: (1) reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solutions of antimony; (4) blood standards prepared as described in Section 9.1. The sequence in which these four kinds of solutions are analysed is as follows: (1) a set of standard solutions of antimony; (2) sample solutions, with reagent blanks and blood standards interspersed; and (3) a second set of standard solutions of antimony. The standard solutions of antimony are used for calibration; the results for the samples are corrected for the amount of antimony found in the reagent blank; and the blood standards are used to determine the efficiency of recovery of known amounts of antimony from blood. The procedure described is for use with a four-cell ASV unit.

9.1 Preparation of Blood Standards

9.1.1 A "normal" blood sample from an individual not suspected of exposure to antimony or a sample from a blood bank is analyzed by the method of standard additions. One-milliliter samples of the blood are placed in each of six Vacutainer tubes. Five of these samples are spiked with known amounts of antimony (5-, 10-, 20-, 30-, and 50-μℓ aliquots of standard solution "C," containing 5, 10, 20, 30, and 50 ng of antimony, respectively). These samples are ultrasonically mixed, transferred to analysis cells, digested, and analyzed by ASV according to the procedure given in Section 8.3. The concentration of antimony in the unspiked "normal" blood is estimated.

9.1.2 Finally, an amount of standard antimony solution is added to 1.0 mℓ of the unspiked "normal" blood to make the total amount of antimony equal to 20 ng. This standard blood solution is subsequently analyzed along with calibration standards and blanks (Section 9.3) to determine the percent recovery of antimony.

9.2 **Initial Analysis of Four Standards.** Four standards of 20 ng of antimony, prepared by adding 20 μℓ of standard solution "C" to 5 mℓ of the hydrazine dihydrochloride-acid solution, should be analyzed first. Each of the four standards should be analyzed with a different electrode. A single point may be taken to describe the calibration curve which has been determined to be linear up to 500 ng of antimony.

9.3 **Analysis of Samples and Controls.** The sample unknowns in the analysis cells are then analyzed with reagent blanks and blood standards (Section 9.1.2) interspersed at the rate of one blank for every 30 samples and one blood standard for every 10 samples. One reagent blank and one blood standard should be analyzed with each electrode during the analysis of a set of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 20 ng of antimony will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA for each electrode since the peak heights may vary significantly from electrode to electrode. The response factor for a particular electrode is the amount of antimony in the standard (20 ng) divided by the average of the two peak height values obtained for that electrode. For a 30-min plating time, a typical response factor is 0.75 ng/μA.

10. Calculations

10.1 The amount of antimony in a sample is calculated from the peak height (in μA) as measured according to Section 8.3.5, the response factor, and a correction factor in the following manner;

$$\text{Amount (ng)} = \frac{\text{peak height } (\mu\text{A})}{C} \times \text{response factor (ng/}\mu\text{A)}$$

where: C = a correction factor equal to the average fraction of antimony recovered from blood standards with the particular electrode used.

Any amount of antimony found in the reagent blank should be subtracted from the sample value to give the correct amount (in ng) of antimony in the sample.

10.2 The concentration of antimony in a blood sample may by expressed as μg/mℓ:

$$\mu\text{g/m}\ell = \frac{\text{corrected weight (in ng) of antimony in sample}}{\text{volume (in m}\ell\text{) of blood} \times 1000\ (\text{ng/}\mu\text{g})}$$

11. **Reference**

Environmental Sciences Associates, Inc., Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Divison of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

INDIUM IN BLOOD

Physical and Chemical Analysis Branch
Analytical Method

Analyte: Indium	Method No.: P&CAM 194
Matrix: Blood	Range: 0.01 to 5 μg/mℓ in 200 μℓ of blood
Procedure: Anodic stripping voltammetry	Precision (CV): 0.10 (analytical) at 0.5 μg/mℓ
Date Issued: 4/15/73	Classification: E (Proposed)
Date Revised: 1/29/76	

1. **Principle of the Method**

A sample of blood is wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The residue is dissolved in an acetate buffer solution and the buffered sample solution is analyzed by anodic stripping voltammetry (ASV) with a composite mercury-graphite electrode (CMGE). In principle, a hanging mercury drop electrode (HMDE) can also be used. However, optimum analysis parameters may vary significantly and must be established for each electrode system. The method as presented is designed for use with the CMGE.

2. **Range and Sensitivity**

2.1 Indium is not normally present in the reagent blank in detectable amounts. Therefore, the sensitivity is limited only by the baseline current in the ASV analysis. The detection limit is estimated to be 2 ng, which corresponds to 0.01 μg/mℓ in a 200-μℓ sample of blood.

2.2 The linear range of the electrode response is estimated to extend from the detection limit to 1 μg of indium, which corresponds to 5 μg/mℓ in a 200-μℓ sample of blood. The range may be extended by analyzing a smaller blood sample or by diluting the sample digestate with acetate buffer and taking an aliquot of the diluted solution for the ASV determination.

3. **Interferences**

3.1 In the ASV analysis, the indium peak occurs between lead and cadmium peaks. Consequently, microgram amounts of lead or cadmium interfere with the determination of indium. At lead levels greater than 2 μg, the indium peak occurs as a shoulder on the lead peak and measurements of indium peak heights become inaccurate.

3.2 Incomplete digestion of a sample, indicated by a yellowish coloration, may lead to peak broadening and anomalous results.

4. **Precision and Accuracy**

4.1 Several processes determine the reproducibility of the overall analytical method: (1) sampling, (2) shipping, (3) preparation of samples, and (4) analysis. The variations introduced by sampling blood at industrial sites and shipping the collected samples to a distant laboratory have not been evaluated. The coefficient of variation for the preparation and analysis steps has been empirically determined to be 0.10 over the range of 20 to 1000 ng, which corresponds to 0.1 to 5 $\mu g/m\ell$ in a 200-$\mu\ell$ sample of blood, and 0.20 over the range of 2 to 20 ng, which corresponds to 0.01 to 0.1 $\mu g/m\ell$ in a 200-$\mu\ell$ sample of blood.

4.2 The estimated percent error in a measurement, neglecting any contribution from sampling and shipping, is less than about 10% of the "true" value in the range of 20 ng to 1 μg, or 0.1 to 5 $\mu g/m\ell$ in a 200-$\mu\ell$ sample of blood.

5. **Advantages and Disadvantages of the Method**

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 Also, the matrix, in which the digested samples are analyzed, the acetate buffer solution, allows the accurate determination of lead, cadmium, and copper, as well as indium.

5.4 One disadvantage is that relatively high lead and cadmium concentrations interfere with the determination of indium. (See Section 3.)

6. **Apparatus**

6.1 Vacutainer tubes (5 mℓ), heparinized, low-lead content; Becton, Dickinson, and Co., or equivalent.

6.2 Model W-140-E Sonifier, Heat Systems-Ultrasonics, Inc., or an equivalent ultrasonic mixer with a microprobe tip having a diameter no larger than 0.2 in.

6.3 Pyrex test tubes (20 x 100 mm) for digesting blood samples and subsequent use as analysis cells.

6.4 Digestion racks for the analysis cells.

6.5 Micro pipettes (20, 50, 100, 200, and 500 $\mu\ell$), Eppendorf or equivalent.

6.6 Model SA 2011 Single-Cell Anodic Stripping Voltammeter with composite mercury-graphite electrode, Environmental Sciences Associates, Inc., Burlington, Mass., or equivalent. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations. Instructions for the preparation and reconditioning of the CMGE are included in the instruction manual.

6.7 Potentiometric recorder (100-mV range).

6.8 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric flasks, volumetric pipettes, and graduated pipettes of appropriate sizes.

7. Reagents

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in indium. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 **Acid Ashing Solution.** Prepare the acid ashing solution by adding 24 volumes of concentrated nitric acid to 24 volumes of perchloric acid (70%) and 1 volume of concentrated sulfuric acid.

7.5 **Acetate Buffer Solution (1*M* NaAc, 4 *M* LiCl, pH 5.2).** To prepare 1 liter of this reagent, dissolve 82 g of sodium acetate, 170 g of lithium chloride, and 21 mℓ of 70% $HClO_4$ in water in a 1-liter volumetric flask and fill to the mark with water. Remove trace metals from the resulting solution electrolytically with a system such as the Model 2014 PM Reagent Cleaning System, Environmental Sciences Associates, Inc., or equivalent. The acetate buffer maintains the pH of the resulting sample solution near 5.2, a pH favorable for indium determination. The relatively high concentration of chloride ions minimizes interference from lead and cadmium.

7.6 **Indium (In(III)), Standard Solution "A" (10 mg/mℓ).** Weigh accurately an amount of pure indium shot (99.99% pure) near 1 g and transfer the weighed indium to a 100-mℓ volumetric flask. Dissolve the metal in a 30 mℓ of concentrated nitric acid and dilute to the mark with water.

7.7 **Indium (In(III)), Standard Solution "B" (100 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "A" to a 100-mℓ volumetric flask containing 2 to 3 mℓ of concentrated nitric acid and dilute to the mark with water. Prepare fresh daily to prevent loss of indium or contamination.

7.8 **Indium (In(III)), Standard Solution "C" (1 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "B" to a 100-mℓ volumetric flask containing 2 to 3 mℓ of concentrated nitric acid and dilute to the mark with water. Prepare fresh daily to prevent loss of indium or contamination.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any indium from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 220 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove, and allow them to cool. Complete decontamination requires four repetitions of this procedure. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom and allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water.

8.2 **Collection and Shipping of Samples.** The samples are collected in Vacutainer tubes according to established procedures. The samples should be shipped to the laboratory in the sampling containers in a box with a minimum of 0.25 in. of foam packing to prevent breakage.

8.3 Analysis of Samples

8.3.1 Rinse the ultrasonic mixer tip thoroughly in distilled deionized water. Place the tip into a blood sample within 2 cm of the bottom of the Vacutainer tube, and mix at the maximum permissible power for 20 to 30 sec. If a sample in a Vacutainer tube sits for more than 10 min after it has been ultrasonically mixed, remix it before taking an aliquot for analysis. Pipette 200 μℓ of the blood into an analysis cell. Rinse the pipette once with distilled deionized water and add the rinse to the cell. Duplicate 200-μℓ aliquots of the blood sample should be taken for analysis.

8.3.2 Into the analysis cell containing 200 µℓ of blood, pipette 500 µℓ of the acid ashing solution.

8.3.3 Place the cell in a digestion rack on a hot plate operating at 220 to 240°C. Carry out the digestion in a hood. The sample will initially turn yellow but will clear in about 10 to 20 min. A reflux line will be established about halfway up the cell. At the end of 20 min, there will be approximately 100 to 200 µℓ of acid left in the bottom of the cell, with most of the nitric acid having evaporated. The digestion should be continued for an additional 20 to 30 min until the acid is almost completely evaporated.

8.3.4 Remove the cell from the hot plate, cool it, and add 4.5 mℓ of the acetate buffer solution.

8.3.5 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the buffered solution under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -1100 mV
- Auto-hold cutoff potential, 980 mV
- Plating time, 30 min.
- Sweep rate, +60 mV/sec
- Current range, 0.05 to 2 mA (full scale)

The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of indium plated on the electrode may occur. As the sample is stripped, the cadmium peak (-675 mV) will occur first, followed by the indium peak (-650 mV), and then the lead peak (-510 mV). A baseline is drawn by connecting with a straight line the flat portion of the curve after the cadmium peak with the flat portion before the lead peak. At high concentrations of lead, a baseline is drawn by extending the shoulder of the lead peak from the end of the indium peak to its beginning. The peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions: (1) reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described below in Section 8.3; (3) standard solutions of indium in acetate buffer; (4) blood standards prepared as described below in Section 9.1. The sequence in which these four kinds of solutions is analyzed is as follows: (1) a set of standard solutions of indium in acetate buffer; (2) sample solutions, with reagent blanks and blood standards interspersed at the rate of one blank and one blood standard for every ten samples; and (3) a second set of standard solutions of indium in acetate buffer. The standard solutions of indium in acetate buffer are used for calibration; the results for the samples are corrected for the amount of indium found in the reagent blank; and the blood standards are used to determine the efficiency of recovery of known amounts of indium from blood. The procedure described is for use with a four-cell ASV unit.

9.1 Preparation of Blood Standards

9.1.1 A "normal" blood sample from an individual not suspected of exposure to indium or a sample from a blood bank is analyzed by the method of standard additions. One-milliliter samples of the blood are placed in each of five Vacutainer tubes. Four of these samples are spiked with known amounts of indium (20-, 50-, 100-, and 200-$\mu\ell$ aliquots of standard solution "C", containing 20, 50, 100, and 200 ng of indium, respectively). These samples are ultrasonically mixed, transferred to analysis cells, digested, and analyzed by ASV according to the procedure given in Section 8.3. The concentration of indium in the unspiked "normal" blood is then estimated.

9.1.2 Finally, an amount of standard indium solution is added to 200 $\mu\ell$ of the unspiked "normal" blood to make the total amount of indium equal to 100 ng. This standard blood solution is subseqently analyzed along with calibration standards and blanks (Section 9.3) to determine the percent recovery of indium.

9.2 **Initial Analysis of Four Standards.** Four standards of 100 ng of indium, prepared by adding 100 $\mu\ell$ of standard solution "C" to 4.5 mℓ of acetate buffer solution, should be analyzed first. Each of the four standards should be analyzed with a different electrode. At the level of 100 ng, the contributions from blanks should be negligible, and a single point may be taken to describe the calibration curve for each electrode, which has been determined to be linear through 1 μg.

9.3 **Analysis of Samples and Controls.** With every ten samples, a blood standard (Section 9.1.2) and a reagent blank are analyzed by the ASV procedure given in Section 8.3. At least one reagent blank and one blood standard should be analyzed with each electrode that is used for analysis of samples.

9.4 Final Analysis of Standards. The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 100 ng of indium will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA. The overall response factor is the amount of indium in each standard (100 ng) divided by the average of the eight peak heights (in μA). However, it will be necessary to calculate an individual response factor for an electrode if the average of the two peak height values for an individual electrode differs from the overall average of the eight peak heights by more than 10%. For example, if the average for the eight peak heights is 210 μA, and the average of the two values for one of the electrodes is 185 μA, then the response factor to be used for that electrode is 100 ng/185 μA, or 0.54 ng/μA. The overall response factor in this example is 100 ng/210 μA, or 0.48 ng/μA.

10. Calculations

10.1 The amount of indium in a sample is calculated from the indium peak height (in μA), as measured according to Section 8.3.5, and the response factor in the following manner:

Amount (in ng) = peak height (in μA) x response factor (in ng/μA)

Any amount of indium found in the reagent blank should be subtracted from the sample value to give the correct weight (in ng) of indium in the sample. The average recovery of indium from blood standards is usually greater than 90%. However, it may be necessary to include a factor in the above equation to correct for incomplete recovery.

10.2 The concentration of indium in a blood sample may be expressed as μg/mℓ of indium in blood:

$$\mu g/m\ell = \frac{\text{corrected weight (in ng) of indium in sample}}{\text{volume (in m}\ell\text{) of blood x 1000 (ng/}\mu g\text{)}}$$

11. Reference

Environmental Sciences Associates, Inc. Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Division of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

LEAD IN BLOOD

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Lead	**Method No.:** P&CAM 195
Matrix: Blood	**Range:** 0.04 to 5 μg/mℓ in 100 μℓ of blood
Procedure: Anodic stripping voltammetry	**Precision** (CV): 0.05 (analytical) at 1 μg/mℓ
Date Issued: 4/15/73	**Classification:** E (Proposed)
Date Revised: 1/29/76	

1. **Principle of the Method**

A sample of whole blood is wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The residue is dissolved in dilute perchloric acid. The resulting solution is analyzed for lead (Pb(II)) by anodic stripping voltammetry (ASV) with a composite mercury-graphite electrode (CMGE). In principle, a hanging mercury drop electrode (HMDE) can also be used. However, optimum analysis parameters may vary significantly and must be established for each electrode system. The method as presented is designed for use with the CMGE.

2. **Range and Sensitivity**

2.1 The sensitivity is limited by the reagent blank. A reasonable detection limit appears to be two times the standard deviation of the reagent blank. Statistically, the probability is 95% that lead is present in the sample if the signal is higher than this limit. Blanks for lead are usually 6 ± 2 ng. By the stated criterion, the detection limit is 4 ng, which corresponds to 0.04 μg/mℓ in a 100-μℓ sample.

2.2 The linear range of the electrode response is estimated to extend from the detection limit to 500 ng of lead, which corresponds to 5 μg/mℓ in a 100-μℓ sample. The range may be extended by diluting the sample digestate with dilute perchloric acid and taking an aliquot of the diluted solution for the ASV determination.

3. **Interferences**

3.1 Thallium forms a shoulder on the cathodic edge of the lead peak. However, the thallium peak does not affect the height of the lead peak until thallium and lead are present in roughly the same concentrations. If the presence of a significant concentration of thallium is suspected in a sample, as in a sample from the blood of

workers in certain smelting or roasting industries, the sample should be analyzed for thallium. Thallium is determined by making the digested sample solution 0.01 *M* in EDTA and analyzing the resulting solution by ASV at the same instrument settings as those recommended for the lead analysis. A typical response factor for thallium is 4 ng/μA when the sample plating time is 30 min. Since the presence of EDTA prevents the ASV response to lead but does not affect the response to thallium, the peak height obtained for thallium alone may be subtracted from the peak height obtained for both lead and thallium to estimate the response due to lead alone.

3.2 Incomplete digestion of a sample, indicated by a yellowish coloration, may lead to peak broadening and anomalous results.

4. **Precision and Accuracy**

4.1 Several processes determine the reproducibility of the overall analytical method: (1) sampling, (2) shipping, (3) preparation of samples, and (4) analysis. The variation introduced by sampling blood in industrial plants has not been evaluated. It has been found that precision is not significantly affected by the shipping of standard blood samples that have been ultrasonically mixed prior to shipping. The contributions from the preparation and analysis steps may be estimated for the entire analytical range from the following empirical relation:

$$CV = \frac{0.02}{[Pb]} + 0.03$$

where: CV = the estimated coefficient of variation of a measurement neglecting any contribution from sampling.

[Pb] = the measured lead concentration (in μg/mℓ) in a 100-$\mu\ell$ sample.

The factor 0.02 in the numerator of the first term of this expression reflects the scatter in the reagent blank, which corresponds to a standard deviation of 2 ng in a 100-$\mu\ell$ sample, or 0.02 μg/mℓ. The second term represents the normal statistical variations in the preparation and analysis steps. (See Reference 11.2.)

4.2 The percent error for the overall sampling and analytical method has been determined to be 5% in the determination of a level of 60 ng of lead in a 100-$\mu\ell$ blood sample, which corresponds to a concentration of 0.6 μg/mℓ. (See Reference 11.3.)

5. **Advantages and Disadvantages of the Method**

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 One disadvantage is that the current-voltage peaks for thallium and lead are not resolved when their concentrations are comparable.

6. **Apparatus**

6.1 Vacutainer tubes (5 mℓ), heparinized, low-lead content; Becton, Dickinson, and Co., or equivalent.

6.2 Micro capillary tubes, "deleaded," heparinized; Environmental Sciences Associates, Inc., or equivalent.

6.3 Model W-140-E Sonifier, Heat Systems-Ultrasonics, Inc., or an equivalent ultrasonic mixer with a microprobe tip having a diameter no larger than 0.2 in.

6.4 Pyrex test tubes (20 x 100 mm) for digesting blood samples and subsequent use as analysis cells.

6.5 Digestion racks for the analysis cells.

6.6 Micro pipettes (20, 50, 100, 150, and 300 μℓ), Eppendorf or equivalent.

6.7 Model SA 2011 Single-Cell Anodic Stripping Voltammeter with composite mercury-graphite electrode, Environmental Sciences Associates, Inc., Burlington, Mass., or equivalent. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations. Instructions for the preparation and reconditioning of the CMGE are included in the instruction manual.

6.8 Potentiometric recorder (100-mV range).

6.9 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric flasks, volumetric pipettes, and graduated pipettes of appropriate sizes.

7. **Reagents**

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in lead. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 **Acid Ashing Solution.** Prepare the acid ashing solution by adding 327 volumes of concentrated nitric acid to 653 volumes of perchloric acid (70%) and 20 volumes of concentrated sulfuric acid.

7.5 Perchloric acid solution (0.35%, v/v, in water).

477

7.6 **Lead (Pb(II)), Standard Solution "A" (10 mg/mℓ).** Transfer 1.5987 g of lead nitrate ($Pb(NO_3)_2$) to a clean 100-mℓ volumetric flask. Dissolve the salt in approximately 30 mℓ of water, add 3 mℓ of concentrated nitric acid and dilute to the mark with water.

7.7 **Lead (Pb(II)), Standard Solution "B" (100 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "A" to a 100-mℓ volumetric flask containing 1 mℓ of concentrated nitric acid and dilute to the mark with water.

7.8 **Lead (Pb(II)), Standard Solution "C" (1 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "B" to a 100-mℓ volumetric flask containing 1 mℓ of concentrated nitric acid and dilute to the mark with water. Prepare a fresh solution daily to avoid loss of lead or contamination.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any lead from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 220 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the hot plate for a period of 60 to 90 min, remove, and allow them to cool. Complete decontamination requires four repetitions of this procedure. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom. Allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water.

8.2 **Collection and Shipping of Samples.** The samples are collected in Vacutainer tubes according to established procedures. Micro samples from the ear or finger are collected in capillary tubes according to standard procedures. The samples should be shipped to the laboratory in the sampling containers in a box with a minimum of 0.25 in. of foam packing to prevent breakage.

8.3 Analysis of Samples

8.3.1 Sample Preparation

- **Macro Sample.** Rinse the ultrasonic mixer tip thoroughly in distilled deionized water. Place the tip into a blood sample within 2 cm of the bottom of the Vacutainer tube, and mix at the maximum permissible power for 20 to 30 sec. If a sample in a Vacutainer tube sits for more than 10 min after it has been ultrasonically mixed, remix it before taking an aliquot for analysis. Pipette 100 $\mu\ell$ of the blood into an analysis cell. Rinse the pipette once with distilled deionized water and add the rinse to the analysis cell. Then rinse the pipette four times with distilled deionized water and discard the rinse water. (Certain batches of micro pipettes may have an appreciable number of tips contaminated with lead; consequently, it is best to use the same tip throughout a series of samples and to rinse it between each sample.) Duplicate 100-$\mu\ell$ blood samples should be taken for analysis.

- **Micro Samples.** Force the blood from a 100-$\mu\ell$ capillary tube into an analysis cell. Rinse the capillary tube twice with 100 $\mu\ell$ of distilled deionized water. Add the rinses to the analysis cell. When available, duplicate 100-$\mu\ell$ blood samples in capillary tubes should be analyzed.

8.3.2 Into the analysis cell containing 100 $\mu\ell$ of blood, pipette 300 $\mu\ell$ of acid ashing solution.

8.3.3 Place the cell in digestion rack on a hot plate operating at 220 to 240°C. Carry out the digestion in a hood. The sample will initially turn yellow but will clear in about 10 min. A reflux line will be established about halfway up the cell. At the end of 30 min, there will be approximately 100 to 200 $\mu\ell$ of acid left in the bottom of the cell, with most of the nitric acid having evaporated. The digestion should be continued for an additional 30 to 60 min until the acid is almost completely evaporated.

8.3.4 Remove the cell from the hot plate, cool it, and add 4.5 mℓ of 0.35% $HClO_4$.

8.3.5 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the sample solution under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -780 mV.
- Auto-hold cutoff potential, 900 mV.
- Plating time, 10 to 30 min. (The plating time for samples and standards must be the same.)
- Sweep rate, +60 mV/sec.
- Current range, 0.2 to 1 mA (full scale).

The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of lead plated on the electrode may occur. As the sample is stripped, a cadmium peak (-600 mV) may occur first, followed by the lead peak (-430 mV). A baseline is drawn by connecting with a straight line the flat portions of the curve before and after the lead peak. The peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions: (1) reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solution of lead; (4) blood standards prepared as described below in Section 9.1. The sequence in which these four kinds of solution are analyzed is as follows: (1) a set of standard solutions of lead; (2) sample solutions, with reagent blanks and blood standards interspersed at the rate of one blank and one blood standard for every ten samples; and (3) a second set of standard solutions of lead. The standard solutions of lead are used for calibration; the results for the samples are corrected for the amount of lead found in the reagent blank; and the blood standards are used to determine the efficiency of recovery of known amounts of lead from blood. The procedure described is for use with a four-cell ASV unit.

9.1 Preparation of Blood Standards

9.1.1 A "normal" blood sample from an individual not suspected of exposure to lead or a sample from a blood bank is analyzed by the method of standard additions. One hundred-microliter samples of the blood are placed in each of five Vacutainer tubes. Four of these samples are spiked with known amounts of lead (20-, 50-, 100-, and 150-μℓ aliquots of standard solution "C," containing 20, 50, 100, and 150 ng of lead, respectively). These samples are ultrasonically mixed, transferred to analysis cells, digested, and analyzed by ASV according to the procedure given in Section 8.3. The concentration of lead in the unspiked "normal" blood is then estimated.

9.1.2 Finally, an amount of standard lead solution is added to 100 μℓ of unspiked "normal" blood to make the total concentration of lead equal to 1 μg/mℓ. This standard blood solution is subsequently analyzed along with calibration standards and blanks (Section 9.3) to determine the percent recovery of lead.

9.2 **Initial Analysis of Four Standards.** Four standards of 100 ng of lead, prepared by adding 100 μℓ of standard solution "C" to 4.5 mℓ of 0.35% $HClO_4$ solution, should be be analyzed first. (Standards of 50 ng may be used.) Each of the four standards should be analyzed with a different electrode. At the level of 50 to 100 ng, the contributions from blanks should be negligible, and a single point may be taken to describe the calibration curve for each electrode, which has been determined to be linear through 500 ng.

9.3 **Analysis of Samples and Controls.** With every ten samples, a blood standard (Section 9.1.2) and a reagent blank are analyzed by the ASV procedure given in Section 8.3. At least one reagent blank and one blood standard should be analyzed with each electrode that is used for analysis of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 100 ng of lead will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA. The overall response factor is the amount of lead in each standard (100 ng) divided by the average of the eight peak heights (in μA). However, it will be necessary to calculate an individual response factor for an electrode if the average of the two peak height values for an individual electrode differs from the overall average of the eight peak heights by more than 10%. For example, if the average for the eight peak heights is 105 μA, and the average of the two values for one of the electrodes is 92 μA, then the response factor to be used for that electrode is 100 ng/92 μA, or 1.09 ng/μA. The overall response factor in this example is 100 ng/105 μA, or 0.95 ng/μA.

10. **Calculations**

10.1 The amount of lead in a sample is calculated from the lead peak height (in μA), as measured according to Section 8.3.5, and the response factor in the following manner:

$$\text{Amount (in ng)} = \text{peak height (in } \mu\text{A)} \times \text{response factor (in ng/}\mu\text{A)}$$

Any amount of lead found in the reagent blank should be subtracted from the sample blank to give the correct amount (in ng) of lead in the sample. The average recovery of lead from blood standards is usually greater than 90%. However, it may be necessary to include a factor in the above equation to correct for incomplete recovery.

10.2 The concentration of lead in a blood sample may be expressed as μg/mℓ:

$$\mu\text{g/m}\ell = \frac{\text{corrected weight (in ng) of lead in sample}}{\text{volume (in m}\ell\text{) of sample} \times 1000\ (\text{ng/}\mu\text{g})}$$

11. **References**

11.1 Environmental Sciences Associates, Inc., Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Division of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

11.2 Matson, W.R., R.M. Griffin, and T.J. Sapienza, "A Total System and Quality Control Procedure for Micro Blood Lead Analysis by Anodic Stripping Voltammetry (ASV)," presented at the Laboratory Director's Conference, Department of Health, Education, and Welfare, Center for Disease Control, August 23, 1974.

11.3 Matson, W.R., Environmental Sciences Associates, personal communication, 1975.

ARSENIC IN URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Arsenic	**Method No.:** P&CAM 196
Matrix: Urine	**Range:** 16 to 600 μg/ℓ in 1 mℓ of urine
Procedure: Anodic stripping voltammetry	**Precision (CV_T):** 0.08 over the above range
Date Issued: 10/5/73	**Classification:** E (Proposed)
Date Revised: 1/29/76	

1. **Principle of the Method**

A sample of ultrasonically mixed urine is wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The resulting As(V) is reduced to As(III) with an acidic solution of sodium bisulfite and ferrous perchlorate. An acid solution of hydrazine dihydrochloride is added to prevent air oxidation of Fe(II) ions. The chemically reduced sample is then analyzed by anodic stripping voltammetry (ASV) with a composite gold-graphite electrode (CGGE).

2. Range and Sensitivity

2.1 The sensitivity is limited by the reagent blank and the background characteristics of the electrode. A reasonable detection limit appears to be two times the standard deviation of the reagent blank. Statistically, the probability is 95% that arsenic is present in the sample if the signal is higher than this limit. Blanks for arsenic are usually 15 ± 8 ng. The detection limit of the method is thus estimated to be about 16 ng/mℓ of arsenic in a 1.0-mℓ sample of urine.

2.2 The linear range of the electrode response is estimated to extend from the detection limit to 600 ng of arsenic. This upper limit corresponds to 600 μg/ℓ of arsenic in a 1.0-mℓ sample of urine. The range may be extended by analyzing a smaller urine sample or by diluting the sample digestate with dilute acid after the reduction step and taking a portion of the diluted solution for analysis.

3. Interferences

3.1 Antimony, bismuth, and tin will form resolvable shoulders on the cathodic edge of the arsenic peak. Mercury will form a shoulder on the anodic edge of the arsenic peak. Tin has been found not to interfere at concentrations up to 100 times that of arsenic. Mercury, bismuth, and antimony do not seriously affect the arsenic signal at concentrations up to ten times that of arsenic. Higher concentration ratios of mercury,

bismuth, and antimony have not been tested. However, mercury should present little, if any, problem at higher concentrations since it is usually lost during digestion. If the concentrations of bismuth and antimony are suspected to be significant, as in samples taken in metallurgical industrial environments, the samples should also be analyzed with a composite mercury-graphite electrode (CMGE) at a plating potential of -350 mV to determine the amounts of bismuth and antimony present.

3.2 Copper and arsenic are not completely resolved at high levels of both metals. The available data suggest that when the absolute quantity of copper exceeds 1 μg, the linear range of electrode response to arsenic will decrease.

3.3 Incomplete digestion of a sample, indicated by a yellowish coloration, may lead to peak broadening and anomalous results.

4. **Precision and Accuracy**

All of the factors that affect the accuracy and precision of the total method for the determination of arsenic in urine have not been fully evaluated. An estimate of the coefficient of variation is 0.08; also, it is estmated that results of the analysis of sample unknowns should fall within 10% of the "true" values.

5. **Advantages and Disadvantages of the Method**

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 One disadvantage is that the current -voltage peaks for copper and arsenic are not completely resolved when their concentrations are comparable.

5.4 Another disadvantage is the occurrence of large variations in response from electrode to electrode.

6. **Apparatus**

6.1 Polyethylene bottles (180 mℓ) with screw caps.

6.2 Model W-140-E Sonifier, Heat Systems–Ultrasonics, Inc., or an equivalent ultrasonic mixer with a probe tip at least 0.5 in. in diameter.

6.3 A urinometer (or hydrometer capable of measuring specific gravities in the range of 1.000 to 1.040 with an uncertainty of only ±0.001 units).

6.4 Pyrex test tubes (20 x 100 mm) for digesting urine samples and subsequent use as analysis cells.

6.5 Digestion racks for the analysis cells.

6.6 Micro pipettes (10, 50, 100, 200, 300, and 500 $\mu\ell$, and 1 mℓ), Eppendorf or equivalent.

6.7 Disposable pipettes, graduated (1 and 5 mℓ).

6.8 Potentiometric recorder (100-mV range).

6.9 Assorted laboratory glassware, including beakers, graduated cylinders, and volumetric flasks of appropriate sizes.

6.10 **Anodic Stripping Voltammeter.** A suitable single-cell instrument with a composite mercury-graphite electrode (Model SA 2011) is available from Environmental Sciences Associates, Burlington, Mass. Equivalent instrumentation may be used. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations.

6.11 **Modification of the Model SA 2011.** The slight modification described below is suggested to improve the mixing of the high-salt-content solutions necessary for the determination of arsenic. This modification is best performed while the test electrode is out of the cell for routine maintenance. Throw the plate-strip switch to "strip" with the cell selector on "EXT." Disconnect and remove the test electrode. Place the test electrode in a beaker of water to keep it wet. With thumb and forefinger, grasp the bent end of the nitrogen bubbler and pull it down and away from the cell head until all of the platinum inside the tubing has been drawn past the retaining loop on the bottom of the reference electrode. Straighten the bend in the Teflon-covered platinum. With the platinum tip of the counter electrode, push the platinum wire in the nitrogen bubbler tube further up inside the bubbler tube so that the solution end of the tube is empty for the last 1 to 3 mm of its length. Rebend the platinum and tubing to the original configuration and return the bubbler tube to its original position and configuration. Replace the test and counter electrodes, reconnect all wire junctions broken, and throw the plate-strip switch back to "plate." This procedure provides an unobstructed gas-solution interface so that micro precipitation due to evaporation of solution trapped inside of the bubbler will not lead to reduced stirring rates.

6.12 **Composite Gold-Graphite Electrode (CGGE).** A CGGE may be prepared from a new waxed graphite electrode or one which has been used as a composite mercury-graphite electrode.

6.12.1 If the waxed graphite electrode has previously been used as a CMGE, withdraw the electrode from the cell head and remove the mercury coat by wiping with a damp soft paper towel. Place the electrode back in the cell head and electrolytically remove the remaining mercury by holding the potential of the electrode at +1.0 V in 8 mℓ of a nitrogen-stirred solution of 1 *N* HNO_3 for 15 min. Remove the nitric acid solution and rinse the electrode thoroughly in a 250-mℓ beaker of distilled deionized water. Remove the electrode from the cell head, wipe dry, and resurface the electrode. The resurfacing is accomplished by gently pinching the polished end of the electrode in 4/0 emery paper between thumb and forefinger of one hand while rotating the electrode with the other hand. If the electrode is rotated a large number of times as it withdrawn from the emery paper, a light gray silvery surface approximating that of spun aluminum will be obtained. Wipe the resurfaced area very gently with the dampened edge of a soft paper towel to remove any particles of graphite clinging to the refinished surface. Carefully place the electrode back into position in the cell head.

6.12.2 If the electrode is new, then resurface as described above; hold at +1.0 V in 8 mℓ of a nitrogen-stirred solution of approximately 1 *N* HNO_3, and rinse well with distilled deionized water.

6.12.3 Immerse the electrodes to be gold plated in analysis cells, each of which contains 5 mℓ of 5 *N* HCl and 100 μℓ of the gold plating solution. (See Section 7.14.) Stir for 60 sec with the nitrogen bubbler on and then turn off the nitrogen. Set the plating potential to -300 mV and throw the plate-strip switch to "plate." Plate the electrodes for 10 to 12 min from an unstirred solution and then for 60 sec from a stirred solution. Arrest plating by immersing the electrodes in the hydrazine dihydrochloride-acid solution. At the end of the plating step the electrodes may range in color from dull brown to bright yellow. Most of the electrodes will be a dull greenish yellow.

6.12.4 When they are not in use, store the electrodes at a potential of +300 mV in a solution consisting of 5 mℓ of 5 *N* HCl and 0.3 mℓ of the ferrous perchlorate reagent. Before use, soak each electrode in 5 mℓ of the ferrous perchlorate reagent for 5 min at -200 mV.

7. Reagents

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in arsenic. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly vacuum distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 **Acid Ashing Solution.** Prepare the acid ashing solution by adding 24 volumes of concentrated nitric acid to 24 volumes of perchloric acid (70%) and 1 volume of concentrated sulfuric acid.

7.5 **Sodium Bisulfite Solution (1 *N*).** Add 10.4 g of sodium bisulfite to a 100-mℓ volumetric flask, dissolve in water, and dilute to the mark.

7.6 Hydrazine dihydrochloride.

7.7 **Ferrous Perchlorate Reagent.** There are two alternate methods of preparing this reagent.

7.7.1 Add 100 mg of iron (electrolytic grade) to a 50-mℓ beaker. Add 5 mℓ of distilled deionized water and 9 mℓ of 70% perchloric acid. Warm gently to aid in dissolution. After dissolution, transfer the contents of the beaker to a 100-mℓ volumetric flask containing 0.5 g of hydrazine dihydrochloride. Rinse the beaker several times with water and add the washings to the volumetric flask. Dilute to the mark with water.

7.7.2 Add 0.650 g of ferrous perchlorate, $Fe(ClO_4)_2 \cdot 6H_2O$), and 9 mℓ of 70% perchloric acid to a 100-mℓ volumetric flask containing 0.5 g of hydrazine dihydrochloride in approximately 30 mℓ of water. Dissolve and dilute to the mark with water.

7.8 Hydrochloric acid, concentrated.

7.9 **Hydrazine Dihydrochloride Acid Solution (5 *N* HCl, 0.001 *N* $H_2NNH_2 \cdot (HCl)_2$).** Add 0.105 g of hydrazine dihydrochloride to a 1-liter volumetric flask. Add approximately 400 mℓ of water and swirl to dissolve the salt. Add 417 mℓ of concentrated hydrochloric acid, swirl, and dilute to the mark with water. This solution may be conveniently stored in a polyethylene bottle with a screw cap.

7.10 **Sodium Hydroxide Solution (1.5 *N*).** Add 6 g of sodium hydroxide to a 100-mℓ volumetric flask with a polyethylene stopper, dissolve in water, and dilute to the mark.

7.11 **Arsenic (As(III)), Standard Solution "A" (10 mg/mℓ).** Transfer 1.320 g of arsenic trioxide (As_2O_3) to a clean 100-mℓ volumetric flask. Add 35 mℓ of 1.5 *N* NaOH. Immediately after dissolution of the arsenic trioxide, acidify the solution with 25 mℓ of concentrated hydrochloric acid and cool to room temperature. Then dilute to the mark with water.

7.12 **Arsenic (As(III)), Standard Solution "B" (100 μg/mℓ).** Add approximately 80 mℓ of water and 2 mℓ of concentrated hydrochloric acid to a 100-mℓ volumetric flask. Swirl to achieve a homogeneous solution and then add 1 mℓ of standard solution "A." Dilute to the mark with water.

7.13 **Arsenic (As(III)), Standard Solution "C" (1 μg/mℓ).** Repeat the procedure for preparing the standard solution "B," but add 1 mℓ of standard solution "B" instead of standard solution "A." During the first two months, prepare standard solution "C" daily and standard solution "B" weekly. Pour out the old solution and use the same flask for making up the new solution. After the two-month period for conditioning the flasks, prepare standard solution "C" weekly, standard solution "B" monthly, and standard solution "A" semiannually.

7.14 **Gold Plating Solution.** Place 1.00 g of high purity gold metal in a 100-mℓ beaker. In a hood, add 2.5 mℓ of concentrated nitric acid and 10 mℓ of concentrated hydrochloric acid to the beaker. Warm gently until all of the gold has dissolved. Add 5 mℓ more of concentrated hydrochloric acid and allow the beaker to cool. Quantitatively transfer the contents of the beaker to a clean 100-mℓ volumetric flask. Rinse the beaker several times with 15-mℓ portions of water and add each rinse to the volumetric flask. Dilute to the mark with water. This solution now contains 10 mg/mℓ of gold in acid solution.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any arsenic from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 220 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove and allow them to cool. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom. Allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water. Polyethylene bottles used in sample collection are cleaned by soaking in 2.5% perchloric acid for 24 hr. The bottles should not be rinsed with concentrated acid as this may cause sample loss.

8.2 **Collection and Shipping of Samples.** Collect the samples in precleaned 180-mℓ polyethylene bottles. Ship the samples in a box with a minimum of 0.25 in. of foam packing to prevent shock that may lead to opening of the screw caps and resultant leakage.

8.3 Analysis of Samples

8.3.1 Shake the urine sample thoroughly, and allow the foam to settle. Mix ultrasonically at 4 A (dc) for 60 sec. Measure the specific gravity of the sample with a urinometer or suitable hydrometer. (If the specific gravity is less than 1.010, discard the sample. Obtain another sample from the subject if desired.) Pipette 1 mℓ of sample into an analysis cell and then add 800 μℓ of the acid ashing solution to the cell. Duplicate 1-mℓ aliquots of the urine sample should be taken for analysis.

8.3.2 Place the cell in a digestion rack on a hot plate operating at 100 to 120°C. Carry out the digestion in a hood. The sample will initially turn brown and will evaporate to about half-volume in 10 min. Adjust the temperature of the hot plate to 220 to 240°C. A reflux line will be established about halfway up the cell. At the end of 20 min, there will be approximately 100 to 200 μℓ of acid left in the bottom, with most of the nitric acid having evaporated. The digestion should be continued for an additional 20 to 30 min until the acid is almost completely evaporated.

8.3.3 Add to the cell 1 mℓ of 1 *N* $NaHSO_3$, 200 μℓ of the ferrous perchlorate reagent, and 300 μℓ of concentrated hydrochloric acid. Heat the sample in a hood on a hot plate at 90 to 95°C for 15 min. Do not evaporate the solution to dryness. Allow the cell to cool to room temperature and dilute the sample to 5 mℓ with the hydrazine dihydrochloride-acid solution.

8.3.4 Following the manufacturer's instructions for operation of the Anodic StrippingVoltammeter, analyze the sample solution with the CGGE under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -200 mV
- Auto-hold cutoff potential, 720 mV
- Plating time, 15 min
- Sweep rate, +60 mV/sec
- Current range, 0.5 to 1.0 mA (full scale)

The nitrogen stirring should be on during the plating step and should be turned off 15 sec prior to the stripping step. The stirring rate should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of arsenic plated on the electrode may occur. As the sample is stripped, the arsenic peak (+195 mV) occurs first, and the copper-iron peak occurs second. After the auto-hold cutoff potential is reached, remove the cell and rinse the electrode by swishing the cellhead assembly up and down while immersed in 230 mℓ of distilled deionized water containing 10 mℓ of concentrated hydrochloric acid. Place the next sample on the electrode cell head and reset the potential of the cell to the plating potential. Turn the nitrogen stirring on and reset the timer.

8.3.5 A baseline is drawn by connecting with a straight line the minimum point between the copper and arsenic peaks with the flat portion of the curve preceding the arsenic peak. The peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions; (1) reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solutions of arsenic; (4) urine standards prepared as described below in Section 9.1. The sequence in which these four kind of solutions are analyzed is as follows: (1) a set of standard solutions of arsenic; (2) sample solutions, with reagent blanks interspersed at the rate of one blank and one urine standard for every ten samples; and (3) a second set of standard solutions of arsenic. The standard solutions of arsenic are used for calibration; the results for the samples are corrected for the amount of arsenic found in the reagent blank; and the urine standards are used to determine the efficiency of recovery of known amounts of arsenic from urine. The procedure described is for use with a four-cell ASV unit.

9.1.1 A "normal" urine sample from an individual not suspected of exposure to arsenic is analyzed by the method of standard additions. One-milliliter samples of the urine are place in each of five analysis cells. Four of these samples are spiked with known amounts of arsenic (50-, 100-, 200-, and 500-μℓ aliquots of standard solution "C," containing 50, 100, 200, and 500 ng of arsenic, respectively). These sample are digested and analyzed by ASV according to the procedure given in Section 8.3 and the concentration of arsenic in the unspiked "normal" urine is estimated.

9.1.2 Finally, an amount of standard arsenic solution is added to 1 mℓ of unspiked "normal" urine to make the total amount of arsenic equal to 100 ng. This standard urine solution is subsequently analyzed along with calibration standards and blanks (Section 9.3) to determine the percent recovery of arsenic.

9.2 **Initial Analysis of Four Standards.** Solutions consisting of 5 mℓ of the hydrazine dihydrochloride-acid reagent and 200 μℓ of ferrous perchlorate reagent are placed in each of four cells and analyzed by the procedure described in Section 8.3.4. The plating of each cell solution should be started sequentially at 1-min intervals. After stripping is completed, add 100 ng of arsenic (100 μℓ of standard arsenic solution "C") and plate and strip once again. A single point may be taken to describe the calibration curve, which has been determined to be linear over the range 25 to 600 ng of arsenic.

9.3 **Analysis of Samples and Controls.** With every ten samples, a urine standard (Section 9.1.2) and a reagent blank are analyzed by the ASV procedure given in Section 8.3. At least one reagent blank and one urine standard should be analyzed with each electrode that is used for analysis of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. These values are used to calculate a response factor in ng/μA for each electrode since the peak heights may vary significantly from electrode to electrode. The response factor for a particular electrode is the amount of arsenic in each standard (100 ng) divided by the average of the two peak height values obtained for that electrode. For a 15-min plating time, typical response factors range from 0.75 to 1.4 ng/μA.

10. Calculations

10.1 The amount of arsenic in a sample is calculated from the peak heights as measured according to Section 8.3.5, the response factor, and the correction factor in the following manner:

$$\text{Amount (ng)} = \frac{\text{peak height } (\mu A)}{C} \times \text{response factor } (\text{ng}/\mu A)$$

where: C = a correction factor equal to the average fraction of arsenic recovered from the urine standards with the particular electrode used. If the correction factor is greater than 0.90, the value of C may be taken to be unity.

Any amount of arsenic found in the reagent blank should be subtracted from the sample value to give the correct amount (in ng) of arsenic in the sample.

10.2 The concentration of arsenic in a urine sample may be expressed as μg/ℓ:

$$\mu g/\ell = \frac{\text{corrected weight (in ng) of arsenic in sample}}{\text{volume (in m}\ell\text{) of sample}}$$

491

10.3 The use of a specific gravity correction factor to normalize values to 1.024, the average specific gravity of urine, has been recommended. (See Reference 11.3.) The preferred method of normalization is to make a straightforward gravity correction as follows:

$$\text{Corrected } \mu g/\ell = \mu g/\ell \times \frac{0.024}{(\text{specific gravity} - 1.000)}$$

Report the uncorrected concentration, the corrected concentration, and the specific gravity of the sample.

11. References

11.1 Environmental Sciences Associates, Inc., Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Divison of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

11.2 "General Method: Arsenic Analysis by Anodic Stripping Voltammetry (ASV)," ESA Methodology M-43, Environmental Sciences Associates, Inc., Burlington, Mass.

11.3 Elkins, H.B., "Concentration Adjustments in Urinalysis," Amer. Ind. Hyg. Ass. J., 35, 559 (1974).

ANTIMONY IN URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Antimony	**Method No.:** P&CAM 197
Matrix: Urine	**Range:** 2 to 500 μg/ℓ in 1 mℓ of urine
Procedure: Anodic stripping voltammetry	**Precision** (CV): 0.08 (analytical) at 20 μg/ℓ
Date Issued: 5/15/73	**Classification:** E (Proposed)
Date Revised: 1/29/76	

1. **Principle of the Method**

A sample of urine is wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The resulting Sb(V) is reduced to Sb(III) with a solution of hydrochloric acid and hydrazine dihydrochloride. The chemically reduced sample is analyzed by anodic stripping voltammetry (ASV) with a composite mercury-graphite electrode (CMGE). In principle, a hanging mercury drop electrode (HMDE) can also be used. However, optimum analysis parameters may vary significantly and must be established for each electrode system. The method as presented is designed for use with the CMGE.

2. **Range and Sensitivity**

2.1 The sensitivity of the method is limited by chemical interferences in the reduction of Sb(V) to Sb(III) and by interferences of copper and bismuth with the ASV determination of antimony. In the absence of copper and bismuth, as in reagent blanks, the detection limit is about 0.5 to 1 ng; however, in typical industrial urine samples, which may contain high concentrations of copper and bismuth, the detection limit is 2 to 4 ng, or 2 to 4 μg/ℓ in a 1.0-mℓ sample of urine.

2.2 The linear range of the electrode response extends from the detection limit to 500 ng of antimony, which corresponds to 500 μg/ℓ in a 1-mℓ sample of urine. The upper limit of the range can be extended to 2.5 mg/ℓ by taking a 200-μℓ urine sample and digesting it in 200 μℓ of the acid ashing solution instead of 1 mℓ. (See Section 8.3.1.)

3. Interferences

3.1 The presence of copper (II) ions in the sample digestate appears to accelerate the oxidation of antimony (III) to antimony (V). This interference is minimized by the presence of an amount of reducing agent in excess of that required for the reduction of antimony (V) to antimony (III) and copper (II) to copper (I).

3.2 Bismuth is stripped at the same potential as antimony. If this interference is suspected, the sample should be analyzed for bismuth after the addition of 20 μℓ of 5% sodium hypochlorite solution to oxidize antimony (III) to antimony (V); a reduction of the peak height by 80% or more signifies that the peak was due to antimony only.

3.3 The formation of an interamalgam complex of copper and antimony during the plating step is minimized by the presence of a relatively large concentration of chloride ions.

3.4 Incomplete digestion of a sample, indicated by a yellowish coloration, may lead to peak broadening and anomalous results.

4. Precision and Accuracy

4.1 Several processes determine the reproducibility of the overall analytical method: (1) sampling, (2) shipping, (3) preparation of samples, and (4) analysis. The variation introduced by sampling urine in industrial plants has not been evaluated. The shipping of samples in polyethylene bottles, as described in Section 8.2, and storage for 3 weeks prior to analysis produce a negligible effect on precision at antimony levels greater than 50 μg/ℓ. The coefficient of variation for the analysis step alone is 0.04 over the range of 10 to 500 ng. Measurements with two different electrodes with different histories may differ by a factor of two; however, the coefficient of variation remains 0.04 for measurements with each electrode. The coefficient of variation for the preparation and analysis steps combined is 0.08 over the range of 20 to 250 ng, which corresponds to 20 to 250 μg/ℓ in a 1.0-mℓ sample of urine.

4.2 The estimated percent error in a measurement, neglecting any contribution from sampling, is less than about 10% of the "true" value in the range of 20 to 250 μg/ℓ.

5. Advantages and Disadvantages of the Method

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 The primary disadvantage is its sensitivity to interferences. (See Section 3.)

5.4 Another disadvantage is the occurrence of large variations in response from electrode to electrode.

6. Apparatus

6.1 Polyethylene bottles (180 mℓ) with screw caps.

6.2 A urinometer (or hydrometer capable of measuring specific gravities in the range of 1.000 to 1.040 with an uncertainty of only ±0.001 units.)

6.3 Pyrex test tubes (20 x 100 mm) for digesting urine samples and subsequent use as analysis cells.

6.4 Digestion racks for the analysis cells.

6.5 Model SA 2011 Single-Cell Anodic Stripping Voltammeter with composite mercury-graphite electrode, Environmental Sciences Associates, Inc., Burlington, Mass., or equivalent. Instruments with more than one analysis cell are available and are recommended for large number of routine determinations. Instructions for the preparation and reconditioning of the CMGE are included in the instrument manual.

6.6 Micro pipettes (5, 10, 20, 50, 100, 200, and 500 μℓ, and 1 mℓ), Eppendorf or equivalent.

6.7 Potentiometric recorder (100-mV range).

6.8 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric flasks, and volumetric pipettes of appropriate sizes.

7. Reagents

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in antimony. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 **Acid Ashing Solution.** Prepare the acid ashing solution by adding 24 volumes of concentrated nitric acid to 24 volumes of perchloric acid (70%) and 1 volume of concentrated sulfuric acid.

7.5 **Hydrazine Dihydrochloride-Acid Solution (5 *N* HCl, 0.001 *N* $H_2NNH_2 \cdot (HCl)_2$).** Add 0.105 g of hydrazine dihydrochloride to a 1-liter volumetric flask. Add approximately 400 mℓ of water and swirl to dissolve the salt. Add 417 mℓ of concentrated hydrochloric acid, swirl, and dilute to the mark with water. This solution may be conveniently stored in a polyethylene bottle with a screw cap. The high concentration of chloride ions is necessary to minimize interference by copper.

7.6 Antimony Reference Standard Solution (1000 μg/mℓ), Fisher Scientific Co., or equivalent.

7.7 **Antimony (Sb(III)), Standard Solution "B" (100 μg/mℓ).** Transfer 10 mℓ of the antimony reference standard solution (1000 μg/mℓ) with a volumetric pipette to a 100-mℓ volumetric flask and dilute to the mark with water. Prepare fresh daily.

7.8 **Antimony (Sb(III)), Standard Solution "C" (1 μg/mℓ).** Transfer 1 mℓ of standard solution "B" with a volumetric pipette to a 100-mℓ volumetric flask and dilute to the mark with water. Prepare fresh daily.

7.9 Sodium Hypochlorite Solution (4 to 6%, w/w).

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any antimony from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 220 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove, and allow them to cool. Then rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom and allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water. Polyethylene bottles used in sample collection are cleaned by rinsing in dilute nitric acid and distilled deionized water. The bottles should not be rinsed with concentrated acid as this may cause sample loss.

8.2 **Collection and Shipping of Samples.** Collect the samples in precleaned 180-mℓ polyethylene bottles. Ship the samples in a box with a minimum of 0.25 in. of foam packing to prevent shock that may lead to opening of the screw caps and resultant leakage.

8.3 Analysis of Samples

8.3.1 Shake the urine sample thoroughly and allow the foam to settle. Measure the specific gravity of each sample with a urinometer or suitable hydrometer. (If the specific gravity is less than 1.010, discard the sample. Obtain another sample from the subject if desired.) Pipette 1 mℓ of sample into an analysis cell and then add 1 mℓ of the acid ashing solution to the cell. Duplicate 1-mℓ aliquots of the urine sample should be taken for analysis.

8.3.2 Place the cell in a digestion rack on a hot plate operating at 100 to 120°C. Carry out the digestion in a hood. The sample will initially turn brown and will evaporate to about half-volume in 10 min. Adjust the temperature of the hot plate to 220 to 240°C. A reflux line will be established about halfway up the cell. At the end of 20 min, there will be approximately 100 to 200 μℓ of acid left in the bottom of the cell, with most of the nitric acid having evaporated. The digestion should be continued for an additional 20 to 30 min until the acid is almost completely evaporated.

8.3.3 Remove the sample from the hot plate, allow it to cool, and add 5 mℓ of the hydrazine dihydrochloride-acid solution to the cell. Allow the solution to run down the sides of the cells to insure that any spattered material is washed down. Then heat the cell to about 90°C for 15 min, remove it from the hot plate, allow it to cool to room temperature, and readjust the volume of the sample to 5 mℓ with the hydrazine dihydrochloride-acid solution if necessary.

8.3.4 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the sample solution under the following conditions (all voltages are given with respect to Ag/AgCl reference electrode):

- Plating potential, -400 mV
- Auto-hold cutoff potential, 200 mV
- Plating time, 15 to 30 min. (The plating time for a set of samples and standards must be the same.)
- Sweep rate, +30 mV/sec
- Current range, 0.1 to 2 mA (full scale)

The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of antimony plated on the electrode may occur. A baseline is drawn by connecting with a straight line the flat portions of the curve before and after the antimony peak, which appears at approximately -180 mV. Peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions: (1) reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solutions of antimony; (4) urine standards prepared as described below in Section 9.1. The sequence in which these four kinds of solutions are analyzed is as follows: (1) a set of standard solutions of antimony; (2) sample solutions, with reagent blanks and urine standards interspersed; and (3) a second set of standard solutions of antimony. The standard solutions of antimony are used for calibration; the results for the samples are corrected for the amount of antimony found in the reagent blank; and the urine standards are used to determine the efficiency of recovery of known amounts of antimony from urine. The procedure described is for use with a four-cell ASV unit.

9.1 Preparation of Urine Standards

9.1.1 A "normal" urine sample from an individual not suspected of exposure to antimony is analyzed by the method of standard additions. One-milliliter samples of the urine are placed in each of four analysis cells. Three of these samples are spiked with known amounts of antimony (5-, 10-, and 20-$\mu\ell$ aliquots of standard solution "C," containing 5, 10, and 20 ng of antimony, respectively). These samples are digested and analyzed by ASV according to the procedure given in Section 8.3 and the concentration of antimony in the unspiked "normal" urine is estimated.

9.1.2 Finally, an amount of standard antimony solution is added to [illegible] mℓ of unspiked "normal" urine to make the total amount of antimony equal to 20 ng. This standard urine solution is subsequently analyzed along with calibration standards and blanks (Section 9.3) to determine the percent recovery of antimony.

9.2 **Initial Analysis of Four Standards.** Four standards of 20 ng of antimony, prepared by adding 20 $\mu\ell$ of standard solution "C" to 5 mℓ of the hydrazine dihydrochloride-acid solution, should be analyzed first. Each of the four standards should be analyzed with a different electrode. A single point may be taken to describe the calibration curve, which has been determined to be linear up to 500 ng of antimony. However, the calibration curves will vary from electrode to electrode, and a curve must be established for each electrode.

9.3 **Analysis of Samples and Controls.** The samples are then analyzed with the reagent blanks and urine standards (Section 9.1.2) interspersed at the rate of one blank for every 30 samples and one urine standard for every 10 samples. One reagent blank and one urine standard should be analyzed with each electrode during the analysis of a set of samples.

9.4 Final Analysis of Standards. The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 20 ng of antimony will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA for each electrode since the peak heights may vary significantly from electrode to electrode. The response factor for a particular electrode is the amount of antimony in each standard (20 ng) divided by the average of the two peak height values obtained for that electrode. For a 30-min plating time, a typical response factor is 0.75 ng/μA.

10. Calculations

10.1 The amount of antimony in a sample is calculated from the peak height as measured according to Section 8.3.4, the response factor, and a correction factor in the following manner:

$$\text{Amount (ng)} = \frac{\text{peak height } (\mu A)}{C} \times \text{response factor } (ng/\mu A)$$

where: C = a correction factor equal to the average fraction of antimony recovered from the urine standards with the particular electrode used.

Any amount of antimony found in the reagent blank should be subtracted from the sample value to give the correct amount (in ng) of antimony in the sample.

10.2 The concentration of antimony in a urine sample may be expressed as μg/ℓ:

$$\mu g/\ell = \frac{\text{corrected weight (in ng) of antimony in sample}}{\text{volume (in m}\ell\text{) of sample}}$$

10.3 The use of a specific gravity correction factor to normalize values to 1.024, the average specific gravity of urine, has been recommended. (See Reference 11.2) The preferred method of normalization is to make a straightforward gravity correction as follows:

$$\text{Corrected } \mu g/\ell = \mu g/\ell \times \frac{0.024}{(\text{specific gravity} - 1.000)}$$

11. References

11.1 Environmental Sciences Associates, Inc., Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Division of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

11.2 Elkins, H.B., "Concentration Adjustments in Urinalysis," Amer. Ind. Hyg. Ass. J., 35, 559 (1974).

GALLIUM IN URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Gallium	**Method No.:** P&CAM 198
Matrix: Urine	**Range:** 10 to 500 μg/ℓ in 2 mℓ of urine
Procedure: Anodic stripping voltammetry	**Precision** (CV): 0.12 (analytical) at 100 μg/ℓ
Date Issued: 4/15/73	**Classification:** E (Proposed)
Date Revised: 1/29/76	

1. **Principle of the Method**

A sample of urine is wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The acidic residue is dissolved in an acetate buffer solution and the buffered solution is analyzed by anodic stripping voltammetry (ASV) with a composite mercury-graphite electrode (CMGE). In principle, a hanging mercury drop electrode (HMDE) can also be used. However, optimum analysis parameters may vary significantly and must be established for each electrode system. The method as presented is designed for use with the CMGE.

2. **Range and Sensitivity**

2.1 The potential required to plate gallium on the CMGE from samples containing less than 20 ng is sufficently cathodic to cause the evolution of hydrogen. Consequently, the ASV response for gallium is not a linear function of the weight of gallium present below the operational detection limit of 20 ng.

2.2 The linear range of the electrode response extends from the detection limit to 1 μg of gallium. However, at amounts near 1 μg, the percent error in estimating values from the calibration curve will be about 10%. The range of 20 ng to 1 μg corresponds to 10 to 500 μg/ℓ in a 2.0-mℓ sample of urine. The range may be extended by analyzing a smaller urine sample or by diluting the sample digestate with acetate buffer and taking an aliquot of the diluted solution for the ASV determination.

3. **Interferences**

3.1 Even small amounts of copper pose a severe and variable interference; copper forms an interamalgam complex with gallium that hinders the stripping of the gallium from the electrode in the ASV procedure. The interference can be diminished by changing the

501

plating potential from -1300 mV to -1400 mV, which yields a higher gallium response in the presence of copper, and by concomitantly using the method of standard additions for the analysis of each sample. However, this approach may lead to interference from iron (see Section 3.2). Another approach is to remove the copper by electrolytically plating it at a potential of -600 mV on one electrode prior to the plating of gallium on a second electrode. This procedure has been shown to markedly improve the ASV response to gallium in the presence of copper.

3.2 At a potential of -1400 mV or more, iron, if present at levels higher than 10 μg in the sample digestate, will be plated on the electrode and will mask the electrode processes for all metals reduced at more anodic potentials.

3.3 Incomplete digestion of a sample, indicated by a yellowish coloration, may lead to peak broadening and anomalous results.

3.4 If present at levels higher than 10 μg in the sample aliquot, zinc will cause a serious interference in the determination of gallium. However, zinc may be removed from the sample digestate by solvent extraction prior to the ASV analysis. (See Reference 11.3.)

4. Precision and Accuracy

4.1 Several processes determine the reproducibility of the overall analytical method: (1) sampling, (2) shipping, (3) preparation of samples, and (4) analysis. Variations introduced by sampling urine at industrial sites and by shipping the collected samples to a distant laboratory prior to analysis have not been evaulated. The variations introduced by the preparation and analysis steps yield a coefficient of variation of 0.12 over the range of 100 ng to 1 μg, which corresponds to 50 to 500 μg/ℓ of gallium in a 2.0-mℓ sample of urine.

4.2 The estimated percent error in a measurement, neglecting any contribution from sampling and shipping, is generally less than 15% of the "true" value in the concentration range of 50 to 500 μg/ℓ.

5. Advantages and Disadvantages of the Method

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 One disadvantage is that copper, iron. and zinc may pose serious interferences in the ASV determination of gallium with a CMGE.

6. Apparatus

6.1 Polyethylene bottles (180 mℓ) with screw caps.

6.2 A urinometer (or hydrometer capable of measuring specific gravities in the range of 1.000 to 1.040 with an uncertainty of only ±0.001 units).

6.3 Pyrex test tubes (20 x 100 mm) for digesting urine samples and subsequent use as analysis cells.

6.4 Digestion racks for the analysis cells.

6.5 Micro pipettes (10, 50, 100, 200, and 500 μℓ), Eppendorf or equivalent.

6.6 Model SA 2011 Single-Cell Anodic Stripping Voltammeter with composite mercury-graphite electrode, Environmental Sciences Associates, Inc., Burlington, Mass., or equivalent. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations. Instructions for the preparation and reconditioning of the CMGE are included in the instruction manual.

6.7 Potentiometric recorder (100-mV range).

6.8 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric flasks, volumetric pipettes, and graduated pipettes of appropriate sizes.

7. Reagents

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in gallium. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 **Acid Ashing Solution.** Prepare the acid ashing solution by adding 24 volumes of concentrated nitric acid to 24 volumes of perchloric acid (70%) and 1 volume of concentrated sulfuric acid.

7.5 **Acetate Buffer Solution (0.5 *M* NaAc, 4 *M* LiCl, pH 4 to 5).** To prepare 1 liter of this reagent, dissolve 41 g of sodium acetate, 170 g of lithium chloride, and 15 to 35 mℓ of 70% $HClO_4$ in water in a 1-liter volumetric flask and fill to the mark with water. The pH of the solution should be 4 to 5. Remove trace metals from the resulting solution electrolytically with a system such as the Model 2014PM Reagent Cleaning System, Environmental Sciences Associates, Inc., or equivalent.

509

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7.6 **Aqua Regia.** Prepare aqua regia by mixing 1 volume of concentrated nitric acid (doubly distilled) with 4 volumes of concentrated hydrochloric acid.

7.7 **Gallium (Ga(III)), Standard Solution "A" (10 mg/mℓ).** Weigh accurately about 1 g of pure gallium metal and transfer it to a 100-mℓ volumetric flask. Dissolve the metal in 10 mℓ of aqua regia and dilute to the mark with water.

7.8 **Gallium (Ga(III)), Standard Solution "B" (100 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "A" to a 100-mℓ volumetric flask containing 10 mℓ of concentrated hydrochloric acid and dilute to the mark with water. Prepare fresh daily to prevent loss of gallium or contamination.

7.9 **Gallium (Ga(III)), Standard Solution "C" (1 μg/mℓ).** With a 1-mℓ volumetric pipette transfer 1 mℓ of standard solution "B" to a 100-mℓ volumetric flask containing 10 of concentrated hydrochloric acid and dilute to the mark with water. Prepare f daily to prevent loss of gallium or contamination.)

7.10 **EDTA (Ethylenediaminetetraacetic Acid) Solution (0.1 *M*).** Dissolve 37.2 g of t disodium salt of EDTA in water in a 1-liter volumetric flask and dilute to the ma with water.

8. **Procedure**

8.1 **Cleaning of Equipment**

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any gallium from the surface and immediate sursurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 200 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove, and allow to cool. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom and allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water. Polyethylene bottles used in sample collection are cleaned by rinsing in dilute nitric acid and distilled deionized water. The bottles should not be rinsed with concentrated acid as this may cause sample loss.

8.2 **Collection and Shipping of Samples.** Collect the samples in precleaned 180-mℓ polyethylene bottles containing 1 mℓ of 0.1 *M* EDTA. Ship the samples in a box with a minimum of 0.25 in. of foam packing to prevent shock that may lead to opening of the screw caps and resultant leakage.

8.3 **Analysis of Samples**

8.3.1 Shake the urine sample thoroughly and allow the foam to settle. Measure the specific gravity of the sample with a urinometer or a suitable hydrometer. (If the specific gravity is less than 1.010, discard the sample. Obtain another sample from the subject if desired.) Pipette 2 mℓ of sample into an analysis cell and then add 500 μℓ of acid ashing solution to the cell. Duplicate 2-mℓ aliquots of the urine sample should be taken for analysis.

8.3.2 Place the cell in a digestion rack on a hot plate operating at 100 to 120°C. Carry out the digestion in a hood. The sample will initially turn brown and will evaporate to about half-volume in 10 min. Adjust the temperature of the surface of the hot plate to 220 to 240°C. A reflux line will be established about halfway up the cell. At the end of 20 min, there will be approximately 100 to 200 μℓ of acid left in the bottom of the cell, with most of the nitric acid having evaporated. The digestion should be continued for an additional 20 to 30 min until the acid is almost completely evaporated.

8.3.3 Remove the sample from the hot plate, cool it, and add 4.5 mℓ of the acetate buffer solution.

8.3.4 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the buffered solution under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -1300 mV.
- Auto-hold cutoff potential, 1150 mV.
- Plating time, 30 min.
- Sweep rate,+60 mV/sec.
- Current range, 0.1 to 2 mA (full scale).

The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of gallium plated on the electrode may occur. As the sample is stripped, a zinc peak (-1150 mV) will occur first, followed by the gallium peak (-1050 mV), and then a cadmium peak (-720 mV). A baseline is drawn by connecting

with a straight line the flat portions of the curve before and after the gallium peak. The peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions: (1) reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solutions of gallium in acetate buffer; (4) urine standards prepared as described below in Section 9.1. The sequence in which these four kinds of solutions are analyzed is as follows: (1) a set of standard solutions of gallium in acetate buffer; (2) sample solutions, with reagent blanks and urine standards interspersed at the rate of one blank and one urine standard for every ten samples; and (3) a second set of standard solutions of gallium in acetate buffer. The standard solutions of gallium in acetate buffer are used for calibration; the results for the samples are corrected for the amount of gallium found in the reagent blank; and the urine standards are used to determine the efficiency of recovery of known amounts of gallium from urine. The procedure described is for use with a four-cell ASV unit.

9.1 Preparation of Urine Standards

9.1.1 A "normal" urine sample from an individual not suspected of exposure to gallium is analyzed by the method of standard additions. Two-milliliter samples of the urine are placed in each of six analysis cells. Five of these samples are spiked with known amounts of gallium (10-, 50-, 100-, 200-, and 500-$\mu\ell$ aliquots of standard solution "C," containing 10, 50, 100, 200, and 500 ng of gallium respectively). These samples are digested and analyzed by ASV according to the procedure given in Section 8.3 and the concentration of gallium in the unspiked "normal" urine is estimated.

9.1.2 Finally, an amount of standard gallium solution is added to 2.0 mℓ of unspiked "normal" urine to make the total amount of gallium equal to 200 ng. This standard urine solution is subsequently analyzed along with calibration standards and blanks (Section 9.3) to determine the percent recovery of gallium.

9.2 **Initial Analysis of Four Standards.** Four standards of 200 ng of gallium, prepared by adding 200 $\mu\ell$ of standard solution "C" to 4.5 mℓ of acetate buffer solution, should be analyzed first. Each of the four standards should be analyzed with a different electrode. At the level of 200 ng, the contributions from blanks should be negligible, and a single point may be taken to describe the calibration curve for each electrode.

9.3 **Analysis of Samples and Controls.** With every ten samples, a urine standard (Section 9.1.2) and a reagent blank are analyzed by the ASV procedure given in Section 8.3. At least one reagent blank and one urine standard should be analyzed with each electrode that is used for analysis of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 200 ng of gallium will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA. The overall response factor is the amount of gallium in the standard (200 ng) divided by the average of the eight peak heights (in μA). However, it will be necessary to calculate an individual response factor for an electrode if the average of the two peak height values for an individual electrode differs from the overall average of the eight peak heights by more than 10%. For example, if the average for the eight peak heights is 210 μA, and the average of the two values for one of the electrodes is 185 μA, then the response factor to be used for that electrode is 200 ng/185 μA, or 1.08 ng/μA. The overall response factor in this example is 200 ng/210 μA, or 0.95 ng/μA.

10. Calculations

10.1 The amount of gallium in a sample is calculated from the gallium peak height (in μA), as measured according to Section 8.3.4, and the response factor in the following manner:

Amount (in ng) = peak height (in μA) x response factor (in ng/μA)

Any amount of gallium found in the reagent blank should be subtracted from the sample value to give the correct amount (in ng) of gallium in the sample. The average recovery of gallium from urine standards is usually greater than 90%. However, it may be necessary to include a factor in the above equation to correct for incomplete recovery.

10.2 The concentration of gallium in a urine sample may be expressed as μg/ℓ:

$$\mu g/\ell = \frac{\text{corrected weight (in ng) of gallium in sample}}{\text{volume (in m}\ell\text{) of sample}}$$

10.3 The use of a specific gravity correction factor to normalize values to 1.024, the average specific gravity of urine, has been recommended. (See Reference 11.2.) The preferred method of normalization is to make a straightforward gravity correction as follows:

$$\text{Corrected } \mu g/\ell = \mu g/\ell \times \frac{0.024}{(\text{specific gravity} - 1.000)}$$

Report the uncorrected concentration, the corrected concentration, and the specific gravity of the sample.

11. References

11.1 Environmental Sciences Associates, Inc., Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Division of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

11.2 Elkins, H.B., "Concentration Adjustments in Urinalysis," Amer. Ind. Hyg. Ass. J., 35, 559 (1974).

11.3 Davis, P.H., and E.D. Moorhead, "Phase-Selective Anodic Stripping Analyses of Gallium in Spiked Bovine Tissue," Anal. Lett., 8, 387 (1975).

INDIUM IN URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Indium	**Method No.:** P&CAM 199
Matrix: Urine	**Range:** 1 to 500 μg/ℓ in 2 mℓ of urine
Procedure: Anodic stripping voltammetry	**Precision** (CV): 0.10 (analytical) at 50 μg/ℓ
Date Issued: 5/15/73	**Classification:** E (Proposed)
Date Revised: 1/29/76	

1. **Principle of the Method**

A sample of urine is wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The residue is dissolved in an acetate buffer solution that is 1 *M* in NaAc and 4 *M* in LiCl. The buffered sample solution is analyzed by anodic stripping voltammetry (ASV) with a composite mercury-graphite electrode (CMGE). In principle, a hanging mercury drop electrode (HMDE) can also be used. However, optimum analysis parameters may vary significantly and must be established for each electrode system. The method as presented is designed for use with the CMGE.

2. **Range and Sensitivity**

2.1 Indium is not normally present in the reagent blank in detectable amounts. Therefore, sensitivity is limited only by the baseline current in the ASV analysis. The detection limit is estimated to be 2 ng, which corresponds to 1 μg/ℓ in a 2-mℓ sample of urine.

2.2 The linear range of the electrode response is estimated to extend from the detection limit to 1 μg of indium, which corresponds to 500 μg/ℓ in a 2-mℓ sample of urine. The range may be extended by analyzing a smaller urine sample or by diluting the sample digestate with acetate buffer and taking an aliquot of the diluted solution for the ASV determination.

3. **Interferences**

3.1 In the ASV analysis, the indium peak occurs between lead and cadmium peaks. Consequently, microgram amounts of lead or cadmium interfere with the determination of indium. At lead levels greater than 2 μg, the indium peak occurs as a shoulder on the lead peak and measurements of indium peak heights become inaccurate. Interferences by cadmium can be reduced with no loss of response to indium by changing the plating potential from -1000 mV to -900 mV and changing the auto-hold cutoff potential from 980 mV to 780 mV.

3.2 Incomplete digestion of a sample, indicated by a yellowish coloration, may lead to peak broadening and anomalous results.

4. **Precision and Accuracy**

4.1 Several processes determine the reproducibility of the overall analytical method: (1) sampling, (2) shipping, (3) preparation of samples, and (4) analysis. The variations introduced by sampling urine at industrial sites and shipping the collected samples to a distant laboratory have not been evaluated. The coefficient of variation for the preparation and analysis steps has been empirically determined to be 0.10 over the range of 20 to 1000 ng, which corresponds to 10 to 500 $\mu g/\ell$ in a 2-mℓ sample of urine, and 0.20 over the range of 2 to 20 ng, which corresponds to 1 to 10 $\mu g/\ell$ in a 2-mℓ sample of urine.

4.2 The estimated percent error in a measurement, neglecting any contribution from sampling and shipping, is less than about 10% of the "true" value in the range of 20 ng to 1 μg, which corresponds to 10 to 500 $\mu g/\ell$ in a 2-mℓ sample of urine.

5. **Advantages and Disadvantages of the Method**

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 Also, the matrix in which the digested samples are analyzed, the acetate buffer solution, allows the accurate determination of lead, cadmium, and copper, as well as indium.

5.4 One disadvantage is that relatively high lead and cadmium concentrations interfere with the determination of indium. (See Section 3.)

6. **Apparatus**

6.1 Polyethylene bottles (180 mℓ) with screw caps.

6.2 A urinometer (or hydrometer capable of measuring specific gravities in the range of 1.000 to 1.040 with an uncertainty of only ±0.001 units).

6.3 Pyrex test tubes (20 x 100 mm) for digesting urine samples and subsequent use as analysis cells.

6.4 Digestion racks for the analysis cells.

6.5 Micro pipettes (10, 50, 100, 200, and 500 $\mu\ell$), Eppendorf or equivalent.

6.6 Model SA 2011 Single-Cell Anodic Stripping Voltammeter with composite mercury-graphite electrode, Environmental Sciences Associates, Inc., Burlington, Mass., or equivalent. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations. Instructions for the preparation and reconditioning of the CMGE are included in the instrument manual.

6.7 Potentiometric recorder (100-mV range).

6.8 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric flasks, volumetric pipettes, and graduated pipettes of appropriate sizes.

7. Reagents

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in indium. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 **Acid Ashing Solution.** Prepare the acid ashing solution by adding 24 volumes of concentrated nitric acid to 24 volumes of perchloric acid (70%) and 1 volume of concentrated sulfuric acid.

7.5 **Acetate Buffer Solution (1 *M* NaAc, 4 *M* LiCl, pH 5.2).** To prepare 1 liter of this reagent, dissolve 82 g of sodium acetate, 170 g of lithium chloride, and 21 mℓ of 70% $HClO_4$ in water in a 1-liter volumetric flask and fill to the mark with water. Remove trace metals from the resulting solution electrolytically with a system such as the Model 2014 PM Reagent Cleaning System, Environmental Sciences Associates, Inc., or equivalent. The acetate buffer maintains the pH of the resulting sample solution near 5.2, a pH favorable for indium determination. The relatively high concentration of chloride ions minimizes interference from lead and cadmium.

7.6 **Indium (In(III)), Standard Solution "A" (10 mg/mℓ).** Weigh accurately an amount of pure indium shot (99.99% pure) near 1 g and transfer the weighed indium to a 100-mℓ volumetric flask. Dissolve the metal in 30 mℓ of concentrated nitric acid and dilute to the mark with water.

7.7 **Indium (In(III)), Standard Solution "B" (100 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "A" to a 100-mℓ volumetric flask containing 2 to 3 mℓ of concentrated nitric acid and dilute to the mark with water. Prepare fresh daily to prevent loss of indium or contamination.

7.8 **Indium (In(III)), Standard Solution "C" (1 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "B" to a 100-mℓ volumetric flask containing 2 to 3 mℓ of concentrated nitric acid and dilute to the mark with water. Prepare fresh daily to prevent loss of indium or contamination.

7.9 **EDTA (Ethylenediaminetetraacetic Acid) Solution (0.1*M*).** Dissolve 37.2 g of the disodium salt of EDTA in water in a 1-liter volumetric flask and dilute to the mark with water.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any indium from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 220 to 240 °C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove, and allow them to cool. Complete decontamination requires four repetitions of this procedure. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom and allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water. Polyethylene bottles used in sample collection are cleaned by rinsing in dilute nitric acid and distilled deionized water. The bottles should not be rinsed with concentrated acid as this may cause sample loss.

8.2 **Collection and Shipping of Samples.** Collect the samples in precleaned 180-mℓ polyethylene bottles containing 1 mℓ of 0.1 *M* EDTA. Ship the samples in a box with a minimum of 0.25 in. of foam packing to prevent shock that may lead to opening of the screw cap and resultant leakage.

8.3 Analysis of Samples

8.3.1 Shake the urine sample thoroughly and allow the foam to settle. Measure the specific gravity of the sample with a urinometer or suitable hydrometer. (If the specific gravity is less than 1.010. discard the sample. Obtain another sample from the subject, if desired.) Pipette 2 mℓ of each sample into an analysis cell and then add 500 μℓ of acid ashing solution to the analysis cell. Duplicate 2-mℓ aliquots of the urine sample should be taken for analysis.

8.3.2 Place the cell in a digestion rack on a hot plate operating at 100 to 120°C. Carry out the digestion in a hood. The sample will initially turn brown and will evaporate to about half-volume in 10 min. Adjust the temperature of the hot plate to 220 to 240°C. A reflux line will be established about halfway up the cell. At the end of 20 min, there will be approximately 100 to 200 $\mu\ell$ of acid left in the bottom of the cell, with most of the nitric acid having evaporated. The digestion should be continued for an additional 20 to 30 min until the acid is almost completely evaporated.

8.3.3 Remove the sample from the hot plate, cool it, and add 4.5 mℓ of the acetate buffer solution.

8.3.4 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the buffered solution under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -1100 mV
- Auto-hold cutoff potential, 980 mV
- Plating time, 30 min.
- Sweep rate, +60 mV/sec
- Current range, 0.05 to 2 mA (full scale)

The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of indium plated on the electrode may occur. As the sample is stripped, the cadmium peak (-720 mV) will occur first, followed by the indium peak (-650 mV), and then the lead peak (-530 mV). A baseline is drawn by connecting with a straight line the flat portion of the curve after the cadmium peak with the flat portion before the lead peak. At high concentrations of lead, a baseline is drawn by extending the shoulder of the lead peak from the end of the indium peak to its beginning. The peak height is measured by dropping a perpendicular from the apex to the baseline.

513.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions: (1) reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solutions of indium in acetate buffer; (4) urine standards prepared as described in Section 9.1. The sequence in which these four kinds of solutions are analyzed is as follows: (1) a set of standard solutions of indium in acetate buffer; (2) sample solutions, with reagent blanks and urine standards interspersed at the rate of one blank and one urine standard for every ten samples; and (3) a second set of standard solutions of indium in acetate buffer. The standard solutions of indium in acetate buffer are used for calibration; the results for the samples are corrected for the amount of indium found in the reagent blank; and the urine standards are used to determine the efficiency of recovery of known amounts of indium from urine. The procedure described is for use with a four-cell ASV unit.

9.1 Preparation of Urine Standards

9.1.1 A "normal" Urine sample from an individual not suspected of exposure to indium is analyzed by the method of standard additions. Two-milliliter samples of the urine are placed in each of six analysis cells. Five of these samples are spiked with known amounts of indium (10-, 50-, 100-, 200-, and 500-$\mu\ell$ aliquots of standard solution "C," containing 10,50, 100, 200, and 500 ng, respectively). These samples are digested and analyzed by ASV according to the procedure given in Section 8.3 and the concentration of indium in the unspiked "normal" urine is estimated.

9.1.2 Finally, an amount of standard indium solution is added to 2 mℓ of unspiked "normal" urine to make the total amount of indium equal to 100 ng. This standard urine solution is subsequently analyzed along with calibration standards and blanks (Section 9.3) to determine the percent recovery of indium.

9.2 **Initial Analysis of Four Standards.** Four standards of 100 ng of indium, prepared by adding 100 $\mu\ell$ of standard solution "C" to 4.5 mℓ of acetate buffer solution, should be analyzed first. Each of the four standards should be analyzed with a different electrode. At the level of 100 ng, the contributions from blanks should be negligible, and a single point may be taken to describe the calibration curve for each electrode, which has been determined to be linear through 1 μg.

9.3 **Analysis of Samples and Controls.** With every ten samples, a urine standard (Section 9.1.2) and a reagent blank are analyzed by the ASV procedure given in Section 8.3. At least one reagent blank and one urine standard should be analyzed with each electrode that is used for analysis of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 100 ng of indium will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA. The overall response factor is the amount of indium in each standard (100 ng) divided by the average of the eight peak heights (in μA). However, it will be necessary to calculate an individual response factor for an electrode if the average of the two peak height values for an individual electrode differs from the overall average of the eight peak heights by more than 10%. For example, if the average for the eight peak heights is 210 μA, and the average of the two values for one of the electrodes is 185 μA, then the response factor to be used for that electrode is 100 ng/185 μA, or 0.54 ng/μA. The overall response factor in this example is 100 ng/210 μA, or 0.48 ng/μA.

10. Calculations

10.1 The amount of indium in a sample is calculated from the indium peak height (in μA), as measured according to Section 8.3.4, and the response factor in the following manner:

Amount (in ng) = peak height (in μA)x response factor (in ng/μA)

Any amount of indium found in the reagent blank should be subtracted from the sample value to give the corrected weight (in ng) of indium in the sample. The average recovery of indium from urine standards is usually greater than 90%. However, it may be necessary to include a factor in the above equation to correct for incomplete recovery.

10.2 The concentration of indium in a urine sample may be expressed as μg/ℓ:

$$\mu g/\ell = \frac{\text{corrected weight (in ng) of indium in sample}}{\text{volume (in m}\ell\text{) of sample}}$$

10.3 The use of a specific gravity correction factor to normalize values to 1.024, the average specific gravity of urine, has been recommended. (See Reference 11.2.) The preferred method of normalization is to make a straightforward gravity correction as follows:

$$\text{Corrected } \mu g/\ell = \mu g/\ell \times \frac{0.024}{(\text{specific gravity} - 1.000)}$$

11. References

11.1 Environmental Sciences Associates, Inc. Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Division of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

11.2 Elkins, H.B., "Concentration Adjustments in Urinalysis," **Amer. Ind. Hyg. Ass. J., 35,** 559 (1974).

515

LEAD IN URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Lead	Method No.: P&CAM 200
Matrix: Urine	Range: 4 to 1000 μg/ℓ in 1 mℓ of urine
Procedure: Anodic stripping voltammetry	Precision (CV): 0.06 (analytical) at 200 μg/ℓ
Date Issued: 4/15/73	
Date Revised: 1/29/76	Classification: E (Proposed)

1. **Principle of the Method**

A sample of urine is wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The residue is dissolved in dilute perchloric acid. The resulting solution is analyzed for lead (Pb(II)) by anodic stripping voltammetry (ASV) with a composite mercury-graphite electrode (CMGE). In principle, a hanging mercury drop electrode (HMDE) can also be used. However, optimum analysis parameters may vary significantly and must be established for each electrode system. The method as presented is designed for use with the CMGE.

2. **Range and Sensitivity**

2.1 The sensitivity is limited by the reagent blank. A reasonable detection limit appears to be two times the standard deviation of the procedure blank. Statistically, the probability is 95% that lead is present in the sample if the signal is higher than this limit. Blanks for lead are usually 6 ± 2 ng. By the stated criterion, the detection limit is 4 ng, which corresponds to 4 μg/ℓ in a 1-mℓ sample of urine.

2.2 The linear range of the electrode response is estimated to extend from the detection limit to 1000 ng of lead, which corresponds to 1000 μg/ℓ in a 1-mℓ sample of urine. Near the level of 1000 ng, the response to lead begins to lose linearity. More accurate results can be obtained by decreasing the plating time from 30 to 10 min when concentrations in 1-mℓ samples are near 1000 μg/ℓ. The range may be extended by analyzing a smaller urine sample or by diluting the sample digestate with dilute perchloric acid and taking an aliquot of the diluted solution for the ASV determination.

3. Interferences

3.1 Thallium forms a shoulder on the cathodic edge of the lead peak. However, the thallium peak does not affect the height of the lead peak until thallium and lead are present in roughly the same concentrations. If the presence of a significant

concentration of thallium is suspected in a sample, as in a sample from the urine of workers in certain smelting or roasting industries, the sample should be analyzed for thallium. Thallium is determined by making the digested sample solution 0.01 *M* in EDTA (ethylenediaminetetraacetic acid) and analyzing the resulting solution by ASV at the same instrument settings as those recommended for the lead analysis. A typical response factor for thallium is 4 ng/μA when the plating time is 30 min. Since the presence of EDTA prevents the ASV response to lead but does not affect the response to thallium, the peak height obtained for thallium alone may be subtracted from the peak height obtained for both lead and thallium to estimate the response due to lead alone.

3.2 Incomplete digestion of a sample, indicated by a yellowish coloration, may lead to peak broadening and anomalous results.

4. Precision and Accuracy

4.1 Several processes determine the reproducibility of the overall analytical method: (1) sampling, (2) shipping, (3) preparation of samples, and (4) analysis. The variation introduced by sampling urine in industrial plants has not been evaluated. It has been found that precision is not significantly affected by the shipping of urine in polyethylene bottles. The variations introduced by the preparation and analysis steps may be estimated for the entire analytical range from the following empirical relation:

$$CV = \frac{2}{[Pb]} + 0.05$$

where: CV = the estimated coefficient of variation of a measurement neglecting any contribution from sampling or shipping.

[Pb] = the measured lead concentration (in μg/ℓ) in a 1-mℓ sample.

The factor 2 in the numerator of the first term of this expression reflects the scatter in the reagent blank. which corresponds to a standard deviation of 2 ng in a 1 mℓ sample, or 2 μg/ℓ. The second term represents the normal statistical variations in the preparation and analysis steps. (See References 11.1 and 11.3.)

4.2 The accuracy of the overall sampling and analytical method has not been evaluated. However, it is estimated that the percent error in a measurment, neglecting any contribution from sampling and shipping, is less than about 10% in the determination of levels near 200 ng of lead in 1-mℓ urine samples, which corresponds to concentrations near 200 μg/ℓ.

518

5. **Advantages and Disadvantages of the Method**

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 One disadvantage is that the current-voltage peaks for thallium and lead are not resolved when their concentrations are comparable.

6. **Apparatus**

6.1 Polyethylene bottles (180 mℓ) with screw caps.

6.2 A urinometer (or hydrometer capable of measuring specific gravities in the range of 1.000 to 1.040 with an uncertainty of only ± 0.001 units).

6.3 Pyrex test tubes (20 x 100 mm) for digesting urine samples and subsequent use as analysis cells.

6.4 Digestion racks for the analysis cells.

6.5 Micro pipettes (10, 50, 100, 200, and 500 μℓ, and 1 mℓ), Eppendorf or equivalent.

6.6 Model SA 2011 Single-Cell Anodic Stripping Voltammeter with composite mercury-graphite electrode, Environmental Sciences Associates, Inc., Burlington, Mass., or equivalent. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations. Instructions for the preparation and reconditioning of the CMGE are included in the instruction manual.

6.7 Potentiometric recorder (100-mV range).

6.8 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric flasks, volumetric pipettes, and graduated pipettes of appropriate sizes.

7. **Reagents**

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in lead. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 **Acid Ashing Solution.** Prepare the acid ashing solution by adding 327 volumes of concentrated nitric acid to 653 volumes of perchloric acid (70%) and 20 volumes of concentrated sulfuric acid.

7.5 Perchloric acid solution (0.35% v/v in water).

7.6 **Lead (Pb(II)), Standard Solution "A" (10 mg/mℓ).** Transfer 1.5987 g of lead nitrate ($Pb(NO_3)_2$) to a clean 100-mℓ volumetric flask. Dissolve the salt in approximately 30 mℓ of water, add 3 mℓ of concentrated nitric acid, and dilute to the mark with water.

7.7 **Lead (Pb(II)), Standard Solution "B" (100 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "A" to a 100-mℓ volumetric flask containing 1 mℓ of concentrated nitric acid and dilute to the mark with water.

7.8 **Lead (Pb(II)), Standard Solution "C" (1 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "B" to a 100-mℓ volumetric flask containing 1 mℓ of concentrated nitric acid and dilute to the mark with water. Prepare fresh daily to avoid loss of lead or contamination.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any lead from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 220 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove, and allow them to cool. Complete decontamination requires four repetitions of this procedure. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom and allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water. Polyethylene bottles used in sample collection are cleaned by rinsing in dilute nitric acid and distilled deionized water. The bottles should not be rinsed with concentrated acid as this may cause sample loss.

8.2 **Collection and Shipping of Samples.** Collect the samples in precleaned 180-mℓ polyethylene bottles. Ship the samples in a box with a minimum of 0.25 in. of foam packing to prevent shock that may lead to opening of the screw caps and resultant leakage.

8.3 **Analysis of Samples**

8.3.1 Shake the urine sample thoroughly and allow the foam to settle. Measure the specific gravity of the sample with a urinometer (or suitable hydrometer). (If the specific gravity is less than 1.010, discard the sample. Obtain another sample from the subject if desired.) Pipette 1 mℓ of sample into an analysis cell and then add 1 mℓ of the acid ashing solution to the cell. Duplicate 1-mℓ aliquots of the urine sample should be taken for analysis.

8.3.2 Place the cell in a digestion rack on a hot plate operating at 100 to 120°C. Carry out the digestion in a hood. The sample will initially turn brown and will evaporate to half-volume in 10 min. Adjust the temperature of the hot plate to 220 to 240°C. A reflux line will be established about halfway up the cell. At the end of 25 min, there will be approximately 100 to 200 μℓ of acid left in the bottom of the cell, with most of the nitric acid having evaporated. The digestion should be continued for an additional 20 to 30 min until the acid is almost completely evaporated.

8.3.3 Remove the cell from the hot plate, cool it, and add 4.5 mℓ of 0.35% $HClO_4$.

8.3.4 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the solution under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -780 mV
- Auto-hold cutoff potential, 900 mV
- Plating time, 10 to 30 min, (The plating time for a set of samples and standards must be the same.)
- Sweep rate, +60 mV/sec
- Current range, 0.2 to 1 mA (full scale)

The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of lead plated on the electrode may occur. As the sample is stripped, a cadmium peak (-600 mV) may occur first, followed by the lead peak (-430 mV). A baseline is drawn by connecting with a straight line the flat portions of the curve before and after the lead peak. The peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions: (1) reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solutions of lead and (4) urine standards prepared as described below in Section 9.1. The sequence in which these four kinds of solutions are analyzed is as follows: (1) a set of standard solutions of lead; (2) sample solutions, with reagent blanks and urine standards interspersed at the rate of one blank and one urine standard for every ten samples; and (3) a second set of standard solutions of lead. The standard solutions of lead are used for calibration; the results for the samples are corrected for the amount of lead found in the reagent blank; and the urine standards are used to determine the efficiency of recovery of known amounts of lead from urine. The procedure described is for use with a four-cell ASV unit.

9.1 Preparation of Urine Standards

9.1.1 A "normal" urine sample from an individual not suspected of exposure to lead is analyzed by the method of standard additions. One-milliliter samples of the urine are placed in each of six analysis cells. Five of these samples are spiked with known amounts of lead (10-, 50-, 100-, 200-, and 500-μℓ aliquots of standard solution "C", containing 10, 50, 100, 200, and 500 ng of lead, respectively). These samples are digested and analyzed by ASV according to the procedure given in Section 8.3 and the concentration of lead in the "normal" urine is estimated.

9.1.2 Finally, an amount of standard lead solution is added to 1 mℓ of unspiked "normal" urine to make the total amount of lead equal to 200 ng. This standard urine solution is subsequently analyzed along with calibration standards and blanks (Section 9.3) to determine the percent recovery of lead.

9.2 **Initial Analysis of Four Standards.** Four standards of 200 ng of lead, prepared by adding 200 μℓ of standard solution "C" to 4.5 mℓ of 0.35% $HClO_4$, should be analyzed first. Each of the four standards should be analyzed with a different electrode. At the level of 200 ng, the contributions from blanks should be negligible, and a single point may be taken to describe the calibration curve for each electrode, which has been determined to be linear through 1000 ng.

9.3 **Analysis of Samples and Controls.** With every ten samples, a urine standard (Section 9.1.2) and a reagent blank are analyzed by the ASV procedure given in Section 8.3. At least one reagent blank and one urine standard should be analyzed with each electrode that is used for analysis of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 200 ng of lead will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA. The overall response factor is the amount of lead in the standard (200 ng) divided by the average of the eight peak heights (in μA). However, it will be necessary to calculate an individual response factor for an electrode if the average of the two peak height values for an individual electrode differs from the overall average of the eight peak heights by more than 10%. For example, if the average for the eight peak heights is 210 μA, and the average of the two values for one of the electrodes is 185 μA, then the response factor to be used for that electrode is 200 ng/185 μA, or 1.08 ng/μA. The overall response factor in this example is 200 ng/210 μA, or 0.95 ng/μA.

10. Calculations

10.1 The amount of lead in a sample is calculated from the lead peak height (in μA), as measured according to Section 8.3.4, and the response factor in the following manner:

$$\text{Amount (in ng)} = \text{peak height (in } \mu\text{A)} \times \text{response factor (in ng/}\mu\text{A)}$$

Any amount of lead found in the reagent blank should be subtracted from the sample value to give the correct amount (in ng) of lead in the sample. The average recovery of lead from urine standards is usually greater than 90%. However, it may be necessary to include a factor in the above equation to correct for incomplete recovery.

10.2 The concentration of lead in a urine sample may be expressed as μg/mℓ:

$$\mu g/\ell = \frac{\text{corrected weight (in ng) of lead in sample}}{\text{volume (in m}\ell\text{) of sample}}$$

10.3 The use of a specific gravity correction factor to normalize values to 1.024, the average specific gravity of urine, has been recommended. (See Reference 11.2.) The preferred method of normalization is to make a straightforward gravity correction as follows:

$$\text{Corrected } \mu g/\ell = \mu g/\ell \times \frac{0.024}{(\text{specific gravity} - 1.000)}$$

Report the uncorrected concentration, the corrected concentration, and the specific gravity of the sample.

11. References

11.1 Environmental Sciences Associates, Inc., Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health,Division of Laboratory and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

11.2 Elkins, H.B., Concentration Adjustments in Urinalysis," Amer. Ind. Hyg. Ass. J., 35, 559 (1974).

11.3 Matson, W. R., R.M. Griffin, and T.J. Sapienza, "A Total System and Quality Control Procedure for Micro Blood Lead Analysis by Anodic Stripping Voltammetry (ASV)," Presented at the Laboratory Director's Conference, Department of Health, Education and Welfare, Center for Disease Control, August 23, 1974.

METHYL CHLORIDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Methyl Chloride	**Method No.:** P&CAM 201
Matrix: Air	**Range:** 2 to 1600 mg/m³ in a 10-liter sample
Procedure: Adsorption on activated carbon, desorption with methanol, gas chromatographic analysis	**Precision** (CV_T): 0.13 at 200 mg/m³ in 10-liter samples
Date Issued: 9/6/74	**Classification:** E (Proposed)
Date Revised: 1/29/76	

1. Principle of the Method

A known volume of air is drawn through a small tube containing activated carbon, which adsorbs the methyl chloride present in the air sample. The collected methyl chloride is then desorbed with methanol, and the resulting solution is analyzed by gas chromatography with a flame ionization detector. The height of the methyl chloride peak is compared to the peak heights observed from the injection of standards.

2. Range and Sensitivity

2.1 The detection limit for methyl chloride by gas chromatography with flame ionization detection is about 10 ng. In the desorption procedure, 10 mℓ of methanol is used for extracting methyl chloride from the sorbent, after which a 5-μℓ aliquot is analyzed by gas chromatography. Therefore, 20 μg of methyl chloride collected in a sorbent tube is required to give a detectable response. This sensitivity easily allows the detection of methyl chloride in a 2-liter air sample containing 42 mg/m³ (20 ppm) of methyl chloride (one-fifth the OSHA standard). A 10-liter air sample would provide a more than adequate amount of sample for this concentration.

2.2 When sampling at a rate of 1 ℓ/min from an atmosphere with a methyl chloride concentration of about 1000 mg/m³ (500 ppm), the loading capacity of the 2.0-g section of activated carbon in the sorbent tube is 2 mg when the relative humidity is greater than about 90%, and 16 mg when the relative humidity is less than about 10%. The maximum volume to be sampled under these sampling conditions is 2 liters at high humidity and 16 liters at low humidity. The breakthrough volume may significantly increase with a drop in the sampling rate. If the humidity is unknown or of intermediate value, several tubes should be exposed to various volumes of air.

2.3 On the basis of the experimental results cited in Sections 2.1 and 2.2, a practical range for applicability of the method when sampling from air of low relative humidity is 2 to 1600 mg/m^3 in a 10-liter sample.

3. Interferences

3.1 High humidity decreases the loading capacity of the sorbent tube.

3.2 Any compound which has the same retention time as methyl chloride at the operating conditions described in this method is an interference. Hence, retention time data on a single column, or even on a number of columns, may not provide proof of chemical identity. Often, operating conditions can be modified to eliminate interferences. Samples should be analyzed by an independent method when overlapping gas chromatographic peaks cannot be resolved.

4. Precision and Accuracy

4.1 The precision of the analytical method alone is not easily determined, since transfer of known amounts of methyl chloride to sorbent tubes directly may be subject to large variations. Since methyl chloride is a gas at room temperature, the transfer can most easily be carried out by drawing vapor of known concentration through the tube. Therefore, the precision of the overall sampling and analytical method may be determined. The coefficient of variation of the total method (CV_T) is estimated to be 0.13 at a methyl chloride concentration of 200 mg/m^3 (100 ppm) in 10-liter samples. This value is based on 13 samples.

4.2 Laboratory measurements of methyl chloride at a concentration of 200 mg/m^3 (100 ppm) in air at 25°C yielded, in general, recoveries between 95 and 100%. The sampling rate was 1 ℓ/min.

5. Advantages and Disadvantages of the Method

5.1 The sampling device is small and portable and involves no liquids. The tubes are analyzed by means of gas chromatography, a rapid instrumental method.

5.2 One disadvantage of this method is the inefficiency of activated carbon in collection of methyl chloride gas. A 2-g sorbent layer is required to collect 18 mg of methyl chloride from dry air at ambient temperatures. The 2-g sorbent section with a 0.5-g backup section requires an oversized sorbent tube, which may be somewhat bulky and therefore inconvenient to use with a personal sampling pump.

5.3 When sampling from very humid air, one can easily exceed the 2-mg humid-air loading capacity of the 2-g sorbent section. Under any sampling conditions, if the backup section is found to contain more than 25% of the methyl chloride collected on the front layer, the possibility of sample breakthrough from the tube exists.

5.4 The collection efficiency at 42 to 45°C has been demonstrated to be about 20% lower than at 20 to 25°C.

5.5 The precision of the method is limited by the reproducibility of the pressure drop and, therefore, the flow rates across the tubes. Because the pump is usually calibrated for one particular tube, differences in flow rates can occur when sampling through other tubes and can cause sample volumes to vary.

6. Apparatus

6.1 **Personal Sampling Pump.** The pump should be calibrated with a representative sorbent tube in the sampling line. A dry or wet test meter or a glass rotameter that will determine the appropriate flow rate (< 1 ℓ/min) to within ± 5% may be used for the calibration.

6.2 **Sorbent Tubes.** Each is 14.4 cm long with an i.d. of 9 mm. The 2.0-g section of activated carbon (20/40 mesh) prepared from coconut shells is separated from the 0.5-g backup section of the same sorbent with a plug of glass wool. Both sections are held in place with one glass plug at each end. The pressure drop across the tube is 3 to 5 in. of water at a sampling rate of 1 ℓ/min.

6.3 A gas chromatograph equipped with a flame ionization detector.

6.4 A column (3-ft x 0.125-in. stainless steel) packed with Porapak Q, N, or T (50/80 mesh).

6.5 Glass-stoppered 25-ml flasks.

6.6 A 10-μℓ liquid syringe and a 2-mℓ gas-tight syringe.

6.7 A 1-liter glass flask fitted with a septum and a magnetic stirrer for the preparation of standards of methyl chloride in air. Mylar or Saran bags are needed for the preparation of standards below 1000 mg/m^3 (500 ppm).

7. Reagents

7.1 Methanol, reagent grade.

7.2 Pure methyl chloride gas.

7.3 Purified nitrogen.

7.4 Purified hydrogen.

7.5 Filtered air.

8. **Procedure**

8.1 **Cleaning of Equipment.** All equipment used for the laboratory analysis should be washed with detergent and thoroughly rinsed with tap water and distilled water.

8.2 **Collection and Shipping of Samples**

8.2.1 Immediately before sampling, remove the caps from the ends of the tube.

8.2.2 The smaller section of activated carbon is used as a backup and should be positioned nearest the sampling pump.

8.2.3 The sorbent tube should be placed in a vertical position with the larger section of sorbent pointing up during sampling to minimize channelling of the methyl chloride through the sorbent.

8.2.4 Air being sampled should not be passed through any hose or tubing before entering the sorbent tube.

8.2.5 The flow rate and time (or volume) must be measured as accurately as possible. The sample should be taken at a flow rate of 1 ℓ/min or less to attain the total sample volume required. The minimum and maximum sample volumes that should be collected are 2 liters and 16 liters. This maximum is for air of low humidity. (See Section 2.2.) The minimum volume quoted must be collected if the desired sensitivity is to be achieved.

8.2.6 At the time personal samples are taken, relatively large volumes (> 16 liters) of air also should be sampled through other sorbent tubes. These bulk air samples will be used by the analyst to identify possible interferences before the personal samples are analyzed.

8.2.7 If the temperature and pressure of the atmosphere being sampled is very different from 25°C or 760 mmHg, they should be measured and recorded.

8.2.8 The sorbent tubes should be capped with the supplied plastic caps immediately after sampling. Under no circumstances should rubber caps be used.

8.2.9 One tube should be handled in the same manner as the sample tube (open, seal, and transport), except that no air is sampled through this tube. This tube should be labeled as a blank.

8.2.10 Capped tubes should be packed tightly before they are shipped to minimize tube breakage during shipping.

8.3 **Analysis of Samples**

8.3.1 **Preparation and Desorption of Samples.** Each sample and blank tube is extracted according to the following procedure. To each of two 25-mℓ glass-stoppered flasks add 10 mℓ of methanol. Remove the caps from the ends of the tube, and push the glass wool plugs into the flask. Add the sorbent section slowly to the same flask. The addition of the exposed or unexposed sorbent to methanol will initiate a vigorous evolution of gases from the sorbent. However, there is little or no loss of methyl chloride during this procedure. Stopper the flask, agitate it slightly, and allow it to stand for 3 to 4 min. Extract the backup sorbent layer in the second flask along with the remaining two glass wool plugs.

8.3.2 **Gas Chromatographic Conditions.** The typical operating conditions for gas chromatographic analysis are:

1. Nitrogen carrier gas flow, 40 mℓ/min.

2. Hydrogen gas flow to detector, 40 mℓ/min.

3. Air flow to detector, 300 mℓ/min.

4. Injector temperature, 200°C.

5. Detector temperature, 170°C.

6. Column temperature, 170°C.

8.3.3 **Injection.** The first step in the analysis is the injection of the sample into the gas chromatograph. To eliminate difficulties arising from blowback or distillation within the syringe needle, one should employ the solvent-flush injection technique. The 10-$\mu\ell$ syringe is first flushed with solvent several times to wet the barrel and plunger. Three microliters of solvent is drawn into the syringe to increase the accuracy and reproducibility of the injected sample volume. The needle is withdrawn from the solvent, and the plunger is pulled back about 0.2 $\mu\ell$ to separate the solvent flush from the sample air to be used as a marker. The needle is then immersed in the sample, and a 5-$\mu\ell$ aliquot is withdrawn, taking into consideration the volume of the needle, since the sample in the needle will be completely injected. After the needle is removed from the sample and prior to injection, the plunger is pulled back a short distance to minimize evaporation of the sample from the tip of the needle. Duplicate injections of each sample and standard should be made. No more than a 3% difference in peak height is to be expected.

8.3.4 Methyl chloride gas chromatographic peaks are sufficiently sharp to permit quantitative determination by measurement of peak heights.

8.4 Determination of Desorption Efficiency

8.4.1 **Importance of Determination.** The desorption efficiency of a particular compound can vary from one laboratory to another and also from one batch of activated carbon to another. Thus, it is necessary to determine the percentage of methyl chloride that is removed in the desorption process. Desorption efficiency should be determined on the same batch of sorbent tubes used in sampling.

8.4.2 **Procedure for Determining Desorption Efficiency.** Sorbent tubes from the same batch as that used in obtaining samples are used in this determination. A known volume of methyl chloride gas is injected into a bag containing a known volume of air. The bag is made of Mylar, Saran, or any material that will retain the methyl chloride and not absorb it. It should have a gas sampling valve and a septum injection port. The concentration in the bag may be calculated at room temperature and pressure. A known volume is then sampled through a sorbent tube with a calibrated sampling pump. At least five tubes are prepared in this manner. These tubes are desorbed and analyzed in the same manner as the unknown samples. (See Section 8.3.) Samples taken with a gas-tight syringe from the bag are also injected into the gas chromatograph. The concentration in the bag is compared to the concentration obtained from the tubes. The desorption efficiency equals the difference between the average weight of the tube samples and the weight of the blank divided by the average weight of the methyl chloride in the standard gas samples:

$$\text{Desorption efficiency} = \frac{\text{Weight of sample - Weight of blank}}{\text{Weight in standard gas sample}}$$

9. Calibration and Standards

Gas standards of methyl chloride in air or nitrogen are used for the preparation of calibration curves. If 10-liter air samples are to be taken from a workroom atmosphere containing 40 mg/m³ (20 ppm) to 1000 mg/m³ (500 ppm), amounts of methyl chloride in the range 0.4 to 10.0 mg will be collected for desorption into 10 mℓ of methanol. Injections of 5-$\mu\ell$ aliquots of the samples will represent 0.2 to 5.0 μg of methyl chloride. Exact 1-mℓ gas injections of standards in the range 200 to 5000 mg/m³ (100 to 2500 ppm at 25°C and 760 mmHg pressure) will provide an appropriate calibration curve. Plotting the curve in terms of weight (in mg) of methyl chloride in 10 mℓ of methanol extract (0.4 to 10.0 mg/10 mℓ methanol) will facilitate calculations. Standards more concentrated than 1000 mg/m³ (500 ppm) may be prepared by injecting appropriate volumes of pure methyl chloride gas into the air-filled 1-liter glass flask while stirring the gas in the flask. Standards of lower concentration should be prepared in plastic bags of Saran or Mylar. Filtered air or purified nitrogen should be used in the preparation of standards. Standards should be analyzed when samples are analyzed.

10. **Calculations**

10.1 From the calibration curve, read the weight of methyl chloride (in mg) in the 10-mℓ extraction solution.

10.2 Corrections for the blank must be made for each sample.

$$\text{Correct mg} = mg_s - mg_b$$

where: mg_s = mg found in front section of sample tube.

mg_b = mg found in front section of blank tube.

A similar procedure is followed for the backup sections.

10.3 Add the amounts present in the front and backup sections of the same sample tube to determine the total measured amount of methyl chloride in the sample.

10.4 The total amount is corrected for the desorption efficiency at the level of methyl chloride measured.

$$\text{Corrected amount (in mg)} = \frac{\text{amount (in mg)}}{\text{desorption efficiency}}$$

10.5 The concentration of methyl chloride in air may be expressed in mg/m^3:

$$mg/m^3 = \frac{\text{corrected weight (in mg)}}{\text{volume of air sampled (in ℓ)}} \times 1000\ \ell/m^3$$

10.6 The concentration may also be expressed in terms of parts per million (ppm) by volume:

$$\text{ppm} = mg/m^3 \times \frac{24.45}{\text{M.W.}} \times \frac{760}{P} \times \frac{T+273}{298}$$

where: 24.45 = molar volume (ℓ/mole) at 25°C and 760 mmHg.

M.W. = molecular weight.

P = pressure (mmHg) of air sampled.

T = temperature (°C) of air sampled.

11. References

11.1 Barrett, W.J., H.K. Dillon, and R.H. James, "Sampling and Analysis of Four Organic Compounds Using Solid Sorbents," Southern Research Institute, Birmingham, Alabama, Final Report for Contract No. HSM-99-73-63 to National Institute for Occupational Safety and Health, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1974, pp. 78-92, 115-122.

11.2 White, L.D., D.G. Taylor, P.A. Mauer, and R.E. Kupel, "A Convenient Optimized Method for the Analysis of Selected Solvent Vapors in the Industrial Atmosphere," Amer. Ind. Hyg. Ass. J., 31, 225 (1970).

ACRYLONITRILE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Acrylonitrile	**Method No.:** P&CAM 202
Matrix: Air	**Range:** 40 to 1100 mg/m³ in a 20 - liter sample
Procedure: Adsorption on Carbosieve B, desorption with methanol, gas chromatographic determination	**Precision** (CV_T): 0.10 at 150 to 200 mg/m³ in a 10 - liter sample
Date Issued: 9/6/74	**Classification:** E (Proposed)
Date Revised: 1/29/76	

1. **Principle of the Method**

A known volume of air is drawn through a small tube containing a sorbent (Carbosieve B, a type of pyrolyzed Saran) on which the acrylonitrile is collected. The acrylonitrile is desorbed from the sorbent with methanol and the resulting solution is analyzed by gas chromatography with a flame ionization detector. The area under the acrylonitrile peak is compared with the areas obtained by the injection of standards.

2. **Range and Sensitivity**

2.1 The limit of detection of the sampling and analytical method is estimated to be 100 μg. With the procedure described, 800 μg of acrylonitrile should be present to obtain a peak twice the height of the methanol background. This corresponds to an acrylonitrile concentration of 40 mg/m³ in a 20-liter sample.

2.2 The largest amount of acrylonitrile that can be collected with a sorbent tube is a function of the relative humidity of the air from which it is sampled. These upper limits at specified relative humidities (R.H.) are: 6 mg when R.H. > 95%, 15 mg when R.H. = 50%, and 22 mg when R.H. < 5%. Each value is the approximate number of milligrams of acrylonitrile that the front section of the tube will hold at a specified relative humidity before a significant amount of acrylonitrile is found on the backup section. (The sorbent tube contains two sections of Carbosieve B separated by a section of glass wool [See Section 6.2.].) The maximum volume to be sampled when the acrylonitrile concentration is 225 mg/m³ (100 ppm) is 25 liters at high humidity or 95 liters at low humidity.

3. Interferences

3.1 High humidity decreases the loading capacity of the sorbent tube.

3.2 Any compound that has the same retention time as acrylonitrile at the operating conditions described in this method is an interference. If the possibility of interference exists, separation conditions (column packing, temperature, etc.) must be changed to eliminate the problem. Samples should be analyzed by an independent method when overlapping gas chromatographic peaks cannot be resolved.

4. Precision and Accuracy

4.1 The coefficient of variation (CV) for the analytical procedure has been found to be 0.05 for 4-mg samples of acrylonitrile. The acrylonitrile was transferred directly with a syringe to sorbent tubes and then analyzed according to Section 8.4.

4.2 For the analytical method plus simulated field testing, the coefficient of variation (CV_T) over a range of concentrations from 150 to 200 mg/m^3 in a 10-liter sample was approximately 0.10. Acrylonitrile in dry air was sampled from a laboratory generator through sorbent tubes.

4.3 Under laboratory conditions, measurements resulting from application of the total sampling and analytical method were within 10% of expected values at acrylonitrile concentrations of 150 to 200 mg/m^3 in a 10 - liter sample.

5. Advantages and Disadvantages of the Method

5.1 The sampling device is small and portable, and involves no liquids. The tubes are analyzed by means of gas-liquid chromatography, a rapid instrumental method. Field testing has not been carried out. It is expected that any interferences that may be encountered can be eliminated by altering chromatographic conditions.

5.2 One disadvantage of the method is that the amount of sample that can be taken is limited by the amount that the tube will hold at ambient temperatures before overloading. High relative humidity reduces the loading capacity. When the sample value obtained for the backup section of the sorbent tube exceeds 20% of that found on the front section, the possibility of sample loss exists.

5.3 Also, sampling from an atmosphere where the temperature was 43 to 46°C (109 to 115°F) has demonstrated about a 10% loss in collection efficiency.

5.4 The precision of the method is limited by the reproducibility of the pressure drop across the tubes. This drop affects the flow rate and causes the volume to be imprecise because the personal sampling pump is usually calibrated for one tube only. The pressure drop across a typical sorbent tube is approximately 1 in. of Hg (12 to 13 in. of water) at a sampling rate of 1 ℓ/min.

6. **Apparatus**

6.1 **A Properly Calibrated Personal Sampling Pump.** The pump should be calibrated with a representative sorbent tube in the sampling line. A dry or wet test meter or a glass rotameter that will determine the appropriate flow rate (< 1 ℓ/min) to within ±5% may be used for the calibration.

6.2 **Sorbent Tubes.** The glass tubes have both ends flame sealed. Each is 8 cm long with a 7-mm o.d. and a 5-mm i.d. They contain two sections of 45/60-mesh Carbosieve B, separated by a 2-mm portion of glass wool. Carbosieve B is a pyrolyzed Saran manufactured and distrubuted by Supelco, Inc., Bellafonte, Pennsylvania. The sorbing section contains 150 mg of sorbent, the backup section 50 mg. A 3-mm plug of glass wool is placed between the outlet end of the tube and the backup section. A 3-mm plug of glass wool is also placed in front of the sorbing section.

6.3 Gas chromatograph equipped with a flame ionization detector.

6.4 A stainless steel column (9 ft x 0.125 in.) with 50/80 mesh Porapak N as the stationary phase.

6.5 A recorder and an appropriate means of determining peak area.

6.6 Vials with plastic caps (2 dram).

6.7 Gas chromatographic liquid syringe (10 μℓ).

6.8 Volumetric pipettes of appropriate sizes.

7. **Reagents**

7.1 Methanol, reagent grade.

7.2 Purified nitrogen.

7.3 Prepurified hydrogen.

7.4 Filtered compressed air.

7.5 Acrylonitrile, > 99% pure.

7.6 **Acrylonitrile, Standard Solution (10 mg/mℓ).** Dilute 1.000 g of acrylonitrile to 100 mℓ with methanol. This standard is stable for at least 2 weeks at ambient temperatures.

8. Procedure

8.1 **Cleaning of Equipment.** All equipment used for the laboratory analysis should be washed with detergent and thoroughly rinsed with tap water and distilled water.

8.2 Collection and Shipping of Samples

8.2.1 Immediately before sampling, break the end of the tube to provide an opening at least one-half the internal diameter of the tube (2 mm).

8.2.2 The smaller section of sorbent is used as a backup and should be positioned nearest the sampling pump.

8.2.3 The sorbent tube should be placed in a vertical position with the larger section of sorbent pointing up during sampling to minimize channeling of acrylonitrile through the sorbent tube.

8.2.4 Air being sampled should not be passed through any hose or tubing before entering the sorbent tube.

8.2.5 The flow rate and time (or volume) must be measured as accurately as possible. The sample should be taken at a flow rate of 1 ℓ/min or less to attain the total sample volume required. The minimum and maximum sample volumes that should be collected for acrylonitrile are 10 liters and 95 liters, respectively. This maximum is for air of low humidity. (See Section 2.2.) The minimum volume quoted must be collected if the desired sensitivity is to be achieved.

8.2.6 At the time personal samples are taken, relatively large volumes of air also should be sampled through other sorbent tubes. These bulk air samples will be used by the analyst to identify possible interferences before the personal samples are analyzed.

8.2.7 The temperature and pressure of the atmosphere being sampled should be measured and recorded if either differs greatly from standard conditions (760 mmHg and 25°C).

8.2.8 The sorbent tubes should be capped immediately after sampling. Under no circumstances should rubber caps be used.

8.2.9 One tube should be handled in the same manner as the sample tube (break, seal, and transport), except that no air is sampled through this tube. This tube should be labeled as a blank.

8.2.10 Capped tubes should be packed tightly before they are shipped to minimize tube breakage during shipping.

8.2.11 A sample of the bulk material should be submitted to the laboratory in a glass container with a Teflon-lined cap. This sample should not be transported in the same container as the sorbent tubes.

8.3 Analysis of Samples

8.3.1 **Preparation of Samples.** In preparation for analysis, each sorbent tube is scored with a file in front of the first section of sorbent and broken open. The glass wool and the sorbent in the first (larger) section are transferred to a small stoppered vial. The separating section of glass wool and the second sorbent section are transferred to another vial. The contents of each vial are analyzed separately.

8.3.2 **Desorption of Samples.** Prior to analysis, 2 mℓ of methanol is added to the vial containing the first sorbent section, and 1 mℓ to the vial containing the backup layer. Desorption is complete after 3 to 4 min if the sample is agitated frequently. No loss of sample or desorbing solvent has been noted if the vial is stoppered with a plastic cap during the desorption process.

8.3.3 Gas Chromatographic Conditions. Typical operating conditions for the gas chromatograph are:

1. Nitrogen carrier gas flow, 40 mℓ/min.

2. Hydrogen gas flow to detector, 40 mℓ/min.

3. Air flow to detector, 300 mℓ/min.

4. Injection temperature, 200°C.

5. Column temperature, 170°C.

6. Detector temperature, 170°C. (In general, the detector temperature should be higher than the column temperature. However, the gas chromatograph used in the development of this method required a common temperature for both.)

8.3.4 **Injection.** The first step in the analysis is the injection of the sample into the gas chromatograph. In order to inject as little methanol as possible and, therefore, to maximize resolution of methanol and acrylonitrile, the following injection procedure should be carried out. A 10-$\mu\ell$ syringe is first flushed with methanol and dried. The tip of the needle is then immersed in the sample, the plunger is pushed in and pulled out several times until there are no air bubbles between the plunger and sample liquid, and 2 $\mu\ell$ of sample is withdrawn. The needle is then withdrawn from the solution and the plunger is pulled back until a small volume of air (approximately 0.5 $\mu\ell$) is evident below the sample layer. The sample volume is then read from the calibrated syringe. The sample is then injected, after which the plunger is again pulled back until the volume of sample left in the syringe can be read from the syringe graduations. The net volume injected can then be computed. Duplicate injections of each sample and standard should be made. No more than a 5% difference in area under the sample peak is to be expected.

8.3.5 **Measurement of Area.** The area under the sample peak is measured and the results are read from a standard curve as explained below.

8.4 **Determination of Desorption Efficiency.** Only small differences in desorption efficiency are expected from physical differences between lots of Carbosieve B. This commercial sorbent is reported to be clean and pure and is stored in sealed ampoules after its manufacture. Desorption efficiency is usually near 100% for Carbosieve B Lots K51 and K73.

9. **Calibration and Standards**

It is convenient to express concentrations of standards in terms of mg/2.0 mℓ methanol because the sorbing layer of each sample is desorbed in this amount of methanol. A series of standards, varying in concentration over the range of interest, is prepared from the 10 mg/mℓ standard and analyzed under the same gas chromatographic conditions and during the same time period as the unknown samples. When acrylonitrile concentrations in the air sampled are near the OSHA standard, 45 mg/m^3 (20 ppm), and 20-liter samples are taken, an appropriate set of standards may be prepared by diluting to 100 mℓ with methanol 1-, 2-, 3-, 4-, and 5-mℓ aliquots of the 10 mg/mℓ standard. Injection of 2-$\mu\ell$ aliquots should be carried out as described in Section 8.3.4. Calibration curves are established by plotting concentration in mg/2.0 mℓ *versus* peak area. Since no internal standard is used in the method, standard solutions must be analyzed at the same time that the sample is analyzed. This will minimize the effect of known day-to-day variations of the response of the flame ionization detector.

10. **Calculations**

10.1 Read the weight, in mg, corresponding to each peak area from the standard curve. No volume corrections are needed, because the standard curve is based on mg/2.0 mℓ and the volume of sample injected is identical to the volume of the standards injected.

10.2 Corrections for the blank must be made for each sample.

$$\text{Correct mg} = mg_s - mg_b$$

where: mg_s = mg found in front section of sample tube.

mg_b = mg found in front section of blank tube.

A similar procedure is followed for the backup sections with one exception. Since only 1 mℓ is used in extracting the backup layer, the amount in milligrams of acrylonitrile as read from the standard curve should be divided by 2.

10.3 Add the corrected amounts present in the front and backup sections of the same sample tube to determine the total measured amount of acrylonitrile in the sample.

10.4 The concentration of acrylonitrile in air may be expressed in mg/m^3 :

$$mg/m^3 = \frac{\text{corrected weight (in mg)}}{\text{volume of air sampled (in ℓ)}} \times 1000\ ℓ/m^3$$

10.5 The concentration may also be expressed in terms of parts per million (ppm) by volume:

$$ppm = mg/m^3 \times \frac{24.45}{M.W.} \times \frac{760}{P} \times \frac{T+273}{298}$$

where: 24.45 = molar volume (ℓ/mole) at 25°C and 760 mmHg.

M.W. = molecular weight.

P = pressure (mmHg) of air sampled.

T = temperature (°C) of air sampled.

11. References

11.1 Barrett, W.J., H.K. Dillon, and R.H. James, "Sampling and Analysis of Four Organic Compounds Using Solid Sorbents," Southern Research Institute, Birmingham, Alabama, Final Report for Contract No. HSM 99-73-63 to the National Institute for Occupational Safety and Health, Division of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio 1974, pp. 53-77, 105-114.

11.2 White, L.D., D.G. Taylor, P.A. Mauer, and R.E. Kupel, "A Convenient Optimized Method for the Analysis of Selected Solvent Vapors in the Industrial Atmosphere," Amer. Ind. Hyg. Ass. J., 31, 225 (1970).

NITROGLYCERIN AND ETHYLENE GLYCOL DINITRATE (NITROGLYCOL) IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Nitroglycerin (NG) and Ethylene Glycol Dinitrate (EGDN)	**Method No.:** P&CAM 203
Matrix: Air	**Range:** NG, 0.1 to 100 mg/m^3 in a 10 - liter sample EGDN, 0.01 to 100 mg/m^3 in a 10-liter sample
Procedure: Sorption on Tenax-GC, desorption with ethanol, gas chromatographic determination	**Precision:** (CV_T): EGDN, 0.10 at 2 mg/m^3 in 15 - liter samples; NG, similar precision at 0.3 mg/m^3
Date Issued: 9/6/74	
Date Revised: 1/29/76	**Classification:** E (Proposed)

1. Principle of the Method

The nitrate esters are sorbed on Tenax-GC, a porous organic polymer, as a known volume of air containing the analytes is drawn through a small bed of the sorbent in a glass tube. The nitrate esters are extracted from the sorbent with ethanol; the resulting solution is analyzed by gas chromatography with an electron capture detector. Areas under the nitrate ester peaks from sample unknowns are compared to areas under the peaks from nitrate ester standards.

2. Range and Sensitivity

2.1 The minimum amount of EGDN detectable in a single injection by the gas chromatographic method is substantially smaller than 0.25 ng. This sensitivity easily permits the detection of EGDN in a 10-liter air sample containing 0.01 mg/m^3. The minimum amount of NG detectable in a single injection by the gas chromatographic method is smaller than 2.5 ng. This sensitivity permits the detection of NG in a 100-liter air sample containing 0.01 mg/m^3.

2.2 The capacity of the sorbent tubes is at least 1 mg of either EGDN or NG. Thus, it is unlikely that an excessive amount of sample will ever be collected even when the concentration greatly exceeds the OSHA standard of 1 mg/m^3. This retention of EGDN or NG by the sorbent tubes is not significantly different when samples are taken from atmospheres of low and high relative humidities.

3. **Interferences**

Ethylene glycol mononitrate, if present in high concentration relative to that of EGDN, produces a gas chromatographic peak that tails into the EGDN peak. This interference, although troublesome, does not prohibit a reasonably satisfactory analysis for EGDN. The combined selectivity of Tenax-GC and electron capture detection practically eliminates other interference. Water vapor does not interfere.

4. **Precision and Accuracy**

4.1 The coefficient of variation of the analytical method (CV) for the determination of EGDN is estimated to be about 0.03 at an EGDN concentration of 2 mg/m^3 in 15-liter samples. The precision of the measurement of NG concentrations is approximately the same at a NG concentration of 0.27 mg/m^3.

4.2 The precision of the overall sampling and analytical method has not been adequately determined. It has been estimated that the coefficient of variation for the total method (CV_T) is about 0.10 at an EGDN concentration of 2 mg/m^3 and an NG concentration of 0.3 mg/m^3. Since both EGDN and NG are sorbed on Tenax-GC at ambient temperature with 100% efficiency, it can be expected that the overall precision will be determined by variations in the measurement of sample volume.

4.3 In laboratory measurements not involving a personal sampling pump, the average recovery of EGDN from vapor samples at concentrations of 0.27 to 3.93 mg/m^3 in 30- to 90-liter samples was 104% and that of NG was 102% at a concentration of 0.28 mg/m^3 in 60-liter samples.

5. **Advantages and Disadvantages of the Method**

5.1 The sampling device is small and portable and involves no liquids. The tubes are analyzed by means of gas chromatography, a rapid instrumental method. Interferences in the chromatographic method are minimal.

5.2 The principal difficulties are those associated with the use of electron capture detection. They include the limited linear range of electron capture detectors and operating variables such as the effects of column bleed, moisture, and oxygen. The effects of these variables may be minimized by analyzing standards at the same time as samples. The electron capture detector is about 500 times as sensitive to the nitrate esters as a hydrogen flame ionization detector, and it provides a substantial advantage in specificity.

5.3 The precision of the method is limited by the reproducibility of the pressure drop across the tubes and, therefore, the flow rate through the tubes. Because the pump is usually calibrated for one particular tube, differences in flow rates can occur when sampling through other tubes and can cause sample volumes to vary.

5.4 The method can be applied for a wide range of sample sizes. However, repeated dilutions of the ethanol extract may be necessary to bring the amount of sample injected into the proper range for the electron capture detector.

6. Apparatus

6.1 A properly calibrated personal sampling pump is required. Calibrate the pump with a representative sorbent tube in the sampling line using a wet or dry test meter or a glass rotameter capable of measuring the appropriate flow rates (<1 ℓ/min) within an accuracy of ± 5%.

6.2 The sorbent tubes are 70 mm long and 5-mm i.d. They contain two sections of 35/60-mesh Tenax-GC separated and held in place by glass wool plugs. The front section contains 100 mg of sorbent and the backup section contains 50 mg. Since the pressure drop must be limited to 1 in. of Hg at 1.0 ℓ/min, it is necessary to avoid overpacking with glass wool. (Tenax-GC is distributed by Applied Science Laboratories, Inc., State College, Pa.)

6.3 A gas chromatograph equipped with an electron capture detector, a 2.5-ft by 0.25-in. glass column packed with 10% of OV-17 on 60/80-mesh Gas Chrom Q, and an integrator, or an equivalent means of measuring areas under peaks, are required.

6.4 Volumetric flask and pipettes, a 10-$\mu\ell$ syringe, and other normally available laboratory supplies and equipment are needed.

7. Reagents

The only reagent required is absolute ethanol for extraction of the sorbent. The gas chromatograph requires a supply of helium and an argon-methane mixture, or other carrier or purge gases as required for the particular instrument used.

8. Procedure

8.1 **Cleaning of Equipment.** All glassware for the laboratory analysis should be washed with detergent and thoroughly rinsed with tap water and distilled water. Particular attention should be paid to the cleaning of the microliter syringe with ethanol.

8.2 **Collection and Shipping of Samples**

8.2.1 Immediately before sampling, break the ends of the tube to provide an opening of at least 2 mm.

8.2.2 The smaller section of sorbent is used as a backup and should be positioned nearest the sampling pump.

8.2.3 The sorbent tube should be placed in a vertical position with the larger section of sorbent pointing up during sampling to minimize channeling of EGDN and NG through the sorbent tube.

8.2.4 Air being sampled should not be passed through any hose or tubing before entering the sorbent tube. This is particularly important with the nitrate esters since they are strongly sorbed on most surfaces, including glass.

8.2.5 The flow rate and time (or volume) must be measured as accurately as possible. The sample should be taken at a flow rate of 1 ℓ/min or less to attain the total sample volume required. The minimum volume that must be collected to permit detection of NG at the OSHA standard (or EGDN at a still lower concentration) is only 0.1 liter. At the OSHA standard, sample volumes as large as 1000 liters can be collected without causing trouble in the analysis.

8.2.6 At the time personal samples are taken, relatively large volumes of air also should be sampled through other sorbent tubes. These bulk air samples will be used by the analyst to identify possible interferences before the personal samples are analyzed.

8.2.7 The temperature and pressure of the atmosphere being sampled should be measured and recorded if either should differ greatly from standard values (25°C and 760 mmHg).

8.2.8 The Tenax-GC tubes should be capped with the supplied plastic caps immediately after sampling. Under no circumstances should rubber caps be used.

8.2.9 One tube should be handled in the same manner as the sample tube (break, seal, and transport), except that no air is sampled through this tube. This tube should be labeled as a blank.

8.2.10 Capped tubes should be packed tightly before they are shipped to minimize tube breakage during shipping.

8.3 Analysis of Samples

8.3.1 **Preparation and Desorption of Samples.** The sorbent tube is scored with a file near the front end and is broken open. The first glass wool plug and the front (100-mg) section of the sorbent are transferred to a small vial or test tube. The remaining glass wool plug and the backup section of sorbent are transferred to another small vial. Two milliliters of ethanol is added to each vial, the vials are stoppered, and the contents are shaken for about 1 min. It may be necessary to dilute the ethanol solution before taking a sample aliquot for injection if the concentrations of the nitrate esters are high.

8.3.2 **Gas Chromatographic Conditions.** Typical operating conditions for the gas chromatographic analysis are:

1. Column: 10% OV-17 on 60/80-mesh Gas Chrom Q, 2.5-ft x 0.25-in. glass column.

2. Helium carrier gas, 100 mℓ/min.

3. Argon/methane purge gas, 125 mℓ/min.

4. Column temperature, 130°C.

5. Injection port temperature, 160°C.

6. Detector temperature, 280°C.

8.3.3 **Injection.** The first step in the analysis is the injection of the sample into the gas chromatograph. To eliminate difficulties arising from blowback or distillation within the syringe needle, one should employ the solvent flush injection technique. The 10-μℓ syringe is first flushed with solvent several times to wet the barrel and plunger. Two microliters of solvent are drawn into they syringe to increase the accuracy and reproducibility of the injected sample volume. The needle is removed from the solvent and the plunger is pulled back about 0.4 μℓ to separate the solvent flush from the sample with a pocket of air to be used as a marker. The needle is then immersed in the sample, and a 5-μℓ aliquot is withdrawn to the 7.4 μℓ mark (2 μℓ solvent + 0.4 μℓ air + 5 μℓ sample = 7.4 μℓ). After the needle is removed from the sample and prior to injection the plunger is pulled back a short distance to minimize evaporation of the sample from the tip of the needle. Duplicate injections of each sample and standard are made. No more than a 3% difference in area is to be expected. Automatic sampling devices may also be used.

8.3.4 **Measurement of Area.** The area under the sample peak is measured by an electronic integrator or some other suitable form of area measurement, and preliminary results are read from a standard curve prepared as discussed below.

8.4 **Determination of Desorption Efficiency.** Only small differences in desorption efficiency in the described procedure are to be expected from differences between lots of Tenax-GC. If the efficiency of desorption should be questioned for some other reason, it can be checked following the procedure detailed in Section 8.5 of Analytical Method Number P&CAM 127, entitled "Organic Solvents in Air." However, since it is not possible to measure accurately the small quantities of the nitrate esters that are to be added to sorbent tubes, 15-μℓ aliquots of appropriate ethanol solutions are added. Ethanol is also the solvent used to desorb the nitrate esters from the sorbent.

9. Calibration and Standards

9.1 If pure NG and EGDN are available, standard solutions in ethanol may be prepared and analyzed under the same gas-chromatographic conditions and during the same time period as the unknown samples. In the course of the development of this method, several reference materials were obtained from manufacturers of nitrate ester products. This experience indicated that the following materials can be obtained for the preparation of standards:

- Solutions of known concentrations of NG and EGDN in ethanol were provided by a dynamite manufacturer.

- A sample of analyzed dynamite containing EGDN but no NG was also provided by a dynamite manufacturer. Since dynamite is a heterogenous material, care must be taken to obtain a representative sample.

- Pharmaceutical-grade sublingual NG tablets may be obtained from wholesale drug dealers. These tablets are manufactured to meet United States Pharmacopeia specifications, which require that the actual NG content be between 80 and 112% of the stated value. The actual NG content of a specific lot of tablets may be obtained from the manufacturer. For use, the tablets are weighed, crushed, and extracted with ethanol, the solution is filtered and diluted to a fixed volume, and aliquots are injected.

- An alternate source was a material of a known NG content called "Nitroglycerin Lactose Trituration," provided by a pharmaceutical manufacturer. This material is used for quality control in the manufacture of NG tablets.

9.2 An experimental comparison of the NG and EGDN contents of these standard materials gave a coefficient of variation of 0.04 for the NG contents and 0.02 for the EGDN contents.

9.3 It is necessary that standards be analyzed concurrently with samples to minimize the effects of variations in detector response and other factors. Calibration curves are established by plotting concentration in mg/2.0 mℓ *versus* peak area.

10. Calculations

10.1 Read the weight, in mg, corresponding to each peak area from the standard curve. No volume corrections are needed, because the standard curve is based on mg/2.0 mℓ and the volume of sample injected is identical to the volume of the standard injected.

10.2 Corrections for the blank must be made for each sample.

$$\text{Correct mg} = mg_s - mg_b$$

where: mg_s = mg found in front section of sample tube.

mg_b = mg found in front section of blank tube.

A similar procedure is followed for the backup sections.

10.3 Add the corrected amounts present in the front and backup sections of the same sample tube to determine the total measured amount of EGDN or NG in the sample.

10.4 The concentration of each compound in air may be expressed in mg/m^3:

$$mg/m^3 = \frac{\text{corrected weight (in mg)}}{\text{volume of air sampled (in } \ell)} \times 1000\ \ell/m^3$$

10.5 The concentration may also be expressed in terms of parts per million (ppm) by volume:

$$ppm = mg/m^3 \times \frac{24.45}{M.W.} \times \frac{760}{P} \times \frac{T+273}{298}$$

where: 24.45 = molar volume (ℓ/mole) at 25°C and 760 mmHg.

M.W. = molecular weight.

P = pressure (mmHg) of air sampled.

T = temperature (°C) of air sampled.

11. References

11.1 Barrett, W.J., H.K Dillon, and R.H. James, "Sampling and Analysis of Four Organic Compounds Using Solid Sorbents," Southern Research Insitute, Birmingham, Alabama, Final Report for Contract No. HSM-99-73-63 to National Institute for Occupational Safety and Health, Division of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1974, pp. 28-52, 97-104.

11.2 White, L.D., D.G. Taylor, P.A. Mauer, and R.E. Kupel, "A Convenient Optimized Method for the Analysis of Selected Solvent Vapors in the Industrial Atmosphere," Amer. Ind. Hyg. Ass. J., 31, 225 (1970).

SULFUR DIOXIDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Sulfur Dioxide	**Method No.:** P&CAM 204
Matrix: Air	**Range:** 2 to 625 mg/m³ in 100 liters of air
Procedure: Sorption on Molecular Sieve 5A, thermal desorption, mass spectrometric analysis	**Precision (CV_T):** 0.002 at 13 mg/m³
Date Issued: 1/29/76	**Classification:** E (Proposed)
Date Revised:	

1. **Principle of the Method**

   Air is drawn through a small tube containing Molecular Sieve 5A, which quantitatively sorbs sulfur dioxide. The sulfur dioxide is desorbed from the Molecular Sieve 5A by applying heat while air is flushed through the tube. The amount of sulfur dioxide liberated is measured by mass spectrometric techniques.

2. **Range and Sensitivity**

   2.1 The smallest amount of sulfur dioxide which has been quantitatively sorbed, desorbed, and accurately measured by this method is 205 μg, which corresponds to 2 mg/m³ (0.8 ppm) in a 100-liter sample of air.

   2.2 The range of the method extends from the detection limit to an upper limit determined by the amount of Molecular Sieve 5A used, the volume of air sampled, and the sampling rate. In laboratory studies, it has been found that the capacity of Molecular Sieve 5A for sulfur dioxide is 125 mg/g for quantitative sorption at a sampling rate not greater than 200 mℓ/min. Typically, 0.5 g of Molecular Sieve 5A is used in each sorbent tube, which limits the loading capacity of the tube to 62.5 mg of sulfur dioxide at the 200 mℓ/min sampling rate. In a 100-liter air sample, the upper limit of the range is therefore 625 mg/m³ (240 ppm). The range can be extended by increasing the amount of Molecular Sieve 5A used in the sorbent tube or by decreasing the volume sampled through the sorbent tube. Decreasing the sampling rate may, also, increase the loading capacity of a sorbent tube.

3. **Interferences**

   3.1 In laboratory studies, no direct interferences have been noted in the mass spectrometric procedure. However, any compound or compound fragment which

649

produces a sizable peak with *m/e* 48, such as any of several organic sulfides, will interfere if it is desorbed from the sorbent along with sulfur dioxide.

3.2 The interactions of water vapor and sulfur dioxide that can occur on Molecular Sieve 5A cause a significant negative interference. However, these reactions are precluded by the desiccant action of the section of the sorbent tube containing phosphorus pentoxide, which efficiently removes water from the sampling stream but does not sorb sulfur dioxide.

3.3 Other gases, such as the oxides of nitrogen, are also sorbed by Molecular Sieve 5A. However, conditions for their desorption differ from those for sulfur dioxide. In any case, the mass-sensitive method of analysis eliminates the possibility of interference from most compounds.

4. Precision and Accuracy

4.1 The precision of the overall sampling and analytical method has been determined under laboratory conditions. With a closely regulated sampling rate and a sulfur dioxide standard of 13 mg/m^3 (5ppm) in air, the coefficient of variation for replicate measurements was found to be 0.002.

4.2 The accuracy of the overall sampling and analytical method has been determined under the laboratory conditions specified in Section 4.1. At a concentration of 13 mg/m^3 (5ppm), the mean of replicate measurements was within 2% of the "true" value. Wide variations in concentrations, sampling rate, and sampling time have not been found to influence the quantitative determination of sulfur dioxide. Also, the storage of sorbent tubes for four weeks prior to the desorption and analysis steps has caused no apparent loss of sulfur dioxide.

4.3 No field tests of this method have been carried out. The precision and accuracy of the method under field conditions is expected to be influenced by the reproducibility and reliability of the airflow measurements achieved with personal sampling pumps.

4.4 No collaborative tests have been performed to corroborate results obtained with this method.

5. Advantages and Disadvantages of the Method

5.1 The principal advantage of the method is that the sampling device is small and portable and involves no liquids. Consequently, exposure data integrated with respect to time can be obtained to reflect the actual amount of sulfur dioxide breathed by an individual worker.

5.2 Another advantage is that the quantitative determination of sulfur dioxide is accomplished in a highly reliable manner by the use of the inherently specific and sensitive mass spectrometer.

5.3 An apparent disadvantage is the susceptibility of the thermal desorption gas inlet system to changes in operating conditions that may lead to variations in instrument response (see Section 6.5). For instance, contamination of the variable leak may change the leak rate of the sample into the mass spectrometer. Also, changes in the airflow rate, which may result because of small differences in pressure drop across different sorbent tubes during desorption, may require frequent regulation of the flowmeter to avoid discrepancies in instrument response. (The use of a flow controller instead of the fine metering valve would insure a constant flow regardless of the pressure drop across a sorbent tube.)

5.4 Another disadvantage of the method is that the precision of sampling is limited by the reproducibility of the pressure drop and, therefore, the flow rates across the tubes, Because the personal sampling pump is usually calibrated for one particular tube, differences in flow rates can occur when sampling through other tubes and can cause sample volumes to vary.

5.5 A third disadvantage is the high cost of a mass spectrometer and the accessories necessary for performing this analytical method.

6. Apparatus

6.1 **Personal Sampling Pump.** The pump should be calibrated with a representative sorbent tube in the sampling line. A dry or wet test meter or glass rotameter that will determine the flow rate (typically 50 to 200 mℓ/min) to within ±5% may be used for the calibration.

6.2 **Sorbent Tubes.** Each tube consists of a polyethylene section (a standard drying tube, 0.75 in. o.d. by 1.5 in. long) coupled with a glass section (0.3125 in. o.d. by 3 in. long) as shown in Figure 1. The polyethylene section contains 4 g of granular phosphorus pentoxide, analytical reagent grade; the glass section contains 0.5 g of Molecular Sieve 5A (45/60 mesh) held in place by two glass wool plugs. Since the molecular sieve functions best in the absence of water, the molecular sieve section of each tube is heated to 240°C, and dry air is flushed through it for 20 min. Following conditioning, the desiccant section is coupled with the molecular sieve section by inserting the end of the glass section into the tip of the drying tube as shown in Figure 1. Prior to use, the assembled sorbent tube is protected from atmospheric moisture and other contaminants by capping the open end with plastic caps.

6.3 **Mass Spectrometer.** Any commercially available mass spectrometer with the capability for single-ion monitoring can be used.

6.4 **Gas-Inlet Reservoir.** The inlet reservoir is needed for use as a supplementary reservoir for the introduction of sulfur dioxide into the mass spectrometer during preliminary adjustments of the magnetic and electric sector voltages and the accelerating voltage.

651 551

6.5 **Thermal Desorption Gas Inlet System.** The mass spectrometer is fitted with a gas inlet system such as that shown diagrammatically in Figure 2, which provides a means for liberating sulfur dioxide from sorbent tubes and for introducing the liberated sulfur dioxide into the mass spectrometer. The inlet system includes a source of compressed, dry air that is passed at a known pressure and flow rate through a sorbent tube containing Molecular Sieve 5A. The tube is heated by means of a heating tape to desorb the sulfur dioxide. A constant fraction of the effluent from the sorbent tube is admitted into the mass spectrometer source *via* a variable leak, while the remainder of the effluent is pumped away by a standard vacuum pump. By adjusting the throttle valve and the variable leak, the desired fraction of the effluent from the sorbent tube is introduced into the mass spectrometer. A different sorbent tube can be inserted into the apparatus without disturbing critical settings by simply closing the on-off valve and the postitive shut-off valve shown in Figure 2 before the removal of one tube and insertion of another.

6.6 **Gas-Handling Apparatus.** In the preparation of standards, known quantities of sulfur dioxide can be readily flushed into unexposed sorbent tubes by using a simple gas-handling apparatus such as that shown in Figure 3. Standard gas mixtures of sulfur dioxide in air in the concentration range of interest are introduced into sorbent tubes through a simple stainless steel manifold equipped with a pressure gauge, flowmeter, metering valve (or flow controller), and a three-way stopcock, which shunts the flow to either a vent or a sorbent tube.

7. Reagents

7.1 Compressed air, dry.

7.2 Standard mixtures of sulfur dioxide in air at concentrations of interest.

8. Procedure

8.1 **Cleaning of Equipment.** No specialized cleaning of equipment is necessary. However, the surfaces of the sorbent tubes, the thermal desorption gas inlet system, and the gas-handling apparatus that may be in contact with sulfur dioxide should be dry and free of dust to avoid loss of sulfur dioxide.

8.2 **Collection and Shipment of Samples.**

8.2.1 Prior to the collection of a sample, it is advisable to estimate the duration of sampling and the sampling rate required to obtain an adequate amount of sulfur dioxide for the analysis, if a preliminary estimate of the sulfur dioxide concentration in the atmosphere to be tested can be made. For concentrations near or below the Threshold Limit Value (TLV) of 13 mg/m^3 (5 ppm), the maximum sampling rate achievable with the personal sampling pump should be used. Typically this rate is 200 mℓ/min. The duration of sampling should be several hours. The amount of sulfur dioxide sampled

must not be less than 205 μg. For concentrations significantly higher than the TLV, much smaller volumes should be sampled; the duration of sampling, the sampling rate, or both, should be lowered. The amount of sulfur dioxide sampled must not exceed 62.5 mg. If the concentration is totally unknown, various sampling rates and sampling times should be used in the exposure of several tubes. Regardless of the concentration of sulfur dioxide in the air, relatively large volumes of air also should be sampled through other sorbent tubes at the same time personal samples are taken. These samples will be used by the analyst to identify possible interferences before the personal samples are analyzed.

8.2.2 Immediately before sampling, remove the protective plastic caps from the ends of each assembled sorbent tube. Connect the end of the glass section of each tube to a personal sampling pump with an appropriate length of flexible tubing and mount the tube vertically with the polyethylene section pointing up to prevent "channelling" of the sulfur dioxide through the molecular sieve.

8.2.3 Switch on the pump, adjust the airflow to the proper rate, and sample through each tube for the selected time. Air being sampled should not be passed through any hose or tubing before entering the polyethylene section of the sorbent tube.

8.2.4 Measure the flow rate and time, or volume, as accurately as possible. If the temperature and pressure of the atmosphere being sampled are significantly different from 25°C or 760 mmHg, they should be measured and recorded.

8.2.5 After sampling, the polyethylene section of the sorbent tube is discarded and the glass section, which contains the Molecular Sieve 5A, is tightly capped with plastic caps.

8.2.6 At least one assembled sorbent tube is handled in a manner similar to that for sample tubes except that no air is sampled through this tube. The plastic caps are removed, the polyethylene section is discarded, and the ends of the glass section are closed with plastic caps. This tube is labeled as a blank.

8.2.7 The capped tubes are packed tightly in a suitable container to minimize breakage during transport to the laboratory.

8.3 Analysis of Samples.

8.3.1 Preliminary Adjustments of the Analysis System. Establish a 100 mℓ/min flow of air through the thermal desorption gas inlet system and gradually open the variable leak until the source pressure of the mass spectrometer reaches the normal operating pressure (typically 10^{-5} to 10^{-6} torr). Obtain a mass spectrum of the air and locate *m/e* 29 ($N_{14}N_{15}^{+}$). Tune the mass

spectrometer to monitor *m/e* 29 by adjusting the magnetic and electric sector voltages and the accelerating voltage. Note the intensity of the *m/e* 29 signal and record it for future reference. During the course of the analyses, the intensity of the *m/e* 29 signal should be checked since it provides an accurate "internal standard" which immediately reflects any change in instrument sensitivity or parameters in the sample inlet such as leak rate into the mass spectrometer. The *m/e* signal intensity is also useful in reproducing conditions for analyses should one be interrupted and not wish to completely recalibrate the instrument. The settings indicated on the numbered dial of a variable leak such as the Granville-Phillips leak are approximate; only a direct measurement such as the intensity of the *m/e* 29 signal provides an accurate indication of the leak rate. Next, while maintaining the source pressure established above, introduce sulfur dioxide into the mass spectrometer from a supplementary reservoir such as the gas-inlet reservoir. Obtain a mass spectrum, locate *m/e* 48 (SO^+),and adjust the magnetic and electric sector voltages so that the instrument is tuned to monitor *m/e* 48. Discontinue the introduction of the sulfur dioxide and, after allowing for equilibration, note the background intensity of the *m/e* 48 signal.

8.3.2 **Analysis Procedure.** With the instrument still tuned on *m/e* 48 and without changing the leak setting or air-metering valve setting, insert a sorbent tube into the desorption manifold, open the on-off valve, and allow about 3 min for the equilibration of airflow. Apply a voltage of about 10 V to the heating tape by adjusting the variable transformer, and open the positive shutoff valve to permit flow through the variable leak. The sulfur dioxide begins to desorb when the temperature of the exterior of the tube reaches 80°C. This should occur about 3 min after the initiation of heating. Desorption of the sulfur dioxide and mass spectrometric detection produces a Gaussian-shaped peak on the strip chart recorder. The area under the resulting peak is directly proportional to the quantity of sulfur dioxide desorbed. It is strongly suggested that the *m/e* 29 signal be checked periodically to insure that subtle changes in conditions in the thermal desorption gas inlet system or mass spectrometer have not taken place during measurements. The air-flow rate, the leak rate into the mass spectrometer, and the heating procedure must be identical for the analysis of unknown samples and solid sorbent standards as prepared according to Section 9.1.

9. Calibration and Standards

9.1 Preparation of Solid Sorbent Standards. Using the gas-handling apparatus shown in Figure 3, prepare each of several solid sorbent standards over the concentration range of interest. Allow sulfur dioxide in air from one of the standard mixtures to flow through the glass section only of an unexposed sorbent tube for an accurately measured time interval at a pressure of 740 to 750 mmHg. The amount of sulfur dioxide sorbed is calculated as follows:

Amount (in μg) = sulfur dioxide concentration in standard mixture (mg/m^3) x flow rate ($m\ell$/min) x sampling time (min) x 0.001 ($\ell/m\ell$).

9.2 Construction of Calibration Curve. The solid sorbent standards are analyzed according to the procedure detailed in Sections 8.3.1 and 8.3.2. The areas under the resulting peaks are measured, and a plot of peak area as a function of amount (in μg) of sulfur dioxide in a standard tube is made.

10. Calculations

10.1 The amount (in μg) of sulfur dioxide found in a sample, corresponding to the area under the analysis peak, is read directly from the calibration curve prepared as described in Section 9.2.

10.2 The average amount (in μg) of sulfur dioxide found in the unexposed tubes (blanks) is subtracted from the amount in each unknown sample as read from the calibration curve to give the correct amount (in μg) of sulfur dioxide in the sample.

10.3 The concentration of sulfur dioxide in air may be expressed in mg/m^3 :

$$mg/m^3 = \frac{\text{corrected weight (in } \mu g)}{\text{volume of air sampled (in } \ell)}$$

10.4 The concentration may also be expressed in terms of parts per million (ppm) by volume:

$$ppm = mg/m^3 \times \frac{24.45}{M.W.} \times \frac{760}{P} \times \frac{T+273}{298}$$

where: 24.45 = molar volume (ℓ/mole) at 25°C and 760 mmHg.

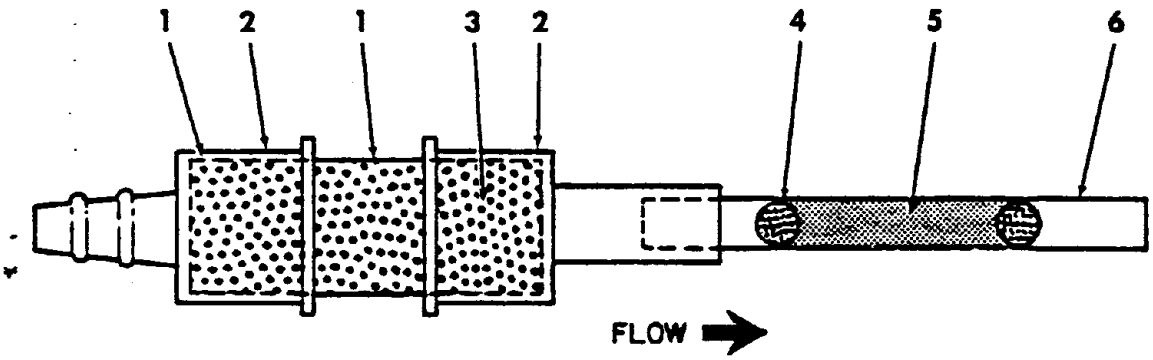
M.W. = molecular weight.

P = pressure (mmHg) of air sampled.

T = temperature (°C) of air sampled.

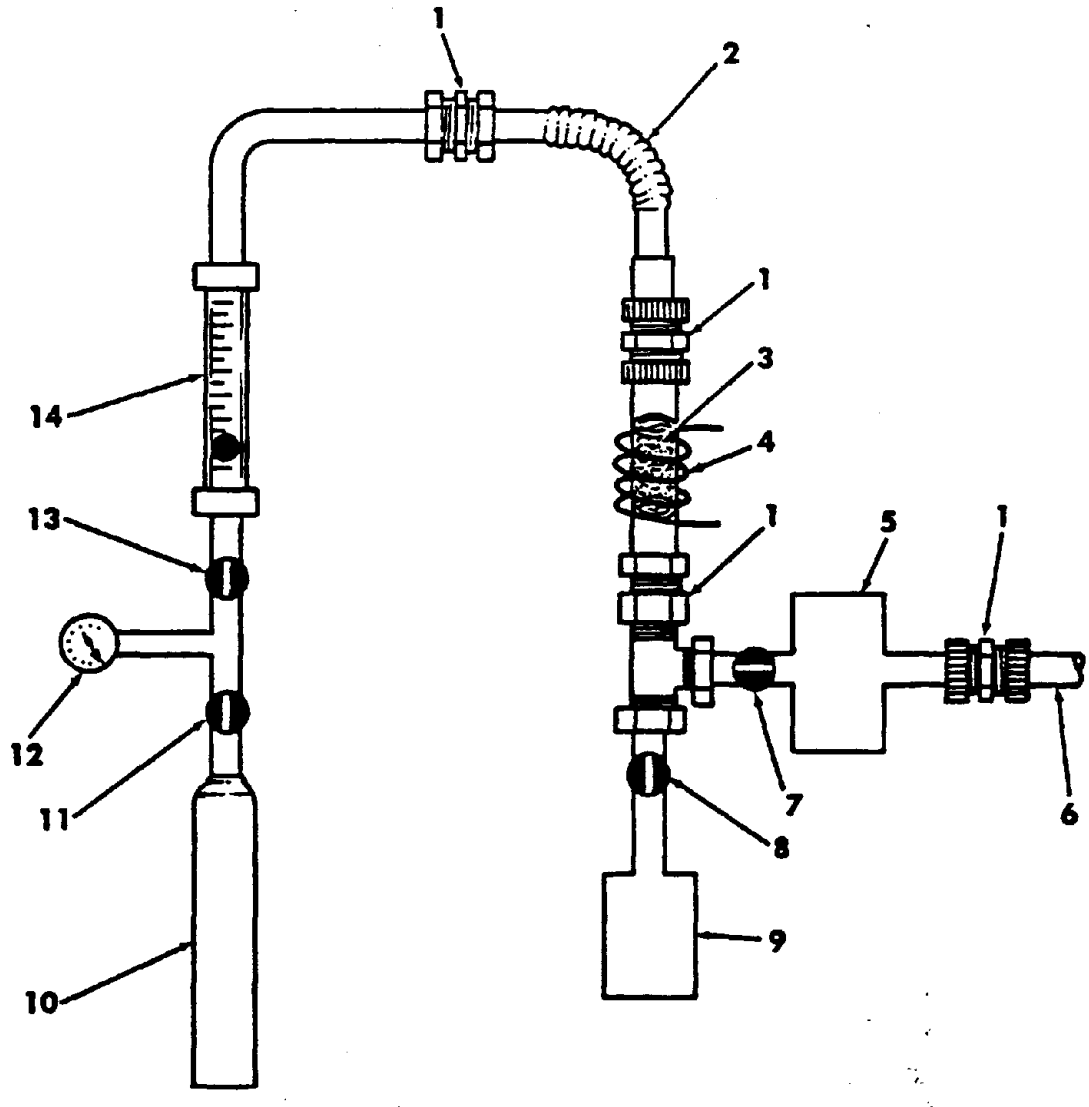
11. **Reference**

Tiernan, T.O., B.M. Hughes, C. Chang, R.P. Clow, and M.L. Taylor, Aerospace Research Laboratories. USAF Systems Command, Wright Patterson Air Force Base, Ohio, "Development and Evaluation of Solid Sorbents for Monitoring Work Place Air Pollutants," Final Report to National Institute for Occupational Safety and Health, Interagency Agreement No. NIOSH-1A-73-11, December 31, 1973.



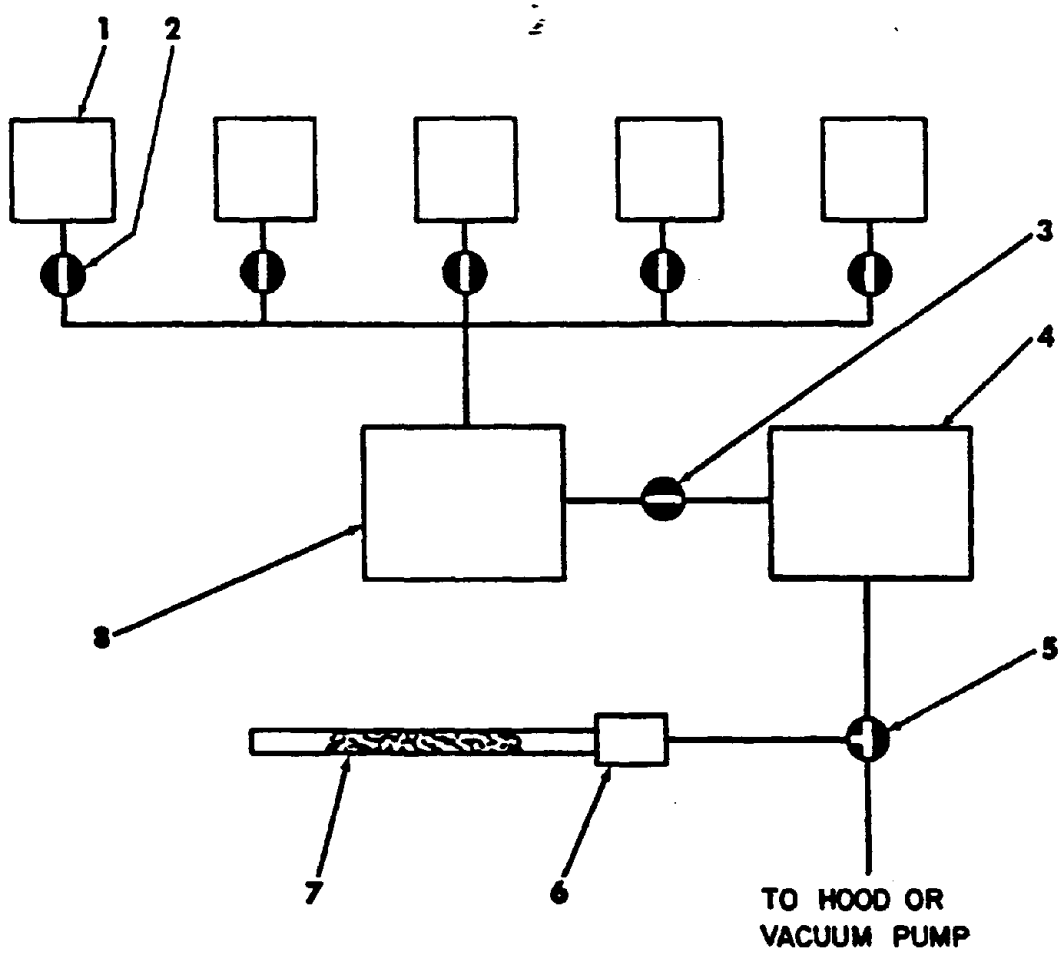
1. Polyethylene tube (0.75 in. o.d. and 1.5 in. long)
2. Polyethylene tips for a standard drying tube. One tip serves as an adaptor for coupling the polyethylene tube to the glass tube.
3. Phosphorus pentoxide (4 g)
4. Glass wool plugs
5. Molecular Sieve 5A (0.5 g)
6. Pyrex tube (0.3125 in. o.d. by 3 in. long)

Figure 1. Assembled Sorbent Tube



1. Standard stainless steel fittings
2. Flexible bellows (0.25 in. o.d. by 4 in. long)
3. Sorbent tube, glass section only
4. Heating tape connected to a variable transformer
5. Variable leak (Granville-Phillips Co., or equivalent)
6. Pyrex tubing (0.25 in. o.d.) connected to mass spectrometer
7. Positive shutoff valve
8. Throttle valve
9. Standard vacuum pump preceded by a cold trap
10. Compressed, dry air with a two-stage regulator followed by a bed of silica gel desiccant
11. On-off valve
12. Pressure gauge (-1 to 1 atm)
13. Fine metering valve or flow controller (0 to 600 mℓ/min)
14. Flowmeter (0 to 200 mℓ/min)

Figure 2. Thermal Desorption Gas Inlet System



1. Standard mixtures of sulfur dioxide in air (5 each)
2. On-off valve (5 each)
3. Fine metering valve or flow controller (0 to 600 mℓ/min)
4. Flowmeter (0 to 200 mℓ/min)
5. Three-way glass-Teflon stopcock
6. Standard stainless steel union
7. Sorbent tube, glass section only
8. Pressure gauge (-1 to 1 atm)

Figure 3. Gas-Handling Apparatus for Preparing Solid Sorbent Standards

AMMONIA IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	NH_3	**Method No.:**	P&CAM 205
Matrix:	Air	**Range:**	20-135 ppm
Procedure:	Midget impinger, colorimetric	**Precision:**	Unknown
Date Issued:	1/1/75		
Date Revised:		**Classification:**	C (Tentative)

1. **Principle of the Method**

   1.1 Ammonia is collected in a dilute sulfuric acid solution in a midget impinger to form ammonium sulfate.

   1.2 Nessler reagent is used to produce a yellow-brown complex.

   1.3 The ammonia concentration is determined by reading the absorbance of the yellow-brown solution at 440 nm and comparing it with a standard curve. Absorption peak may shift with concentration.

2. **Range and Sensitivity**

   2.1 The Nessler reagent is said to be sensitive to as little as 0.002 mg of ammonia.

   2.2 The range of application of this method is 0.10 mg to 0.80 mg of ammonia in a 10-liter air sample, about one-third to twice the present limit of 35 mg/m^3 of air.

3. **Interferences**

   3.1 Ammonium salts will react with Nessler reagent to give a false high reading. These can be removed by filtration of the air before its passage into the midget impinger.

   3.2 Other interferences have not been reported.

4. **Precision and Accuracy**

   The precision and accuracy of this method have not been reported.

5. **Advantages and Disadvantages of the Method**

   5.1 The method is sensitive, but ammonium salts can interfere.

   5.2 The method does not distinguish between free and combined ammonia.

6. **Apparatus**

   6.1 Battery operated personal air sampling pump, capable of drawing 1 liter of air per minute through 10 ml of absorbing solution.

   6.2 Impingers, standard midget, all glass, calibrated.

6.3 Beckman Model B spectrophotometer, or equivalent.

6.4 Spectrophotometer cells, 1 cm.

6.5 Volumetric flasks, 50 ml, 100 ml, 200 ml, 1000 ml, glass stoppered.

6.6 Pipettes, 0.5 ml, 1.0 ml, 5 ml, 10 ml, 20 ml.

6.7 Graduated cylinders, 25 ml, 50 ml, 100 ml.

7. Reagents

7.1 All reagents should be analytical reagent grade.

7.2 Double distilled water free of ammonia.

7.3 Potassium iodide.

7.4 Mercuric chloride.

7.5 Potassium hydroxide.

7.6 Ammonium sulfate.

7.7 Nessler Reagent. Dissolve 35 grams of mercuric chloride in 500 ml of hot water. Filter and allow to cool. Dissolve 62.5 grams of potassium iodide in 260 ml of cold water. Gradually add the mercuric chloride solution to 250 ml of the iodide solution until a slight permanent red precipitate is formed. Dissolve the precipitate with the remaining iodide solution and again add mercuric chloride slowly until a red precipitate remains.

Dissolve 150 grams of potassium hydroxide in 250 ml of distilled water. Add this solution to the potassium iodide-mercuric chloride solution and make up to 1 liter with distilled water. Stir thoroughly and allow to stand a day or so, and decant the clear liquid.

Warning. The Nessler reagent should be handled with caution because of its toxicity and corrosive properties.

7.8 Standard Ammonium Sulfate Solution. Dissolve 77.6 mg of ammonium sulfate in distilled water and make up to 1 liter. 1 ml of this solution contains 20 μg of ammonia. Discard solution after a week.

7.9 Absorbing Solution. Dilute 2.8 ml of concentrated sulfuric acid (18M) to 1 liter with distilled water to form 0.1 N sulfuric acid.

8. Procedure

8.1 Sampling. Add 10 ml of absorbing solution to each midget impinger including one for the blank. Attach the bubbler to a personal air sampling pump and draw air through the bubbler at a rate of 1 lpm for 10-15 min. Record the volume of air sampled.

8.2 Color Development. Dilute the sample to 50 ml with distilled water in a volumetric flask and shake well. Take 1 ml of this solution and make up to 50 ml with distilled water in another volumetric flask. Shake. Add 2 ml of Nessler reagent to the latter flask and determine absorbance after 10 min at 440 nm in a spectrophotometer using a 1-cm cell. Treat the blank in the same manner as the sample.

9. Calibration and Standards

Dilute samples of 5 ml, 10 ml, 20 ml, 30 ml, and 40 ml of the standard ammonium sulfate solution and process in the same manner as described in Section 8.2. Determine light absorbance in a spectrophotometer at 440 nm. Prepare a standard curve of absorbance versus micrograms of ammonia.

10. Calculations

$$\mu g\ NH_3/m^3 = (w/v) \times 1000$$

where:

w = μg of ammonia from the standard curve minus blank value, and

v = Volume of air in liters, corrected to 25°C and 760 Torr.

11. References

11.1 Goldman, F. H. and M. B. Jacobs, Chemical Methods of Industrial Hygiene, Interscience Publishers, New York, 1953.

11.2 Elkins, H. B., The Chemistry of Industrial Toxicology, 2nd Ed., John Wiley and Sons, New York, 1959.

TOTAL PARTICULATE AROMATIC HYDROCARBONS (TpAH) IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	TpAH	**Method No.:**	P&CAM 206
Matrix:	Air	**Range:**	Lower limit, 3 nanograms benzo(a)pyrene
Procedure:	Sampling with glass fiber filter, extract ultrasonically, enrich and measure with HPLC	**Precision:**	± 1.33% RSD (Analytical)
Date Issued:	1/1/75	**Classification:**	E (Proposed)
Date Revised:			

1. **Principle of the Method**

   Airborne particles collected from polluted atmospheres on glass fiber filters are extracted ultrasonically in the presence of silica powder (11.1-3). The TpAH in the filtered extract are separated by high speed liquid chromatography on a column of Corasil II with a non-polar solvent, and the absorbance is measured by a UV detector at 254 nm. Compounds responding to the detector are shown in Tables 1, 2, 3, and 4. The extract is suitable also for the analysis of the aliphatic hydrocarbons (11.4).

2. **Range and Sensitivity**

   2.1 Minimum reproducible level of standard benzo(a)pyrene at 254 nm is approximately 3 nanograms.

   2.2 The minimum detectable TpAH (in terms of benzo(a)pyrene) for particulates collected on one glass fiber filter of approximately 452 cm^2 is approximately 5 micrograms, or 3.3 nanograms/m^3 of air if 1500 m^3 of air are sampled in the ambient atmosphere.

   2.3 The upper range of TpAH concentrations can be increased by dilution of the extract and/or analyzing smaller samples. Sensitivity for low concentrations can be increased by injecting larger samples into the chromatograph. Thus, very high levels of TpAH can be measured.

3. **Interferences**

   3.1 Any compound which is not retained on the silica column and absorbs light at 254 nm is measured in this procedure.

   Fluorene and some of its analogues and derivatives listed in Table 2, and polychloro derivatives of some di- and tricyclic hydrocarbons in Table 3 are examples of such compounds.

   3.2 Amino, carbonyl, hydroxy and nitro compounds elute after the PAH, so do not interfere. See Table 2.

   3.3 Carbazoles and aldehydes are either retained or have retention times larger than the PAH, except N-alkyl substituted derivatives, which elute with the PAH. See Table 4.

3.4 Oxygenated compounds, some phenols and aza and imino-heterocyclics (except some members of the indole series) are retained. Examples are benzoquinone, o-ethylphenol, acridine, and quinoline.

3.5 Most interfering compounds have quite low peak area/μg values, which decreases their significance, as shown in Tables 2, 3, and 4.

TABLE 1

Elution of PAH[a]

Compound	% Eluted Through Column	PA*/μg $\times 10^{-3}$
Mono-, dicyclics		
Benzene	99	0.4
N-Hexylbenzene	100	0.5
N-Heptylbenzene	100	0.7
Naphthalene	101	0.7
Azulene	93	3.0
Tricyclics		
Anthracene	100	36.0
9-Methylanthracene	99	15.0
Xanthene	102	1.3
Phenoxathiin	92	0.2
Phenanthrene	100	10.0
Tetracyclics		
Naphthacene	95	4.7
Chrysene	105	4.5
Pyrene	96	3.6
4-Methylpyrene	100	1.7
1,3-Dimethylpyrene	96	0.9
Triphenylene	100	9.0
Benz(a)anthracene	96	4.3
7,12-Dimethylbenz(a)anthracene	102	3.3
Pentacyclics		
Dibenz(a,h)anthracene	96	0.6
Benzo(a)pyrene	100	5.3
Benzo(e)pyrene	92	2.2
Picene	99	5.0
Perylene	96	5.8
Hexacyclics		
Benzo(ghi)perylene	99	1.8
Anthanthrene	93	2.6
Dibenzo(fg, op)naphthacene	93	0.6
Coronene	91	0.5
Dibenzo(g,p)chrysene	96	1.0
Naphtho(2,1,8-qra) naphthacene[b]	100	0.7

[a]Retention time is approximately 2 minutes. [b]Or naphtho(2,3-a)pyrene. PA = Peak Area.

4. Precision and Accuracy

4.1 Homogeneous glass fiber samples containing air particulates were analyzed by Soxhlet and ultrasonic extraction. See Table 5. The relative standard deviation for 6 ultrasonic extracts was $\pm$ 1.33% and for 4 Soxhlet extracts $\pm$ 26.1%. The ratio of ultrasonic to Soxhlet recovery was 1.14.

4.2 Recovery of PAH added to glass fiber filter blanks and extracted ultrasonically was 95% for anthracene; 97.5% for phenanthrene; and 98.2% for benzo(a)pyrene (Table 6).

5. Advantages and Disadvantages of the Method

5.1 The extraction is done at room temperature. Complete extraction of the TpAH is assured by the fine shredding of the glass fibers and the breaking up of clumps of particulates.

5.2 Only a relatively small sample of air particulates is required. Complete analysis time is well under an hour, most of which is waiting time.

5.3 Most of the polar constituents are removed by adsorption in the homogenizing vessel. The remainder are removed by the fast simple chromatographic analysis.

5.4 The method can accommodate a wide range of hydrocarbon pollution concentrations, since sample extract volumes ranging from 0.1 to 2 ml can be chromatographed.

5.5 Time and work are saved by not weighing the particulates or soluble organics.

TABLE 2

Elution of Fluorene, Analogues and Derivatives

Compound	t,Min.	% Eluted Through Column	PA/μg $\times 10^{-3}$
Fluorene	2.0	100	2.9
Dibenzothiophene	2.0	96	1.8
Dibenzofuran	2.0	98	0.3
Fluoranthene	2.0	95	2.5
Benzo(k)fluoranthene	1.8	97	1.4
Benzo(b)fluoranthene	1.0	99	1.6
2-Ethylfluorene	1.0	95	1.6
11H-Benzo(b)fluorene	1.0	110	5.6
2-Nitrofluorene	4.8	104	0.2
2,5-Dinitrofluorene	7.0	71	0.3
9-Fluorenol	8.5	14	0.2
3,6-Dinitrodibenzoselenophene	18.2	38	0.2
3-Aminofluorene	18.2	68	0.4
4-Fluorenecarboxylic acid		Retained on column	
2-Hydroxyfluorene		Retained on column	
2-Nitro-7-hydroxyfluorene		Retained on column	
Fluorenone		Retained on column	

TABLE 3

Elution of Polychloro Derivatives of Di- and Tricyclic Hydrocarbons[a]

Compound	% Eluted Through Column	PA/μg $\times 10^{-3}$
1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane (p,p'DDD)	94	0.02
1,1-Dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE)	97	0.50
1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane (p,p'DDT)	85	0.02
Aroclor 1260 (chlorinated biphenyls, 60% chlorine)	100	0.13
Aroclor 5432 (chlorinated triphenyls, 32% chlorine)	104	0.61
Halowax 1099 (mixture of tri- and tetrachloro naphthalenes, 52% chlorine)	101	0.25
1,2,3,4,5,6,7,8-Octachloronaphthalene	97	0.64
2,3,4,5,6,2',3',4',5',6'-Decachlorobiphenyl	95	0.19
1,2,3,4,5,6,7,8-Octachlorodibenzofuran	93	0.33
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin	98	0.85
Tetradecachloro-p-terphenyl	95	0.22

[a]Retention times from 1 to 2 min.

5.6 A disadvantage is that a blank correction must be made for the fiber glass filter. Also, care must be taken to avoid evaporation of the extract to dryness.

5.7 A further disadvantage is that the ultrasonic extraction must be done in a sonabox to reduce the unacceptably high noise level.

6. Apparatus

6.1 Sonifier Cell Disruptor, 20 kHz power ultrasonic generator capable of dialing 70 watts accurately, with a 1.27-cm (½-inch) horn disruptor and Sonabox.

6.2 Liquid Chromatograph, with stainless steel column 2.6 × 300 mm, UV Detector with 254 nm filter and loop injector with a capacity ranging from 0.1 to 2 ml.

6.3 Strip Chart Recorder with Disc Integrator.

6.4 An approved and calibrated personal sampling pump for collection of particulate matter. Any vacuum pump whose flow can be determined accurately to within 1 lpm or less.

TABLE 4

Elution of Some Indoles, Carbazoles and Aromatic Aldehydes

Compound	t_R Min.	% Eluted Through Column	PA*/μg $\times 10^{-6}$
Indole	5.3	82	1.1
Carbazole	11.8	67	0.7
4-H-Benzo(def)carbazole	8.0	98	2.0
11-H-Benzo(a)carbazole	14.5	55	3.0
7-H-Dibenzo(c,g)carbazole	18.0	92	2.1
N-Phenylcarbazole	2.3	74	1.8
N-Ethylcarbazole	2.5	98	0.5
5-Methyl-5, 10-dihydroindeno(1,2-b) indole	2.8	103	1.9
2,3-Dimethylindole	5.3	90	5.5
2-Methylcarbazole	6.8	100	0.8
2-Hydroxycarbazole		Retained on column	
N-Ethyl-3-aminocarbazole		Retained on column	
Benzaldehyde	12.8	56	1.1
2-Naphthaldehyde	8.2	78	0.3

*Peak Area

TABLE 5

Comparison of Ultrasonic and Soxhlet Extractions

	Ultrasonic		Soxhlet	
Sample No.	PA/μg	% Eluted*	PA/μg	% Eluted*
1	0.575	51	0.449	28
2	0.562	53	0.509	—
3	0.567	50	0.500	—
4	0.579	48	0.545	31
5	0.560	44	—	—
6	0.573	44	—	—
Average	0.569	49	0.509	30
Rel. Std. Dev.	±1.33%		±26.1%	
Ultrasonic/Soxhlet Recovery = 1.14				

*Refers to % of TpAH in the extracted material.

TABLE 6

Recovery of Added PAH

Compound	Sample, μg	Peak Area: Sonified Filter + Std.	Peak Area: Standard Solution	% Recovery
Anthracene	0.035	1005	1055	95.0
Phenanthrene	0.147	1155	1185	97.5
Benzo(a)pyrene	0.355	1846	1880	98.2

6.5 Column Bypass.

6.6 Fisher Filtrator and medium sintered glass filter.

6.7 U.S. Standard Sieve Series No. 120, with 125-micron openings.

7. Reagents

7.1 Cyclohexane, ACS spectroanalyzed, distilled once from glass.

7.2 Polynuclear aromatic hydrocarbons.

7.3 Glass powder, spherical, non-wettable, 38-53 microns in diameter.

7.4 Corasil II.

8. Procedure

8.1 Extraction

8.1.1 The 1.27-cm horn of the sonifier cell disruptor is supported in a sonabox to reduce noise. The sonifying vessel is a beaker 3.8 cm I.D. × 10 cm tall. The end of the horn is set about 0.6 cm above the bottom of the beaker to insure adequate "stirring" of the mixture and equal exposure to areas of intense cavitation. Approximately 16 square cm of the exposed glass fiber filter and blank are cut into roughly 1.3-cm squares to facilitate shredding. The sonifying vessel is surrounded by an ice water bath up to the level of the solvent mixture.

8.1.2 Homogeneous replicate samples of approximately 16 square cm of exposed and blank glass fiber filters are prepared and adjusted to exactly 100 mg. This weight necessarily includes both the particulates and the glass fiber. These samples were used to maximize parameters and for comparison of ultrasonic and Soxhlet extractions, shown in Table 5.

8.1.3 Samples for routine analysis are not weighed. Only the areas of the sample (16 square cm) and the whole filter, the volume of air sampled and the volume of extract injected need to be determined. Sample at rate of at least 2 lpm for 1 hr or more.

8.1.4 Extraction Procedure. The sample, 60 ml cyclohexane, and 5 ml silica powder are placed in the sonifying vessel, and sonified for 8 min at 70 watts. The supernatant is decanted into the sintered glass filter supported on a Fisher Filtrator. Cyclohexane is added to the sonifying vessel to the level of the original mixture (usually about 50 ml). Sonification is carried on for an additional 4 minutes. The contents are filtered and combined with the first fraction, and rinsed with 50 ml cyclohexane. The filtrates and rinsings are collected in an Erlenmeyer flask and evaporated to about 5 ml, transferred quantitatively to a 10-ml volumetric flask and made to the mark.

8.1.5 Sample and blank filters (8.1.2) are extracted by Soxhlet with 80 ml cyclohexane for 6-8 hr, for comparison with the ultrasonic extraction. See Table 5. After filtering, the extracts are evaporated in the same manner as the ultrasonic extracts.

8.1.6 The glass fiber filters used for air sampling should be as free as possible of soluble compounds which absorb at 254 nm. It may be necessary to flash fire or extract them and care should be taken to avoid contaminating them.

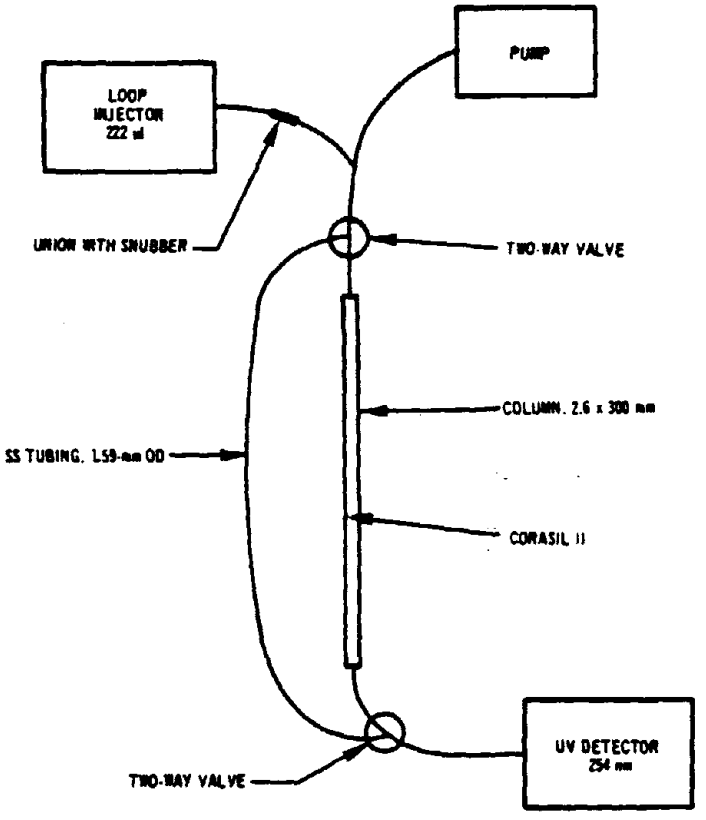


FIGURE 1. Schematic of Chromatographic System

8.2 Chromatographic Separation

8.2.1 A schematic of the chromatographic system is shown in Figure 1. The stainless steel column is 2.6 × 300 mm; the packing is Corasil II; the eluent is cyclohexane. Two two-way valves are installed in the chromatographic line, one before the column, the other after it. They are connected with stainless steel tubing. This enables the sample to be pumped through either the column or the tubing (column bypass) into the UV detector. A union with snubber may be placed in the line to prevent clogging.

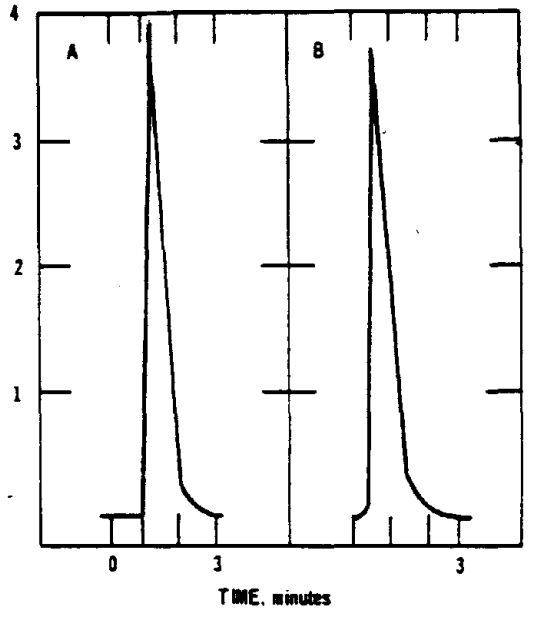


FIGURE 2. Chromatograms of benzo(ghi)perylene, 0.629 μg in cyclohexane, through the column (A) and through the column bypass (B). Stationary phase, Corasil II; eluent, cyclohexane; flow rate, 1.6 ml/minute. Peak area on column, 1165; on column bypass, 1180; recovery on column, 99%; peak area/μg, 1850.

8.2.2 To test the performance of the column, the percent of PAH which elutes is calculated from the peak areas through the column and the column bypass. Typical chromatograms from column and tubing are shown in Figure 2. Recovery of benzo(ghi)perylene was 99%. The percent of other hydrocarbons which eluted through the column ranged from 91 to 105, Table 1.

8.3 **Analysis Procedure**

8.3.1 An appropriate volume of extract is injected through the loop injector. A flow rate of 1.6 ml/min gives a pressure drop of less than 800 PSI. The peak area is measured with a disk integrator, driven by 0 to 10 servo strip chart recorder with a 0.5 in/min chart speed. The PAH elute in 3 to 5 min. Benzo(a)pyrene is used as the standard. Polar compounds are retained on the column. Samples can be chromatographed every 5 to 10 minutes.

8.3.2 The column bypass is also used to determine the percent of PAH in the organic material of the extract. Chromatograms of sample extracts made on the column and column bypass are shown in Figure 3. On the basis of absorbance measurements at 254 nm, approximately 50% of the organic material in the unchromatographed extract is PAH. This procedure is not necessary for routine analyses, but is helpful in elucidating the analytical situation in research samples.

8.4 **Effects of Storage**

8.4.1 Urban particulates on glass fiber filters stored in the dark in an envelope for one year lost 32% of their benzo(a)pyrene. Losses of some other PAH ranged from 1-88% (11.5).

8.4.2 Benzene-soluble extracts evaporated to dryness and stored in closed bottles in a refrigerator were stable (in terms of benzo(a)pyrene concentrations) for 4 years (11.6).

8.4.3 The ultrasonic extract is stable in the dark at room temperature for several days, longer in the refrigerator. However, losses usually occur after about two weeks.

9. **Calibration and Standards**

The benzo(a)pyrene (BaP) standard is made in cyclohexane and is chromatographed when the samples are run, and repeated whenever a parameter such as solvent lot is changed. Both standard and samples are run at concentrations which do not overload the detector and give reproducible results when diluted. For example, 0.4 μg BaP gave a peak area of about 2000 and fulfilled the above criteria.

The standard is expressed in terms of peak area per microgram (PA/μg). The unit of measurement for the samples is corrected peak area per cubic meter of air (PA/m^3). The BaP equivalent of the TpAH is calculated from these data (10.2). The standard is kept in the dark and is stable for more than 30 days when refrigerated nights and weekends.

10. **Calculations**

10.1 The peak area of the TpAH in a cubic meter of air is given by the equation

$$PA/m^3 = \frac{PA \times A \times B}{V \times a \times b}$$

where:

PA = Peak area, corrected for the blank
V = Volume of air sampled in m^3, corrected to 25°C and 760 Torr
A = Area of whole glass fiber filter in cm^2

520

B = Volume of extract in ml
a = Area of glass fiber filter sample in cm^2
b = Volume of extract injected in ml

10.2 The concentration of the TpAH may be expressed in terms of their equivalent in benzo(a)pyrene.

$$\text{TpAH}(\mu g)/m^3 \text{ air} = \frac{\text{PA}/m^3 \text{ air (See Table 7)}}{\text{PA}/\mu g \text{ benzo(a)pyrene (See Table 1)}}$$

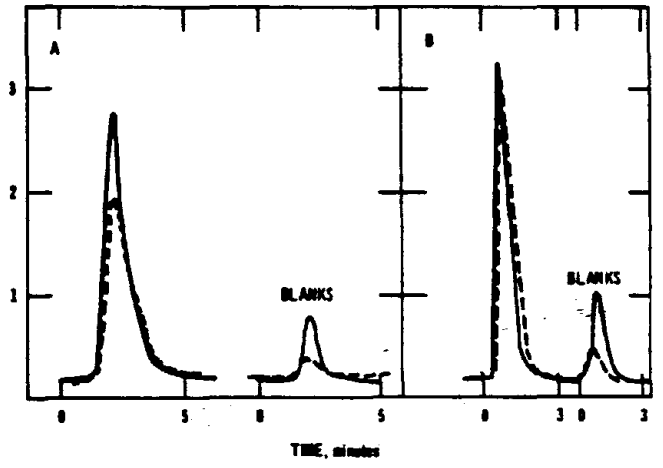


FIGURE 3. Chromatograms of ultrasonic and Soxhlet extracts of composited sample No. 1, Table 6 and blanks, through the column (A) and through the column bypass (B). Stationary phase, Corasil II; eluent, cyclohexane; flow rate, 1.6 ml/min. Solid lines are ultrasonic extracts; broken lines are Soxhlet extracts. Extracts were diluted x 3.3 for column bypass.

TABLE 7

Analysis of Particulate Samples

Description	Corrected Peak Area	m^3 Air Sampled	PA/m^3 Air[a]	TpAH[b] (μg)/m^3 Air
Urban I	1200	1500	1120	0.211
Urban II	620	1500	580	0.109
Urban III	545	1500	509	0.096
Mt. Storm	0	1673	0	0.000

[a]See Calculations—Section 10.1. [b]See Calculations—Section 10.2.

11. References

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LEAD IN BLOOD AND URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Lead	**Method No.:**	P&CAM 208
Matrix:	Blood, urine	**Range:**	0.05 - > 1.5 μg per gram of blood or per ml of urine
Procedure:	Chelation, extraction, AA	**Precision:**	Relative standard deviation ± 5%, (analytical)
Date Issued:	1/1/75		
Date Revised:		**Classification:**	E (Proposed)

1. Principle of the Method

Whole blood is collected in a lead-free sampling tube containing heparin anticoagulant; urine is collected in a polyethylene bottle containing nitric acid preservative. Blood is hemolyzed, the lead complexed with ammonium pyrrolidine dithiocarbamate and the complex extracted into methyl isobutyl ketone. Urine is similarly extracted, with hemolysis not required. The lead content of the organic phase is determined by atomic absorption spectrometry at 283.3 nm using an air-acetylene flame (11.1).

2. Range and Sensitivity

2.1 This method is applicable to the determination of lead in blood and urine in an optimum range of 0.1-1.5 μg lead per g blood or per ml urine.

2.2 The sensitivity of the method is 0.05 μg lead per ml methyl isobutyl ketone (MIBK) solution for a 1% change in transmittance (0.0044 absorbance units).

2.3 The detection limit of the method is 0.05 μg lead per g blood or per ml urine.

3. Interferences

3.1 Phosphate buffers, EDTA, and other complexing agents used as anticoagulants or preservatives may cause reduced recoveries. Addition of citrate will eliminate phosphate interferences (11.2) and excess calcium will prevent interference by EDTA (11.3).

3.2 Blood specimens stored at 18°C and above for 5 days or more may show poor lead recoveries, typically 20-80% (11.2).

3.3 Ammonium pyrrolidine dithiocarbamate (APDC) is an unstable reagent; extraction efficiency should be checked before use.

3.4 The Pb-APDC complex in MIBK is unstable and must be analyzed within two hours of extraction.

4. Precision and Accuracy

4.1 Relative standard deviations of about 5% at 0.45 μg lead per g blood are obtained (11.2).

575

4.2 Analytical data obtained by this procedure agree closely with that obtained by several other analytical procedures, including the carbon rod atomizer (11.4), Delves cup (11.5), and anodic stripping voltammetry (11.6).

5. Advantages and Disadvantages of the Method

The method is rapid and does not require a high level of technical skill. It is not necessary to ash the sample with a mixture of nitric and perchloric acid to destroy the organic matter. Over 99% extraction of lead by MIBK solvent from blood and urine samples has been achieved without wet ashing (11.3). Addition of a non-ionic surfactant, Triton X-100, to the APDC solution allows aspiration of the MIBK solvent layer into the flame of the atomic absorption spectrometer. The only known disadvantage of this method is the need for AA spectrometer equipment.

6. Apparatus

6.1 Sample Collection

6.1.1 Heparinized sample collection tubes, 10 ml, certified to contain less than 0.1 μg lead per tube (Vacutainer®, Becton-Dickinson, Rutherford, New Jersey, or equivalent).

6.1.2 Polyethylene or polystyrene syringes, 5 ml.

6.1.3 Polyethylene bottles, 25 ml and 2 l.

6.1.4 25 × 100 mm swab tubes, or equivalent, fitted with polyethylene caps.

6.2 Glassware. Borosilicate glassware should be used throughout the analysis. The glassware must be washed with 1:1 HNO_3 and rinsed with distilled water. Beakers, pipettes and auxiliary glassware will be required.

6.3 Equipment

6.3.1 Centrifuge: 2000 rpm

6.3.2 Vortex mixer (Vortex-Genie, Scientific Industries, Inc., New York or equivalent)

6.3.3 Atomic absorption spectrometer

6.3.4 Weighing syringe, polypropylene syringe with Teflon coated plunger

6.3.5 Lead hollow cathode lamp

6.3.6 Air supply, filtered to remove oil and water, and with a minimum pressure of 40 psi

6.3.7 Acetylene gas (Tank should be changed when pressure < 100 psi). The grade recommended by the manufacturer of the atomic absorption spectrometer should be used.

6.3.8 Hydrometer

7. Reagents

7.1 All reagents should be ACS reagent grade or the equivalent. All references to water are double-distilled water or the equivalent.

7.2 Non-ionic surfactant, Triton X-100, octyl phenoxy polyethoxyethanol (Sigma Chemical, St. Louis, Mo.), or equivalent.

7.3 Ammonium pyrrolidine dithiocarbamate (APDC). APDC is unstable and each new batch should be checked for extraction efficiency. The reagent is stable for about 6 months.

7.4 Methylisobutyl ketone (MIBK).

7.5 Lead nitrate $Pb(NO_3)_2$. Dry at 120°C for 4 hours prior to use; cool in a desiccator.

7.6 Nitric acid, concentrated. (Specific gravity = 1.421.)

7.7 MIBK—water saturated. Add 100 ml water to 900 ml MIBK, and saturate by shaking and allowing to stand one hour.

7.8 Triton X-100/APDC solution (TX-APDC): 2.5% Triton X-100, 2% APDC. Add 4 g APDC to about 40 ml water, then add 5 ml Triton X-100 and mix until dissolved. Make up to 200 ml. Prior to use, check the reagent by analyzing standards using both the new and old batches of reagent.

7.9 Calcium chloride, 1.5 M. Dissolve 16.648 g $CaCl_2$ in a few ml water, make up to 100 ml.

8. Procedure

8.1 Sample Collection

8.1.1 At least 4 ml blood is collected by venipuncture in sample collection tubes. Immediately after collection, the tube is shaken vigorously to ensure thorough mixing with the anticoagulant. Samples are stored at 4°C prior to shipping to the laboratory and in the laboratory prior to analysis. During shipping, samples should not be exposed to high ambient temperatures (ideally maintained at temperatures below 25°C). Samples should be promptly analyzed, preferably within 3 days of collection.

8.1.2 Urine samples (preferably 24 hr) are collected in a 2-liter polyethylene bottle containing ca. 0.5 ml concentrated nitric acid. After collection, the sample is thoroughly mixed and an aliquot of about 20 ml is transferred to a 25-ml bottle for shipping to the laboratory.

8.2 Sample Preparation

8.2.1 **Blood.** Shake the sample collection tube, draw the entire sample into a weighing syringe and weigh. Disperse a 2-g aliquot into a swab tube and reweigh. Disperse a second aliquot.

8.2.2 **Urine.** Filter the urine and measure two 2-ml fractions into a swab tube.

8.2.3 Add 0.8 ml TX-APDC solution to each swab tube, cap the tube, and mix on a vortex mixer for 10 sec. Remove the cap, add 2.00 ml MIBK, cap the tube, and shake for 2 min, inverting the tube at regular intervals. Centrifuge at 2000 rpm for 10 min.

NOTES: (1) Poor mixing will lead to low recovery.
(2) Do not allow aqueous phase from the MIBK to be included in the aliquot.
(3) If the patient is receiving EDTA therapy, add 50μl 1.5 M $CaCl_2$ immediately before adding the MIBK.

8.3 Analysis of Samples

8.3.1 Allow the atomic absorption spectrometer and hollow cathode lamp to warm up for 10 min and operate an air-acetylene flame for 10 min (aspirating water) to warm up the burner. Set the wavelength at 283.3 nm and set the instrument parameters according to manufacturer's recommendations.

8.3.2 Aspirate MIBK. This will increase the fuel content of the flame, so it will be necessary to reduce acetylene flow to produce a stoichiometric flame. After a further 30 sec to allow the nebulizer-burner to stabilize, take a background reading.

8.3.3 Aspirate the standards and record the absorbance. Prepare a calibration curve as described in Section 9.1.

8.3.4 Aspirate the samples plus blanks and record absorbances for comparison with the standards. Should the absorbance be greater than 0.35, dilute an appropriate aliquot with MIBK to 10 ml. Be certain to aspirate MIBK between samples. Standards must be aspirated with sufficient frequency to assure the accuracy of the sample determinations. Aspirate a 0.80 μg lead/ml standard every tenth sample.

524

621

9. Calibration and Standards

9.1 Standard Lead Solution

9.1.1 Master Standard (1000 μg lead/ml). Heat lead nitrate for 4 hr at 120°C, cool in a desiccator, dissolve 1.598 g lead nitrate in one liter of 2% nitric acid. This solution is stable for 1 year when stored in a polyethylene bottle.

9.1.2 Working lead standards. Each day, dilute 10.00 ml master standard to 1 liter using a volumetric flask. Aliquot exactly 2, 4, 6, 8, 10 and 15 ml respectively of this diluted solution into 100-ml volumetric flasks and dilute to volume. 2.00 ml standards are analyzed as described in Sections 8.2.3 and 8.3.

9.2 Blank. Blank water samples (2 g) must be carried through the entire procedure.

10. Calculations

10.1 The net signal for each sample, standard, and blank is computed from:

$$\text{Net Signal} = S - \frac{(B_1 + B_2)}{2}$$

where:

S = Sample signal
B_1 = Background signal, read immediately before the sample signal
B_2 = Background signal, read immediately after the sample signal

10.2 A least squares curve of best fit is constructed from the duplicate measurements of standards and blank over the range 0-1.5 μg lead per ml. Blanks should contain less than 0.05 μg lead/ml.

10.3 If a 24-hour urine sample was not collected, the lead level can be corrected to a standard urine specific gravity of 1.024 by multiplying by the factor $\frac{1.024}{S.G.}$, where S.G. is the specific gravity of the urine sample.

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FREE CHLORINE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Chlorine	**Method No.:**	P&CAM 209
Matrix:	Air	**Range:**	0.05-1.0 ppm
Procedure:	Fritted bubbler for sampling colorimetric methyl orange	**Precision:**	± 5% (Analytical)
Date Issued:	1/1/75		
Date Revised:		**Classification:**	C (Tentative)

1. **Principle of the Method**

   1.1 Sampling is performed by passing a measured volume of air through a fritted bubbler containing 100 ml of dilute methyl orange.

   1.2 Near a pH of 3.0, the color of a methyl orange solution ceases to vary with acidity. The dye is quantitatively bleached by free chlorine, and the extent of bleaching can be determined colorimetrically (11.1, 11.6). The optimum concentration range is 0.05-1.0 ppm in ambient air (145 μg to 2900 μg per m^3 at 25°C and 760 Torr).

2. **Range and Sensitivity**

   2.1 The procedure given is designed to cover the range of 5-100 micrograms of free chlorine/100 ml of sampling solution. For a 30-liter air sample, this corresponds to approximately 0.05-1.0 ppm in air, which is the optimum range.

   2.2 Increasing the volume of air sampled will extend the range at the lower end, but only within limits, since 50 liters of chlorine-free air produce the same effect as about 0.01 ppm of chlorine.

   2.3 By using a sampling solution more dilute in methyl orange, a concentration of 1 μg/100 ml of solution may be measured. But beyond this, problems are encountered because of the absorption of ammonia and other gases from the air and by the presence of minute amounts of chlorine-consuming materials even in distilled water.

3. **Interferences**

   Free bromine, which gives the same reaction, interferes in a positive direction (11.4). Manganese (III, IV) in concentrations of 0.1 ppm or above also interferes positively (11.3). In the gaseous state, interference from SO_2 is minimal, but in solution, negative interference from SO_2 is significant. Nitrites impart an off-color orange to the methyl orange reagent. NO_2 interferes positively, reacting as 20% chlorine. SO_2 interferes negatively, decreasing the chlorine by an amount equal to one-third the SO_2 concentration. Ozone may also interfere positively (11.2).

4. **Precision and Accuracy**

   The data available (11.5) indicate that 26 chlorine concentrations produced by two different methods (flowmeter calibrated by KI absorption, and gas-tight syringe) were measured by this procedure with an average error of less than ± 5% of the amount present.

5. **Advantages and Disadvantages of the Method**

5.1 The method is relatively simple with direct bleaching of the methyl orange reagent.

5.2 Interfering substances in the air sample may affect the accuracy of the method. The sampled solutions must be protected from direct sunlight if the color is to be preserved for 24 hr.

6. **Apparatus**

6.1 Spectrophotometer. Suitable for measurement at 505 nm, preferably accommodating 5-cm cells.

6.2 Fritted bubbler. Coarse porosity, of 250-350 ml capacity. A small bubbler of 50-60 ml capacity may be more convenient for industrial hygiene sampling; volumes of reagents are then reduced proportionally.

7. **Reagents**

Reagents must be ACS analytical grade quality. Distilled water should conform to ASTM Standard for Referee Reagent Water.

7.1 Chlorine-demand-free water. Add sufficient chlorine to distilled water to destroy the ammonia. The amount of chlorine required will be about ten times the amount of ammoniacal nitrogen present. In no case should the initial residual be less than 1.0 mg/l free chlorine. Allow the chlorinated distilled water to stand overnight or longer, then expose to direct sunlight for one day or until all residual chlorine is discharged. A UV lamp may also be used to discharge the chlorine.

7.2 Methyl orange stock solution, 0.05%. Dissolve 0.500 g reagent grade methyl orange in distilled water and dilute to 1 liter. This solution is stable indefinitely if freshly boiled and cooled distilled water is used.

7.3 Methyl orange reagent, 0.005%. Dilute 100 ml of stock solution to 1 liter with distilled water. Prepare fresh for use.

7.4 Sampling solution. 6 ml of 0.005% methyl orange reagent is diluted to 100 ml with distilled water and 3 drops (0.15-0.20 ml) of 5.0 N HCl added. One drop of butanol may be added to induce foaming and increase collection efficiency.

7.5 Acidified water. To 100 ml of distilled water, add 3 drops (0.15-0.20 ml) of 5 N HCl.

7.6 Potassium dichromate solution, 0.1000 N. Dissolve 4.904 g anhydrous $K_2Cr_2O_7$, primary standard grade, in distilled water and dilute to 1 liter.

7.7 Starch indicator solution. Prepare a thin paste of 1 g of soluble starch in a few ml of distilled water. Bring 200 ml of distilled water to a boil, remove from heat, and stir in the starch paste. Prepare fresh before use.

7.8 Potassium iodide, reagent grade.

7.9 Sodium thiosulfate solution, 0.1 N. Dissolve 25 g of $Na_2S_2O_3 \cdot 5H_2O$ in freshly boiled and cooled distilled water and dilute to 1 liter. Add 5 ml chloroform as preservative and allow to age for 2 weeks before standardizing as follows: To 80 ml of distilled water, add, with constant stirring, 1 ml conc. H_2SO_4, 10.00 ml 0.1000 N $K_2Cr_2O_7$, and approximately 1 g of KI. Allow to stand in the dark for 6 minutes. Titrate with 0.1 N thiosulfate solution. Upon approaching the end-point (brown color changing to yellowish green), add 1 ml of starch indicator solution and continue titrating to the end-point (blue to light green).

$$\text{Normality } Na_2S_2O_3 = \frac{1.000}{\text{mls of } Na_2S_2O_3 \text{ used}}$$

7.10 Sodium thiosulfate solution, 0.01 N. Dilute 100 ml of the aged and standardized 0.1 N $Na_2S_2O_3$ solution to 1 liter with freshly boiled and cooled distilled water. Add 5 ml chloroform as preservative and store in a glass-stoppered bottle. Standardize frequently with 0.0100 N $K_2Cr_2O_7$.

7.11 Chlorine solution, approximately 10 ppm. Prepare by serial dilution of household bleach (approx. 50,000 ppm), or by dilution of strong chlorine water made by bubbling chlorine gas through cold distilled water. The diluted solution should contain approximately 10 ppm of free (available) chlorine. Prepare 1 liter.

8. **Procedure**

8.1 Place 100 ml of sampling solution in the fritted bubbler. A measured volume of air is drawn through at a rate of 1-2 l/min for a period of time appropriate to the estimated chlorine concentration. Transfer the solution to a 100-ml volumetric flask and make to volume, if necessary, with acidified water. Measure absorbance at 505 nm in 5-cm cells against distilled water as reference.

8.2 The volume of sampling solution, the concentration of methyl orange in the sampling solution, the amount of air sampled, the size of the absorbing vessel and the length of the photometer cell can be varied to suit the needs of the situation as long as proper attention is paid to the corresponding changes necessary in the calibration procedure.

9. **Calibration and Standards**

9.1 Prepare a series of six 100-ml volumetric flasks containing 6 ml of 0.005% methyl orange reagent, 75 ml distilled water, and 3 drops (0.15-0.20 ml) of 5.0 N HCl. Carefully and accurately pipet 0, 0.5, 1.0, 5.0 and 9.0 ml of chlorine solution (approximately 10 ppm) into the respective flasks, holding the pipet tip beneath the surface. Quickly mix and make to volume with distilled water.

9.2 Immediately standardize the 10 ppm chlorine solution as follows: To a flask containing 1 gm KI and 5 ml glacial acetic acid, add 400 ml of chlorine solution, swirling to mix. Titrate with 0.01 N $Na_2S_2O_3$ until the iodine color becomes a faint yellow. Add 1 ml of starch indicator solution and continue the titration to the end-point (blue to colorless). One ml of 0.0100 N $Na_2S_2O_3$ = 0.3546 mg of free chlorine. Compute the amounts of free chlorine added to each flask in 9.1.

9.3 Transfer the standards prepared in 9.1 to absorption cells and measure absorbance vs. micrograms of chlorine to draw the standard curve.

10. **Calculations**

$$\text{ppm } Cl_2 = \frac{\text{mg } Cl_2 \text{ found}}{\text{liters of air sampled}} \times \frac{24.450}{71}$$

For different temperatures and atmospheric pressures, proper correction for air volume should be made to standard conditions of 25°C and 760 Torr.

11. **References**

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ACROLEIN IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Acrolein	**Method No.:**	P&CAM 211
Matrix:	Air	**Range:**	1-30 μg/10 ml reagent
Procedure:	Collection in midget impinger with 1% aqueous $NaHSO_3$; reaction with 4-hexylresorcinol; colorimetry	**Precision:**	± 5% for standards, unknown for air samples
Date Issued	1/1/75		
Date Revised:		**Classification:**	C (Tentative)

1. **Principle of the Method**

   1.1 Air is drawn through two midget impingers containing 1% $NaHSO_3$.

   1.2 The reaction of acrolein with 4-hexylresorcinol in an alcoholic trichloroacetic acid solvent medium in the presence of mercuric chloride results in a blue colored product with strong absorption maximum at 605 nm (11.1-3).

2. **Range and Sensitivity**

   2.1 The absorbances at 605 nm are linear for at least 1-30 μg of acrolein in the 10 ml portions of mixed reagent (11.4).

   2.2 A concentration of 10 ppb of acrolein can be determined in a 50 liter air sample based on a difference of 0.05 absorbance unit from the blank using a 1-cm cell. Greater sensitivity could be obtained by use of a longer path length cell.

3. **Interferences**

   There is no interference from ordinary quantities of sulfur dioxide, nitrogen dioxide, ozone and most organic air pollutants. A slight interference occurs from dienes: 1.5% for 1,3-butadiene and 2% for 1,3-pentadiene. The red color produced by some other aldehydes and undetermined materials does not interfere in spectrophotometric measurement (11.4).

4. **Precision and Accuracy**

   Known standards can be determined to within ± 5% of the true value (11.5-6). No data are available on precision and accuracy of air samples.

5. **Advantages and Disadvantages of the Method**

   5.1 The method is sensitive to the extent that 0.01 ppm of acrolein can be determined in a 50-liter air sample.

   5.2 Both the solid trichloroacetic acid and the solution are corrosive to the skin. Mercuric chloride is highly toxic. This reagent and the former should be handled carefully.

6. **Apparatus**

6.1 Absorbers. All glass standard midget impingers with fritted glass inlets are acceptable. The fritted end should have a porosity approximately equal to that of Corning EC (170-220 μ maximum pore diameter). A train of 2 bubblers is needed.

6.2 Air pump. A pump capable of drawing at least 2 l of air/min for 20 minutes through the sampling train is required. A trap at the inlet to protect the pump from the corrosive reagent is recommended.

6.3 Air metering device. Either a limiting orifice of approximately 1 or 2-liter/min capacity or a glass meter can be used. If a limiting orifice is used, regular and frequent calibration is required.

6.4 Spectrophotometer. This instrument should be capable of measuring the developed color at 605 nm. The absorption band is rather narrow and thus a lower absorptivity may be expected in a broad-band instrument.

7. **Reagents**

7.1 Purity of chemicals. All reagents must be analytical reagent grade. An analytical grade of distilled water must be used.

7.2 $HgCl_2$-4-hexylresorcinol. Dissolve 0.30 g $HgCl_2$ and 25 g 4-hexylresorcinol in 50 ml of 95% ethanol (stable at least 3 weeks in refrigerator).

7.3 TCAA. To a 1-lb. bottle of trichloracetic acid, add 23 ml of distilled water and 25 ml of 95% ethanol. Mix until all the TCAA has dissolved.

7.4 Collection medium. 1% sodium bisulfite in water.

7.5 Acrolein, purified. Freshly prepare a small quantity (less than 1 ml is sufficient) by distilling 10 ml of the purest grade of acrolein commercially available. Reject the first 2 ml of distillate. (The acrolein should be stored in a refrigerator to retard polymerization.) The distillation should be done in a hood because the vapors are irritating to the eyes.

7.6 Acrolein, standard solution "A" (1 mg/ml). Weigh 0.1 g (approximately 0.12 ml) of freshly prepared, purified acrolein into a 100-ml volumetric flask and dilute to volume with 1% aqueous sodium bisulfite. This solution may be kept for as long as a month if properly refrigerated.

7.7 Acrolein, standard solution "B" (10 μg/ml). Dilute 1 ml of standard solution "A" to 100 ml with 1% sodium bisulfite. This solution may be kept for as long as a month if properly refrigerated.

8. **Procedure (11.5-6)**

8.1 **Cleaning of Equipment.** No specialized cleaning of glassware is required. However, since known interferences occur with dienes, cleaning techniques should insure the absence of all organic materials.

8.2 **Collection of Samples.** Two midget impingers, each containing 10 ml of 1% $NaHSO_3$ are connected in series with Tygon tubing. These are followed by and connected to an empty impinger (for meter protection) and a dry test meter and a source of suction. During sampling, the impingers are immersed in an ice bath. Sampling rate of 2 liters/min should be maintained. Sampling duration will depend on the concentration of aldehydes in the air. One hour sampling time at 2 liters/min is adequate for ambient concentrations.

8.3 **Storage of Samples.** After sampling is complete, the impingers are disconnected from the train, the inlet and outlet tubes are capped, and the impingers stored in an ice bath or at 0°C in a refrigerator until analyses are performed. Cold storage is necessary only if the acrolein determination cannot be performed within 4 hr of sampling.

8.4 **Shipping of Samples.** Because of the two hour limit for initiation of color development, it is best to analyze the samples soon after completion of sampling. However, where a refrigerated sample can be shipped and analyzed within 48 hr after collection, then samples can be shipped or carried to a central lab at some distance from the sampling area.

8.5 **Analysis of Samples.** (Each impinger is analyzed separately). To a 25-ml graduated tube, add an aliquot of the collected sample in bisulfite containing no more than 30 μg acrolein. Add 1% sodium bisulfite (if necessary) to a volume of 4.0 ml. Add 1.0 ml of the $HgCl_2$-4-hexylresorcinol reagent and mix. Add 5.0 ml of TCAA reagent and mix again. Insert in a boiling water bath for 5-6 min, remove, and set aside until tubes reach room temperature. Centrifuge samples at 1500 rpm for 5 min to clear slight turbidity. One hour after heating, read in a spectrophotometer at 605 nm against a bisulfite blank prepared in the same fashion as the samples. Determine the acrolein content of the sampling solution from a curve previously prepared from the standard acrolein solutions.

9. **Calibration and Standards**

9.1 Pipette 0, 0.5, 1.0, 2.0, and 3.0 ml of standard solution "B" into glass stoppered test tubes.

9.2 Dilute each standard to exactly 4 ml with 1% aqueous sodium bisulfite.

9.3 Develop color as described above, in section 8.5.

9.4 Plot data on semi-log paper, of absorbance values against micrograms of acrolein in the color developed solution. The color after forming is stable for about 2 hr.

10. **Calculations**

The concentration of acrolein in the sampled atmosphere may be calculated by using the following equations.

$$\mu g/m^3 = \frac{\text{total } \mu g \text{ of acrolein in test sample} \times 1000}{\text{air sample volume in liters}}$$

$$ppm = \frac{\text{total } \mu g \text{ of acrolein in sample}}{2.3 \times \text{sample volume in liters}}$$

Correct the air sample volume to 25°C and 760 Torr.

11. **References**

11.1 Rosenthaler, L. and G. Vegezzi, "Determination of Acrolein", Z Lebensm Unters Forsch 99:352, 1954.

11.2 Cohen, I. R. and A. P. Altshuller, " A New Spectrophotometric Method for the Determination of Acrolein in Combustion Gases and the Atmosphere", Anal Chem 33:726, 1961.

11.3 Altshuller, A. P. and S. P. McPherson, "Spectrophotometric Analysis of Aldehydes in the Los Angeles Atmosphere", J Air Pollut Control Assoc 13:109, 1963.

11.4 Intersociety Committee, Methods of Air Sampling and Analysis, p. 187, American Public Health Association, Washington, D.C., 1972.

11.5 Levaggi, D. A. and M. Feldstein, "The Determination of Formaldehyde, Acrolein, and Low Molecular Weight Aldehydes in Industrial Emissions on a Single Collected Sample", J Air Pollut Control Assoc 20:312, 1970.

11.6 "Tentative Method of Analysis for Low Molecular Weight Aliphatic Aldehydes in the Atmosphere", Health Lab Sci 9:75, 1972.

GASEOUS AND PARTICULATE FLUORIDES

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Gaseous and particulate fluorides	**Method No.:**	P&CAM 212
Matrix:	Air	**Range:**	Lower limit 0.005 mg/m³ air
Procedure:	Collection on membrane filter and alkaline filter, selective ion electrode	**Precision:**	Unknown
Date Issued:	1/1/75		
Date Revised:		**Classification:**	E (Proposed)

1. **Principle of the Method**

   1.1 Particulate material from a measured volume of air is collected by means of a membrane filter.

   1.2 Gaseous fluoride, from the same sample of air, is absorbed by an alkali-impregnated cellulose pad placed immediately behind the membrane filter.

   1.3 The membrane filter and collected solids are made alkaline, ashed, the residue fused with additional alkali, and fluoride determined in a solution of the melt by use of a fluoride ion selective electrode.

   1.4 Gaseous fluoride is determined in an aqueous extract of the cellulose pad, also by means of the fluoride ion selective electrode.

2. **Range and Sensitivity**

   The method is capable of collection and analysis of both particulate and gaseous fluorides over a wide range (11.1-4). The recommended range of the method is 0.005 to 5 mg F^-/m^3 air. The sensitivity has not been established.

3. **Interferences**

   3.1 Because a selective ion electrode responds to ionic activity, insoluble and complexed forms of fluoride must be released by appropriate combinations of fusion, adjustment of pH, and addition of complexing agent.

   3.2 Acidity (pH) and ionic strengths of fluoride standard solutions must be matched to those of samples.

   3.3 Temperature of sample and standard solutions must be controlled within ± 2°C.

4. **Precision and Accuracy**

   Precision and accuracy of this method have not been determined.

5. Advantages and Disadvantages of the Method

5.1 Advantages include simplicity, elimination of distillation or diffusion, speed and specificity.

5.2 No significant disadvantages are known at present.

6. Apparatus

6.1 Personal sampling pump. Mine Safety Appliances Company Model G, or equivalent.

6.2 Filter holder. Plastic holders of the pre-loaded personal monitor type, which accept filters of 37-mm diameter, are preferred. The holder is to be numbered for identification. (Millipore Corporation Field Monitor, Cat. No. MAWP 037 AO, or equivalent. This is supplied with a membrane filter and cellulose back-up pad.)

6.3 Membrane filter of mixed cellulose esters, 0.8 μm pore size, and of diameter to fit the filter holder (6.2).

6.4 Cellulose pad, of size to fit the filter holder (6.2).

6.5 Crucibles, 20 ml, of nickel or Inconel.

6.6 Fluoride ion selective electrode.

6.7 Reference electrode, calomel type, preferably combined with the fluoride ion selective electrode. (Orion Research, Inc. combination fluoride electrode, No. 96-09, or equivalent).

6.8 An electrometer or expanded scale pH meter, with a millivolt scale for measurement of potentials.

6.9 Magnetic stirrer and Teflon coated stirring bars, for 50-ml plastic beakers.

7. Reagents

7.1 Purity of Reagents. Reagent grade chemicals, ACS specifications or equivalent, must be used in all tests. Polyethylene beakers and bottles should be used for holding and storing all fluoride-containing solutions.

7.2 Purity of Water. References to water shall be understood to mean double distilled water.

7.3 Alkaline Fixative Solution. Dissolve 25 g sodium carbonate (Na_2CO_3) in water, add 20 ml glycerol, and dilute to 1 liter.

7.4 Total Ionic Strength Activity Buffer (TISAB). Dissolve 37 g potassium chloride (KCl), 68 g sodium acetate ($NaC_2H_3O_2 \cdot 3\ H_2O$), and 294 g sodium citrate ($Na_3C_6H_5O_7 \cdot 2\ H_2O$) in water, adjust to pH 8 $\pm$ 0.1 with addition of a small amount of 1:1 hydrochloric acid, and dilute to 1 liter.

7.5 Standard Fluoride Solution, 100 mg/liter. Dissolve 0.2211 g sodium fluoride (NaF, dried at 105°C for 2 hr) in water and dilute to volume in a 1-liter volumetric flask.

7.6 Ethanol, denatured. Formula 30 denatured alcohol is satisfactory.

7.7 Borate-Carbonate Fusion Mixture. Intimately mix a 1:2 (w/w) combination of sodium tetraborate ($Na_2B_4O_7$) and sodium carbonate (Na_2CO_3).

8. Procedure

8.1 **Preparation of Monitors.** Disassemble the personal monitor (6.2), removing the membrane filter and cellulose pads. Moisten the pad with a measured volume of alkaline fixative solution (7.3); 0.8 ml is required for a pad of 37-mm diameter. Dry the pad at 105°C for 30-45 min. Preparation of alkali-impregnated pads must be carried out in fluoride-free environment with minimum exposure.

8.2 Reassemble the filter monitor, inserting an impregnated pad and membrane filter, and closing with the filter retaining ring and front cover. The assembly is sealed against air leakage by a wrap of masking tape covering the crevice between retaining ring and back cover. Inlet and outlet openings of the monitor are closed with plastic plugs.

8.3 **Calibration of Personal Monitoring Pump.** Select several of the prepared monitors at random, for calibration of air flow rate with the personal sampling pump (6.1). Connect the monitor exit to the sampling pump by means of a 30-in. length of hose. Connect the flowmeter (preferably a bubble flowmeter or wet test meter) to the inlet port of the monitor. Start the pump and adjust its rate, noting the position of the rotameter ball when a sampling rate of 2.5 liters/min is indicated by use of the calibration flowmeter and a timer.

8.4 **Sampling**

8.4.1 A worker whose exposure is to be evaluated is equipped with a personal monitor connected by a 30-inch length of hose to a belt-supported sampling pump. The monitor is attached to the worker's collar and the cover removed. Air is drawn through the filter at the calibrated rate of 2.5 liters/min and maintained at that rate by occasional checking and adjustment. On termination of sampling, the duration of sampling is noted and the monitor is resealed and returned to the laboratory. A minimum air sample of 250 l should be filtered. This will allow the measurement of at least 1/10 times the TLV, 0.2 mg/m³ (760 Torr, 25°C).

8.4.2 Total particulate loading may be determined, if required, by pre- and post-sampling weighing of the membrane filter.

8.5 **Analysis of Samples**

8.5.1 **Particulate Fluoride.** Carefully remove the membrane filter from the filter holder and place it in a nickel or Inconel crucible containing about 0.5 g borate-carbonate fusion mixture. Transfer any dust from inside the filter cover and retaining ring to the crucible. Drench the filter with ethanol and ignite with a small gas flame. Heat the residue to fusion temperature for 1 to 2 min, then cool and dissolve in a few milliliters of water. Transfer the sample solution into a plastic beaker by means of 25 ml TISAB followed by a rinse of the crucible with a few drops of 1:1 hydrochloric acid. Adjust to pH 8 ± 0.1 by further addition of 1:1 hydrochloric acid. Dilute to 50 ml in a volumetric flask, mix, and bring to standard temperature (25°C). Pour about 20 ml of the solution into a plastic beaker and read the potential while the electrodes are immersed in the gently stirred solution. Convert potential (mv) to fluoride concentration (μg/ml) by means of the calibration chart determined from the standard fluoride series containing borate-carbonate flux (9.3).

8.5.2 **Gaseous Fluoride.** Transfer the impregnated cellulose pad to a 100-ml plastic beaker containing 25 ml water and 25 ml TISAB. Allow the pad to soak for about 30 min with sufficient stirring to reduce it to a pulp. Bring the solution to standard temperature (25°C), insert the electrodes, and read potential of the gently stirred mixture after two minutes. Convert potential (mv) to fluoride concentration (μg/ml) by means of the calibration chart determined from the standard fluoride series (9.5).

9. **Calibration and Standards**

9.1 **Fluoride Standards, Particulates.** Add 1.0 g borate-carbonate fusion mixture to each of four 250-ml beakers containing 10 ml water and 50 ml TISAB. Add various size aliquots (0.1, 1, 5, 10, and 25 ml) of 100 mg/l standard fluoride solution to produce a series of working standards (0.1, 1, 5, 10 and 25 μg F^-/ml). Adjust each solution to pH 8 ± 0.1 with 1:1 hydrochloric acid, transfer to a 100 ml volumetric flask, and dilute to volume with water. These standards may be stored for several months in tightly capped polyethylene bottles, under refrigeration.

9.2 For calibration of the electrode, pour about 20 ml of the working standard solution into a plastic beaker containing a Teflon-coated stirring bar. Adjust solution to within ± 2°C of the 25°C standard temperature. Insert the fluoride and reference electrodes into the constantly stirred solution and record the potential after 2 min. Repeat for each of the working standards.

9.3 Prepare a calibration curve, on three-cycle semi-log paper, relating potential, in millivolts (linear scale), to concentration of fluoride, in μg/ml (log scale). Reproducibility of each point should be ± 1 millivolt. A linear calibration curve is obtained in the 0.1 to 25 μg/ml range, with a slope of between 57 and 59 millivolts per tenfold change in fluoride concentration. If solutions containing less than 0.1 μg/ml are measured, additional standards must be prepared since the calibration curve is not linear at low fluoride concentrations.

9.4 **Fluoride Standards, Gaseous.** Into each of four 250-ml beakers, place 10 ml water and 50 ml TISAB. Add various size aliquots (0.1, 1, 5, 10 and 25 ml) of 100 mg/l standard fluoride solution to produce a series of working standards (0.1, 1, 5, 10 and 25 μg F^-/ml). Adjust each solution to pH 8.0 ± 0.1 with 1:1 hydrochloric acid, transfer to a 100-ml volumetric flask, and dilute to volume with water.

9.5 A separate calibration curve for gaseous fluoride is prepared from potential measurements of these standards (9.4), by the same procedure as used for particulate fluorides (9.2 and 9.3).

10. **Calculations**

10.1 The concentration of particulate fluorides in air, integrated over the sampling period, is calculated as follows:

$$C_p = 0.05 \times \frac{C_1}{V}$$

where:

C_p = concentration of particulate fluoride, in milligrams per cubic meter

C_1 = concentration of fluoride in particulate sample solution, in micrograms per milliliter

V = volume of air sample, in cubic meters, corrected to 25°C and 760 Torr

10.2 The concentration of gaseous fluoride in air, integrated over the sampling period, is calculated as follows:

$$C_G = 0.05 \times \frac{C_2}{V}$$

where:

C_G = concentration of gaseous fluoride, in milligrams per cubic meter

C_2 = concentration of fluoride in gaseous sample solution, in micrograms per milliliter

V = volume of air sample, in cubic meters, corrected to 25°C and 760 Torr

10.3 If desired, gaseous fluoride concentration in air, in milligrams per cubic meter, may be converted to equivalent concentration expressed as parts per million: C_G mg/m³ × 1.29 = C_G ppm.

References

11.1 Elfers, L. A. and C. E. Decker, "Determination of Fluoride in Air and Stack Gas Samples by use of an Ion Specific Electrode", Anal Chem 40:1658, 1968.

11.2 AST, Method D1606-60 (Reapproved 1967), "Standard Method of Test for Inorganic Fluoride in the Atmosphere".

11.3 Weinstein, L. H. and R. H. Mandl, "The Separation and Collection of Gaseous and Particulate Fluorides", VDI Ber No. 164:53-63, 1971.

BIS(CHLOROMETHYL) ETHER (BIS-CME) IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Bis-CME	**Method No.:**	P&CAM 213
Matrix:	Air	**Range:**	0.5 to 10 ppb
Procedure:	Adsorption on Chromosorb 101, desorption with helium through GC column, measurement by mass spectroscopy	**Precision:**	± 10% RSD (Analytical)
Date Issued:	1/1/75	**Classification:**	E (Proposed)
Date Revised:			

1. Principle of the Method

Bis-CME is concentrated from air by adsorption on Chromosorb 101 in a short tube. It is desorbed by heating and purging with helium through a gas chromatography column where it is separated from most interferences. The concentration of bis-CME is measured from the spectroscopic signal at *m/e* 79 and 81.

2. Range and Sensitivity

2.1 The range of the mass spectroscopic signal for the conditions listed corresponds to 0.5 to 10 ppb.

2.2 A concentration of 0.5 ppb of bis-CME can be determined in a 5-liter air sample. Greater sensitivity can be obtained by using a larger sample.

3. Interferences

Interferences resulting from materials having retention times similar to bis-CME or simply background such as ions C_6H_7, $C_5C_1{}^{13}H_6$, C_4H_3Si and Br giving rise to *m/e* 79 can be encountered. However, the DuPont 21-491 mass spectrometer has sufficient resolution to resolve at least three of these ions at *m/e* 79 particularly the $C_5C_1{}^{13}H_6$ and C_6H_7 ions, that are normally encountered in the plant samples. These are completely resolved from the bis-CME $C_2H_4Cl^{35}O$ ion.

4. Precision and Accuracy

4.1 The precision of this method has been determined to be ± 10% of relative standard deviation when different sampling tubes were spiked with 133 ng (corresponding to 1.4 ppb in 20 l of air) at intervals of approximately one hour. These data were obtained using 4 in × ¼ in stainless steel columns packed with 60/80 mesh Chromosorb 101.

4.2 The accuracy of the analysis is approximately ± 10% of the amount reported as determined from repeated analysis of several standards.

5. Advantages and Disadvantages of the Method

5.1 The gas chromatography-mass spectrometry technique interfaced with a Watson-Biemann helium separator is extremely sensitive and specific for the analysis of bis-CME. The gas chromatographic separation yields a retention time that is characteristic for bis-CME, but it is not highly specific for positive assignment of the signal as bis-CME. The mass spectrometer in combination with the gas chromatograph provides the high degree of specificity. The most intense ion in the mass spectrum of bis-CME is formed at *m/e* 79 with its chlorine-37 isotope at *m/e* 81. The chlorine isotopic abundances require that the intensity ratio of peaks at *m/e* 79:81 be approximately 3:1. The mass spectrometer is set to continuously monitor these ions as the sample elutes through the gas chromatograph. It is absolutely necessary that the retention time, observed masses at *m/e* 79 and 81 and the intensity ratio be correct in order to assign the signal to bis-CME.

5.2 Bis-CME, an impurity in chloromethyl methyl ether, has been reported to be a pulmonary carcinogen. Extreme safety precautions should be exercised in the preparation and disposal of liquid and gas standards and the analysis of air samples. Bis-CME may be destroyed in a methanol-caustic solution.

5.3 Sampling must be carried out away from the mass spectrometer.

5.4 GC-MS is not a good approach for continuous monitoring.

6. Apparatus

6.1 Sampling Tubes

6.1.1 The sampling tubes are prepared by packing a 2½ in × ¼ in stainless steel tubing with 1¾ in of 60/80 mesh Chromosorb 101 with glass wool in the ends. These are conditioned overnight at 200°C with helium or nitrogen flow set at 10-30 ml/min. The conditioned sampling tubes are then cooled under flow and capped immediately upon removal. An identification number is scribed on one of the nuts. The stainless steel tubing should be rinsed internally with water, acetone and methylene chloride before packing with Chromosorb 101.

6.1.2 Longer sampling tubes may be prepared with a proportional amount of Chromosorb 101.

6.2 Gas Chromatography Column

A 4 ft × ⅛ in stainless steel separator column is rinsed internally with water, acetone and methylene chloride and air dried. The cleaned tubing is then packed with Chromosorb 101. The column is conditioned overnight at 200°C and 10-30 ml/min helium or nitrogen flow.

6.3 A Watson-Biemann helium separator

6.4 Gas Chromatograph

A Hewlett-Packard 5750 gas chromatograph or equivalent. A gas chromatograph employing a single column oven and a temperature programmer is adequate.

6.5 Mass Spectrometer

A mass spectrometer with a resolution of 1000-2000 and equipped with a repetitive scan attachment can be used in conjunction with a gas chromatograph. A CEC (now DuPont Analytical Instruments Div.) Model 21-491 modified by adding a follower amplifier with a long time constant (0.03 sec.) to the output of the electron multiplier to increase the gain and signal to noise ratio has been found satisfactory for this purpose.

6.6 Syringes

6.6.1 Syringe, 1-ml, gas tight (The Hamilton Co., Inc.).

6.6.2 Syringe, 10-μl (The Hamilton Co., Inc.).

7. **Reagents and Materials**

All reagents must be analytical reagent grade.

7.1 Bis(chloromethyl) ether (Eastman Kodak)

7.2 Acetone

7.3 Dichloromethane

7.4 n-Pentane

7.5 Methanol

7.6 Sodium hydroxide

7.7 Plastic film gas bag, 13-liter. Saran brand (Trademark of The Dow Chemical Company abroad) plastic film gas bag has proven to be satisfactory for this purpose (The Anspec Co., Inc.). Polyethylene film bags are not suitable.

7.8 Chromosorb 101 (Johns-Manville)

8. **Procedure**

8.1 **Cleaning of equipment:** None required.

8.2 **Collection of Samples**

8.2.1 Spot samples may be obtained by attaching the sampling tube to a manual syringe pump. The sample is drawn into the end of the sampling tube with the marked nut.

8.2.2 Continuous sampling of air may be accomplished by attaching a vacuum pump to a throttling valve and manometer. Each sample tube is calibrated so that the pressure drop is determined. Then the flow is established by adjusting the pressure drop with the throttling valve.

8.2.3 Continuous or cumulative samplig valve and calibrated rotameter. The flow is then ing a vacuum pump to a throttlin through the calibrated rotameter. This sampling adjusted with the throttling valve through the calibrated rotameter. This sampling is much preferred to 8.2.2.

8.2.4 For larger sample size, it is important to realize that larger flows of air over a long period of time may cause elution of bis-CME through the sampling tube. It has been shown that a total of 25 l of air can be pumped through a 4 in × ¼ in packing of Chromosorb 101 over a period of 24 hours without any elution of the bis-CME. This corresponds to a flow of 17 cc/min maximum. A maximum air volume of 5 l at a rate of 80 cc/min has been found to be safe for the 2½ in × ¼ in sampling tube.

8.2.5 Bis-CME has been found to be stable in the sampling tubes for at least 10 days when kept tightly closed and at room temperature.

8.3 **Analysis of Samples**

8.3.1 **Instrument Conditions and Set-up.** The mass spectrometer should be set to sweep peaks at *m/e* 79 to 81 with the visicorder speed at 0.8 in/sec for recording the resulting signal.

8.3.2 Hold the temperature of the Watson-Biemann helium separator at 160°C. Set the helium flow at 30 cc/min. Balance the helium flow so that there is no vacuum applied to the outlet of the gas chromatographic column.

8.3.3 Attach the sampling tube to the gas chromatograph via an adapter to the septum nut. The inscribed sample tube nut should be closest to the inlet of the analytical column.

8.3.4 A ¼-in Swagelok Tee fitting is attached on the other end of the sampling tube to accommodate the carrier gas and a silicone rubber septum so that standards may be injected.

8.3.5 After thoroughly checking for leaks, wrap the sampling tube with heat tape and heat to 150°C for 5 min to assure complete desorption of the organic components onto the analytical column which is at ambient temperature.

8.3.6 Temperature program the analytical column to 130°C at 30°C/min and hold to yield a retention time for bis-CME of 8 to 9 min. Turn on recorder 30 sec before elution of bis-CME.

8.3.7 The analytical column should be cooled to ambient temperature before changing sampling tubes.

9. Calibration and Standards

9.1 Preparation of Gas Standard

9.1.1 Rinse out a 13-liter Saran bag with prepurified nitrogen.

9.1.2 Introduce 5 l of prepurified nitrogen through a dry test meter into the Saran bag and cap it with a cork.

9.1.3 Place plastic tape on a Saran bag and inject 1 μl of bis-CME through the tape into the bag. The tape should prevent any cracking of the bag at the injection point.

9.1.4 Tape the injection point at once upon removing the syringe needle from the bag.

9.1.5 Clean the syringe immediately with methanol-caustic solution and then water and acetone.

9.1.6 Knead the bag for approximately 2 min.

9.1.7 Carefully connect the bag with the meter to introduce an additional 5 l of prepurified nitrogen (the end of the Saran tubing inside the bag should be kept tight with a finger from the outside in order to avoid any loss of bis-CME while connecting the bag with the meter).

9.1.8 Knead the bag for additional 2 min to assure complete mixing.

9.1.9 With the 10-liter bag, this procedure can prepare a ppm level standard (e.g., 1 μl of liquid bis-CME yields a 28.3 ppm by volume standard). This standard should not be kept for more than three days.

9.2 Preparation of Liquid Standard

Bis-CME Standard (0.132 μg bis-CME/μl). Dissolve 1 μl of bis-CME liquid in 10 ml n-pentane. This standard solution may be kept for as long as desired if evaporation is avoided.

9.3 Calibration

9.3.1 Inject a 1-ml portion of the 28.3 ppm standard bis-CME (0.132 μg) through a standard septum onto a sampling tube attached to the inlet of the analytical column. This practice may be done routinely after two or three samples to ensure accuracy. Also one μl of the liquid standard (0.132 μg/μl) could be used for the calibration.

9.3.2 Repeat steps as described in Sections 8.3.3-8.3.7.

10. Calculations

10.1 The total ng of bis-CME in the air sampled is determined from the ratio of the peak height of *m/e* 79 of the sample and the standard multiplied by 132.

10.2 Concentration in ppb is calculated as follows:

$$ppb = \frac{\text{ng bis-CME}}{V} \times \frac{24.45}{MW}$$

where:

ng bis-CME = total ng concentration as determined in 10.1
V = volume of air in liters sampled at 25°C and 760 Torr
24.45 = molar volume of an ideal gas at 25°C and 760 Torr
MW = molecular weight of bis-CME, 115

11. References

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11.2 Laskin, S., M. Kuschner, R. T. Drew, V. P. Cauppiello, and N. Nelson, Technical Report, New York University Medical Center, Department of Environmental Medicine, August 1971.

11.3 Leong, K. J., H. N. MacFarland, and W. H. Reese, Arch Environ Health 22:663, 1971.

11.4 Watson, J. T. and K. Biemann, Anal Chem 36:1135, 1964.

11.5 DuPont de Nemour, E. I., Analytical Instrument Division, Monrovia, California.

11.6 Shadoff, L. A., G. J. Kallos, and J. S. Woods, "Analysis for bis-Chloromethyl Ether in Air", Anal Chem 45:2341, 1973.

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595.

LEAD IN AIR OR BLOOD

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Lead	**Method No.:** P&CAM 214
Matrix: Air or blood	**Range:** 0.02 to 0.4 mg/m^3 in a 5-liter sample of air; 0.1 to 2.0 μg/mℓ in a 1-mℓ sample of blood
Procedure: Atomic absorption with a graphite furnace	**Precision** (CV) : 0.057 (analytical)
Date Issued: 1/29/76	**Classification:** E (proposed)
Date Revised:	

1. **Principle of the Method**

   Particulate matter is collected from air on a membrane filter; blood is collected in Vacutainer tubes. The samples are wet-ashed with nitric acid and the residue is taken up in dilute nitric acid. Aliquots are introduced into the graphite furnace and the absorbance is measured at 283.3 nm. Lead is estimated by the method of standard additions.

2. **Range and Sensitivity**

   2.1 The working range is 0.1 to 2 μg of lead, corresponding to 0.02 to 0.4 mg/m^3 in a 5-liter sample of air, or 0.10 to 2.0 μg/mℓ in a 1.0-mℓ sample of blood.

   2.2 The limit of detection of the atomic absorption method is approximately 50 pg for a single sample aliquot delivered to the furnace. For samples with high solids contents, the limit of detection may be larger.

3. **Interferences**

   Flameless atomic absorption is more prone to matrix effects than are flame methods. As a consequence, it is usually necessary to use the method of standard additions, even with digested samples.

4. **Precision and Accuracy**

   4.1 The coefficient of variation of the analytical part of the method, as determined by the analysis of four 5-μℓ aliquots of a single solution, is 0.057.

   4.2 The accuracy of the method has not been determined.

5. Advantages and Disadvantages of the Method

5.1 A major advantage of the graphite furnace method is that it is more sensitive by one or two orders of magnitude than flame atomic absorption. Thus, smaller samples with lower concentrations of lead can be analyzed.

5.2 The main disadvantage is that matrix effects are more prominent than in flame atomic absorption, requiring the use of the method of standard additions. The analysis is, therefore, more time consuming. Also, the equipment is more expensive.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Cellulose ester membrane filters, 0.8-μm pore size, 37-mm diameter, Millipore Type AA, or equivalent.

6.1.2 Filter holder for 37-mm filters, Millipore MAWP 037 AO, or equivalent.

6.1.3 Personal sampling pump, calibrated with a representative filter unit in the line. A wet or dry test meter or a glass rotameter capable of measuring a flow rate of 1.5 ℓ/min to within ± 5% may be used in the calibration.

6.1.4 Thermometer, manometer, and stopwatch.

6.2 Atomic Absorption Spectrophotometer

6.2.1 Atomic absorption spectrophotometer with graphite furnace and tube or rod atomizer. Reproducible control of times and temperatures during the drying, ashing, and atomizing cycles is essential.

6.2.2 Hollow cathode lamp for lead.

6.2.3 Purge gas, high purity argon, in a cylinder equipped with a two-stage, two-gauge pressure regulator and appropriate hose connections. The pressure on the instrument side of the regulator should be 30 psig. Nitrogen cannot be used as the purge gas since cyanogen interferes in the analysis.

6.3 Glassware (borosilicate)

6.3.1 Phillips beakers with watchglass covers, 125 mℓ.

6.3.2 Graduated centrifuge tubes, 15 mℓ.

6.3.3 Volumetric flasks, 10 and 100 mℓ.

6.3.4 Volumetric pipettes, 1 mℓ.

6.3.5 Micropipettes, 10 to 50 μℓ.

6.3.6 Vacutainer tubes, 5 mℓ, heparinized, Becton Dickinson and Company, or equivalent.

6.4 Variable hot plate capable of reaching 140°C.

7. Reagents

All reagents must be at least analytical reagent grade. Water must be doubly distilled or deionized.

7.1 Nitric acid, concentrated, redistilled in glass.

7.2 **Lead Standard Solution "A" (1000 μg/mℓ).** A commercially prepared aqueous lead standard solution may be used; or, the solution may be prepared by dissolving 1.598 g of lead nitrate ($Pb(NO_3)_2$) in 1 liter of 1% (v/v) nitric acid.

7.3 **Lead Standard Solution "B" (10 μg/mℓ).** Transfer 1 mℓ of Standard Solution "A" to a 100-mℓ volumetric flask and dilute to the mark with 5% nitric acid. This solution should be prepared fresh daily and stored in a polyethylene or borosolicate glass container.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 Soak new glassware in chromic acid cleaning solution (saturated solution of sodium dichromate in concentrated sulfuric acid) and rinse thoroughly, first with warm tap water, then with concentrated nitric acid, and finally with double-distilled or deionized water. Clean polyethylene containers by soaking for 24 hr in 5% nitric acid and rinsing thoroughly with distilled or deionized water.

8.1.2 Clean used glassware by soaking in detergent solution and rinsing with warm tap water, distilled water, 5% nitric acid, and finally with double-distilled water.

8.2 Collection and Shipping of Samples

8.2.1 **Air Samples.** With a filter in place, connect the filter holder to the sampling pump and sample the air at 1.5 ℓ/min for 10 min. A 10-min sampling time provides an amount of lead suitable for analyzing a 10-μℓ aliquot when the

599

concentration of lead in air is 0.05 mg/m^3. (See Section 8.3.) A larger sampling time may be used if the sample solution is appropriately diluted before analysis. A blank filter should be submittted with each group of samples; this filter is handled in the the same way as the sample filters except that no air is drawn through it.

8.2.2 **Blood samples.** The samples are collected in Vacutainer tubes according to established procedures.

8.2.3 For shipment to the laboratory, the filters are placed in individual filter holders and the blood samples are packed in a box with foam packing to prevent breakage.

8.3 Analysis of Samples

8.3.1 Remove the filter blanks and filter samples from their containers and place them in 125-mℓ Phillips beakers. For the analysis of blood, pipette a 1.0-mℓ sample into a 125-mℓ Phillips beaker. From this point the procedures are identical for the two types of samples.

8.3.2 Add 2 mℓ of concentrated nitric acid to the sample in the beaker. Cover with a watchglass and heat on a hot plate at 140°C in a hood until nearly dry. Add an additional 1 mℓ of nitric acid and again take to near dryness. Do not allow the residue to ignite.

8.3.3 Transfer the ashed residue quantitatively to a 15-mℓ centrifuge tube by washing several times with small volumes of 1% nitric acid, heating the solution in the beaker after each addition of nitric acid. Cool the solution in the centrifuge tube, dilute it to the 5-mℓ mark with 1% nitric acid, and mix thoroughly.

8.3.4 Remove three 1.0-mℓ portions from the sample solution and place them in small test tubes marked "A," "B," and "C." To tube "A" add an aliquot of lead standard solution (10 μg/mℓ) sufficient to provide an amount of lead equal to that estimated to be present in 1.0 mℓ of sample solution. Then add a volume of double-distilled water equal to the volume of lead standard solution just added. (For example, to 1.0 mℓ of sample containing about 0.2 μg/mℓ of lead, add 20 μℓ of lead standard solution and 20 μℓ of water.) To tube "B" add twice as much lead standard solution as was added to tube "A." To tube "C" add a volume of water equal to the volume of lead standard solution that was added to tube "B." Mix the solutions thoroughly.

8.3.5 Operate the atomic absorption spectrophotometer according to the manufacturer's instructions, using the 283.3-nm resonance line of lead. Automatic background correction is desirable. If it is not available in the instrument used, estimate the nonspecific absorbance by a separate analysis at 283.3 nm, using a deuterium or hydrogen lamp; subtract this value from the sample absorbance. Deliver an aliquot of 5 to 25 µℓ to the graphite furnace with a micropipette and read the absorbance as peak height on a recorder or an electronic peak height readout. Drying, ashing, and atomizing times and temperatures will vary according to the instrument used and other factors but are approximately as follows: dry for 20 sec at 110°C, ash for 15 sec at 400°C, and atomize for 10 sec at 2000°C.

8.3.6 Analyze the "A," "B," and "C" sample solutions prepared as described above in Section 8.3.4. Analyze the least three aliquots of each solution. Also, determine a reagent blank for the amount of nitric acid used in the procedure by direct comparison of the absorbance with the absorbances found for appropriate amounts of lead standards.

9. Calibration and Standards

Since the analysis is performed by the method of standard additions, calibration is an integral part of the analysis.

10. Calculations

10.1 Using high quality, precision graph paper, plot the absorbance values for the sample solutions and the spiked sample solutions against an abscissa labeled "µg of Pb added." The abscissa values to be plotted are the amounts (in µg) of lead added to tubes "A," "B," and "C" (0.0 µg for tube "C"). Draw the best straight line through the three points. The intercept of this line on the negative abscissa is read as the concentration in µg/mℓ of lead in the sample solution (1.0 mℓ of sample solution was analyzed). Alternatively, a least squares calculation may be performed to obtain the intercept value. Subtract the amount of lead found in the reagent blank from the sample value.

10.2 The concentration of lead in air is calculated as follows:

$$\text{mg/m}^3 \text{ (Pb in air)} = \text{µg/mℓ (Pb)} \times \frac{V_{soln}}{V_s}$$

where: µg/mℓ (Pb) = concentration of lead in 1.0 mℓ of solution as read from the graph.

V_{soln} = volume of digested solution, in mℓ. (See Section 8.3.3.)

V_s = volume of air sampled, in liters.

10.3 The concentration of lead in blood is calculated as follows:

$$\mu g/m\ell \text{ (Pb in blood)} = \mu g/m\ell \text{ (Pb)} \times \frac{V_{soln}}{V_s}$$

where: $\mu g/m\ell$ (Pb) = concentration of lead in 1.0 mℓ of solution as read from graph.

V_{soln} = volume of digested solution, in mℓ. (See Section 8.3.3.)

V_s = volume of blood sample, in mℓ.

11. References

11.1 *"Analytical Methods for Atomic Absorption Spectrophotometry,"* Perkin-Elmer Corporation, Norwalk, Conn., 1973.

11.2 *"Analytical Methods for Atomic Absorption Spectrophotometry Using the Graphite Furnace,"* Perkin-Elmer Corporation, Norwalk, Conn., 1973.

11.3 Fernandez, F.J., and D.C. Manning, "Atomic Absorption Analyses of Metal Pollutants in Water Using a Heated Graphite Atomizer," **Atomic Absorption Newsletter**, 10, 65 (1971).

INORGANIC PHOSPHATE IN URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Inorganic phosphate	**Method No.:** P&CAM 215
Matrix: Urine	**Range:** 0.6 to 6 mg/mℓ for a 25-μℓ sample of urine
Procedure: Spectrophotometry	**Precision (CV):** 0.07 (analytical) for a solution containing 60 μg of PO_4^{-3}
Date Issued: 1/29/76	**Classification:** D (Operational)
Date Revised:	

1. Principle of the Method

Inorganic phosphate in urine reacts in an acidic medium with ammonium molybdate and potassium antimonyl tartrate to form an antimony-phosphomolybdate complex. This complex is reduced to a blue complex by ascorbic acid. The absorbance of the solution at 880 nm is proportional to the phosphate concentration.

2. Range and Sensitivity

2.1 The normal range of the method extends from 0.6 mg/mℓ to 6 mg/mℓ of inorganic phosphate (PO_4^{-3}) in urine, corresponding to an absorbance range of approximately 0.075 to 0.75 for a 25-μℓ sample of urine when a 1-cm cell is used to measure the absorbance of the final volume of 50 mℓ of solution. The normal range of inorganic phosphate excreted in urine is 30 to 45 mg/kg of body weight/day. (See Reference 11.1.)Assuming a body weight of 70 kg and a daily urine volume of 1.5 ℓ, a typical concentration of inorganic phosphate is 1.5 to 2.0 mg/mℓ.

2.2 The detection limit is approximately 0.06 mg/mℓ of inorganic phosphate in urine, corresponding to an absorbance of about 0.01.

3. Interferences

3.1 In applications of this method to the analysis of sea and ground water it was shown that no serious interference was caused by Cu(II) at 10 mg/ℓ, Fe(III) at 50 mg/ℓ, silicate at 10 mg/ℓ, or arsenate at 0.02 mg/ℓ. Since arsenate and silicate yield blue complexes similar to that obtained with phosphate, they could interfere at higher concentrations. For example, in cases of exposure to arsenic, urine levels of 0.1 mg/ℓ, or higher, may be encountered; if the arsenic is present in the urine as arsenate, interference can occur. Barium, lead, mercury, and silver can interfere by forming a precipitate. Total salt concentrations up to 20% in sea water cause an error of less than 1% in the determination of phosphate. None of these interferences, however, is expected to be significant in the analysis of urine. (See References 11.2, 11.3, and 11.4.)

3.2 Since organic phosphorus in urine amounts to only about 1% of the total phosphorus (Reference 11.1), the hydrolysis of organic phosphorus compounds may be neglected.

4. **Precision and Accuracy**

From the results of the analyses of 25 standard solutions, each containing 60 μg of inorganic phosphate, the percent error in a measurement was estimated to be less than 1% of the true value and the coefficient of variation was found to be 0.07.

5. **Advantages and Disadvantages of the Method**

5.1 The method is relatively free of interferences.

5.2 This method can be adapted to the determination of phosphorus in water, air, and food products.

5.3 It is necessary to refrigerate samples and reagent solutions, or to use freshly prepared reagent solutions.

6. Apparatus

6.1 A spectrophotometer with the capability for absorbance measurement at 880 nm.

6.2 One-centimeter cuvettes.

6.3 Volumetric flask (50 mℓ) and micropipettes (25 to 500 μℓ).

6.4 A urinometer or suitable hydrometer.

7. Reagents

All reagents should be analytical reagent grade. Solutions should be prepared with distilled water.

7.1 **Sulfuric Acid, 5 *N*.** Dilute 70 mℓ of concentrated sulfuric acid to 500 mℓ.

7.2 **Potassium Antimonyl Tartrate.** Dissolve 1.3715 g of potassium antimonyl tartrate in water and dilute to 500 mℓ. Store in a refrigerator.

7.3 **Ammonium Molybdate.** Dissolve 20 g of ammonium molybdate in 500 mℓ of water. Store in a refrigerator.

7.4 **Ascorbic Acid.** Dissolve 1.76 g of ascorbic acid in 100 mℓ of water. Prepare fresh daily.

7.5 **Mixed Reagent.** To prepare 300 mℓ of reagent, mix 150 mℓ of 5 *N* sulfuric acid and 45 mℓ of ammonium molybdate solution, and then add 90 mℓ of ascorbic acid solution and 15 mℓ of potassium antimonyl tartrate solution. This reagent mixture should be freshly prepared as needed.

7.6 **Phosphate Standard Solution "A" (300 μg PO_4^{-3}/mℓ).** Dissolve 0.4299 g of potassium dihydrogen phosphate in water and dilute to 1 liter. A commercially prepared standard solution of phosphate may be used. Do not mix or store in polyethylene containers since polyethylene absorbs phosphate.

7.7 **Phosphate Standard Solution "B" (30 μg PO_4^{-3}/mℓ).** Transfer 10 mℓ of phosphate standard solution "A" to a 100-mℓ volumetric flask and dilute to the mark with water.

7.8 **Sodium Hydroxide, 10 *N*.** Dissolve 400 g of sodium hydroxide in water, cool, and dilute to 1 liter.

7.9 **Phenolphthalein.** Dissolve 1 g of phenolphthalein in 50 mℓ of alcohol and add 50 mℓ of water.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware should be washed with hot 1:1 hydrochloric acid. Alternatively, the glassware may be filled with concentrated sulfuric acid, allowed to stand for 24 hr, and then rinsed thoroughly with distilled water.

8.2 **Collection and Shipping of Samples.** Collect 5 mℓ of sample in a glass bottle that has been previously cleaned with acid. Measure the specific gravity. If the specific gravity is less than 1.010, discard the sample and obtain another sample from the same person. Acidify each sample with a small volume of nitric or hydrochloric acid sufficient to reduce the pH to below 5. Ship the samples to the laboratory in a suitable container to prevent breakage.

8.3 **Analysis of Samples**

8.3.1 To about 25 mℓ of water in a 50-mℓ volumetric flask add 25 μℓ of urine sample.

8.3.2 Add 25 μℓ of phenolphthalein solution and then add 10 *N* sodium hydroxide until the solution becomes pink. Add 5 *N* sulfuric acid until the pink color disappears.

8.3.3 Add 10 mℓ of the mixed reagent (Section 7.5) and mix thoroughly. Dilute to the mark with water and mix again.

8.3.4 After 30 min, measure the absorbance at 880 nm using a reagent blank in the reference cell.

9. **Calibration and Standards**

9.1 Pipette 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mℓ of phosphate standard solution "B" into 50-mℓ volumetric flasks and proceed as described in Sections 8.3.2 to 8.3.4. Plot absorbance against micrograms of phosphate to obtain the calibration curve.

9.2 Prepare a new calibration curve for each set of samples. Run the standards and a blank along with the samples.

10. **Calculations**

10.1 The amount of phosphate in the sample is read directly from the calibration curve and is corrected for the blank. The concentration of inorganic phosphate in urine is calculated as follows:

$$\text{Phosphate (mg/m}\ell) = \frac{\text{amount in sample } (\mu g)}{\text{volume of sample } (\mu\ell)}$$

10.2 The concentration may be expressed in terms of elemental phosphorus by multiplying the value obtained for phosphate by 0.326:

$$\text{Phosphorus (mg/m}\ell) = \text{phosphate (mg/m}\ell) \times 0.326$$

10.3 The use of a specific gravity factor to normalize values to 1.024, the average specific gravity of urine, has been recommended. (See Reference 11.5.) The preferred method of normalization is to make a straightforward gravity correction as follows:

$$\text{Corrected mg/m}\ell = \text{mg/m}\ell \times \frac{0.024}{\text{specific gravity} - 1.000}$$

Report the uncorrected concentration, the corrected concentration, and the specific gravity of the sample.

11. References

11.1 *"Blood and Other Body Fluids,"* Dittmer, D. S., Editor, Federation of American Societies for Experimental Biology, Washington, D.C., 1971.

11.2 Murphy, J., and J. P. Riley, "A Modified Single Solution Method for the Determination of Phosphate in Natural Waters," Anal. Chim. Acta, 27, 31 (1962).

11.3 *"Methods for Collection and Analysis of Water Samples for Dissolved Minerals and Gases,"* U.S. Geological Survey, 1970.

11.4 *"Methods for Chemical Analysis of Water and Wastes,"* EPA-625/6-74-003, U.S. Environmental Protection Agency, Office of Technology Transfer, Washington, D. C., 1974.

11.5 Elkins, H. B., "Concentration Adjustments in Urinalysis," Am. Ind. Hyg. Assoc. J., 35, 559 (1974).

11.6 Gales, M. E., E. C. Julian, and R. C. Kroner, "Method for Quantitative Determination of Total Phosphorus in Water," J. Am. Water Works Assoc., 8, 1363 (1966).

PHOSPHORIC ACID IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Phosphoric Acid	Method No.: P&CAM 216
Matrix: Air	Range: 0.2 to 4.0 mg/m³ in a 50-liter sample of air
Procedure: Collection on a membrane filter, spectrophotometric determination of phosphate	Precision (CV): 0.043 (analytical) in the range 0.2 to 4.0 mg/m³
Date Issued: 1/29/76	Classification: D (Operational)
Date Revised:	

1. **Principle of the Method**

Particulate matter containing phosphoric acid is collected on a membrane filter. The filter is extracted with water and the dissolved phosphate ion is determined spectrophotometrically by the molybdenum blue method.

2. **Range and Sensitivity**

2.1 The detection limit for the procedure is 2 μg of H_3PO_4 (which gives an absorbance of 0.010), corresponding to 0.2 mg/m³ in a 50-liter sample of air. The sensitivity may be improved by collecting a larger sample.

2.2 The range of the method extends from about 10 to about 200 μg of H_3PO_4, corresponding to 0.2 to 4.0 mg/m³ in a 50-liter sample of air.

3. **Interferences**

Phosphate salts will cause the results for phosphoric acid to be high. Arsenates, if present in sufficiently high amounts, will give high results. No other interferences are to be expected in particulate samples.

4. **Precision and Accuracy**

The recovery of known amounts of phosphate from 28 spiked filters averaged 98.7%. The coefficient of variation was 0.043.

5. **Advantages and Disadvantages of the Method**

The method is relatively simple and precise. There are no important disadvantages.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Cellulose ester membrane filters, 0.8-μm pore size, 37-mm diameter, Millipore Type AA, or equivalent.

6.1.2 Plastic filter holder for 37-mm filters, Millipore MAWP 037 AO, or equivalent.

6.1.3 Personal sampling pump, calibrated with a representative filter unit in the line. A wet or dry test meter or a glass rotameter capable of measuring a flow rate of 1.5 ℓ/min to within ± 5% may be used in the calibration.

6.2 Spectrophotometer, for use at 830 nm, with 1-cm cells.

6.3 Water bath, controlled at 85 to 100°C.

6.4 Volumetric flasks, 50 mℓ and 1 liter.

6.5 Phillips beakers, 125 mℓ.

6.6 Pipettes of various sizes.

7. Reagents

Reagents should be analytical reagent grade and water should be doubly distilled, unless otherwise stated.

7.1 **Phosphate Standard Solution.** Dissolve 0.1389 g of potassium dihydrogen phosphate in water and dilute to 1 liter. This solution contains the equivalent of 100 μg/mℓ of H_3PO_4.

7.2 **Molybdate Solution.** Dissolve 25 g of sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) in 10 *N* sulfuric acid and dilute to 1 liter with 10 *N* sulfuric acid.

7.3 **Hydrazine Sulfate Solution.** Dissolve 1.5 g of hydrazine sulfate in water and dilute to 1 liter.

8. Procedure

8.1 **Cleaning of Equipment.** Wash all glassware with 1:1 HCl, concentrated sulfuric acid, chromic acid, or nitric acid and rinse thoroughly with tap water and distilled water. If phosphate-containing detergents are used for cleaning the glassware, it is especially important to follow the cleaning with warm acid and distilled water rinses.

8.2 **Collection and Shipping of Samples**

8.2.1 With a filter in place, connect the filter holder to the sampling pump and sample the air for 1 hr at 1.5 to 2.0 ℓ/min. The sampling time may be more or less than 1 hr, depending on the concentration of H_3PO_4 expected.

8.2.2 Place the filters in individual filter holders for shipment to the laboratory. The samples are stable for at least 2 weeks.

8.3 **Analysis of Samples**

8.3.1 Remove the filter from its container and place it in a 125-mℓ Phillips beaker. Add 5 mℓ of warm (40-60°C) distilled water, swirl, and decant the water into a 50-mℓ volumetric flask. Repeat this process five times.

8.3.2 Add 5 mℓ of molybdate solution and 2 mℓ of hydrazine sulfate solution to the sample solution in the flask. Dilute to 50 mℓ with water and mix.

8.3.3 Immerse the flask in a boiling water bath for 10 min, remove, and cool rapidly.

8.3.4 Measure the absorbance at 830 nm in a 1-cm cell. Use a reagent blank in the reference cell. If gas bubbles collect on the walls of the cells, release them by gentle tapping.

9. **Calibration and Standards**

9.1 Add 25 mℓ of water to each of seven 50-mℓ volumetric flasks. Pipette known volumes of phosphate standard solution into six of the flasks (0.05, 0.1, 0.2, 0.5, 1.0, and 1.5 mℓ), leaving one as a blank. Continue as described in Section 8.3.2.

9.2 Plot a calibration curve of absorbance *versus* μg of H_3PO_4.

9.3 From the absorbance of the sample solution and the calibration curve, read the amount of H_3PO_4 (in μg) in the sample.

10. **Calculations**

The concentration of H_3PO_4 in air is calculated as follows:

$$\text{mg/m}^3\ (H_3PO_4) = \frac{\mu\text{g of } H_3PO_4 \text{ in sample}}{V_s}$$

where: V_s = volume of air sampled in liters.

11. Reference

Boltz, D.F.,"Total Phosphorus," Chapter 1 in *"Chemical Analysis"* (Elving, P.J., and I.M. Kolthoff, Eds.), Volume 37, *"Analytical Chemistry of Phosphorus Compounds"* (Holmann, M., ed.), Wiley-Interscience, New York, 1972.

BENZENE-SOLUBLE COMPOUNDS IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Benzene Solubles	**Method No.:** P&CAM 217
Matrix: Air particulates trapped on silver membrane and glass fiber filters	**Range:** 20 μg/sample
	Precision (CV): 0.02 (analytical) for 1 mg; 0.23 (analytical)for 20 μg
Procedure: Ultrasonic extraction with benzene, aliquot evaporated to dryness and weighed	**Classification:** D (Operational)
Date Issued: 1/29/76	
Date Revised:	

1. **Principle of the Method**

   Particulate matter suspended in air is collected on a filter. The filter is extracted with benzene with ultrasonic agitation. An aliquot of the extract is evaporated to dryness and the residue is weighed.

2. **Range and Sensitivity**

   The limit of detection is estimated to be 20 μg of benzene-soluble material in a filter sample; this value is two times the standard deviation of values determined for six blank filters. For a 300-liter air sample the limit of detection is, therefore, 0.067 mg/m^3. The range of the method extends upward to any weighable amount greater than 20 μg per sample.

3. **Interferences**

   By definition, the method is inherently free of interferences that could occur in the particulate material collected on a filter. Errors may be caused by extraneous dust picked up in handling the filter or by changes in humidity or temperature during weighing. These errors may be avoided if the sample is properly protected and if temperature and humidity are controlled.

4. Precision and Accuracy

4.1 The analytical precision is dependent upon the amount of residue remaining to be weighed after evaporation of the solvent, as illustrated by the following example. Several benzene extracts of samples of aluminum reduction plant emissions were combined to give a solution containing the equivalent of 1.350 mg of benzene-soluble material per sample; nine aliquots of this solution gave residue weights with a coefficient of variation of 0.02. Benzene extracts of six blank filters gave residue weights with a coefficient of variation of 0.23.

4.2 The accuracy of this method has not been determined.

5. Advantages and Disadvantages

5.1 Ultrasonic agitation has been reported to be more efficient than Soxhlet extraction for dissolving organic materials is particulate samples. For example, the recovery of polycyclic arenes is higher with ultrasonic extraction than with Soxhlet extraction (Reference 11.3).

5.2 A major advantage of the method is that it is simple and direct. The analysis can be made by a technician with minimum training. An experienced technician should be able to analyze a set of 12 samples in 4 hr. If the extraction, evaporation, and weighing are staggered, it should be possible to analyze three sets of 12 samples in an 8-hr day.

5.3 The method is free of interference if temperature and humidity are constant during weighing and if the laboratory atmosphere is dust-free.

5.4 The method is empirical and nonspecific,and measures all substances in a sample that are soluble in benzene. It yields no information on the composition of the benzene-soluble material.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Glass fiber filters, 37 mm, free of organic binder, with an efficiency of at least 99% as determined by the DOP penetration test.

6.1.2 Silver membrane filters, 37 mm, 0.8 μm pore size. Selas Corporation of America, No. FM-37-(0.8), or equivalent.

6.1.3 Disposable plastic filter holder (cassette) for 37-mm filters, Millipore MOOO 037 PO, or equivalent. In order to accommodate the two filters, the cassette must be held together with tape or a shrinkable band.

6.1.4 Personal sampling pump capable of operating for 4 hr at 1.5 ℓ/min. The pump should be calibrated with a representative filter assembly in the line. A wet or dry test meter or a glass rotameter capable of measuring the flow rate of 1.5 ℓ/min to within ± 5% may be used for the calibration.

6.2 **Electrobalance.** Cahn Instruments, Model G-2, or equivalent.

6.3 **Vacuum oven.** Thelco Model 19, GCA Precision Scientific, or equivalent.

6.4 **Ultrasonic bath.** Dynasonics Corporation Model NT-201, 90 kHz, 60 W, or equivalent. An ultrasonic bath operating at a lower frequency and having a higher power output may improve extraction efficiency. (See Reference 11.4.). A test tube support is needed for use in the ultrasonic bath.

6.5 **Test tubes,** 100 mm by 16 mm, with Teflon-lined screw caps.

6.6 **Teflon weighing cups,** Cahn Instruments No. 2034, or equivalent, approximate tare weight, 60 mg.

6.7 Allihn tubes, fine-porosity fritted glass, 30 mℓ.

6.8 Small glass vials with Teflon-lined screw caps.

6.9 Volumetric pipettes, 1 and 5 mℓ.

6.10 Wood applicator sticks, 6 in. long and 1/16 in. diameter.

6.11 Weighing paper, 3.5 by 4.5 in.

6.12 Tweezers.

7. **Reagents**

7.1 Benzene, ACS reagent grade.

7.2 Nitrogen, prepurified.

8. **Procedure**

8.1 **Cleaning of Equipment.** Wash the glassware with detergent solution and rinse with tap water and distilled water. Wash the Teflon weighing cups with reagent grade acetone and allow them to dry.

8.2 Collection and Shipping of Samples

8.2.1 Assemble the filters in the filter holder so that the air being sampled passes first through a glass fiber filter and then through a silver membrane filter. Remove the small plugs from the filter holder and connect the filter holder to the sampling pump by means of an adaptor and a length of tubing. Sample at least 300 liters of air at 1.5 ℓ/min. The optimum sample volume will depend on the type of workroom environment being sampled. Since high concentrations of particulate material or certain types of mists may plug the filters, the flow rate should be checked about once every hour. On completion of sampling, reinsert the small plugs in the inlet and outlet of the filter holder.

8.2.2 With each group of samples prepare at least three blanks consisting of a filter holder with representative filters that have been handled in the same manner as the sample filters, except that no air is drawn through them. The samples and the blanks should be shipped promptly in a light-proof container to prevent photooxidation or damage during transit.

8.3 Analysis of Samples The glass fiber and silver membrane filters are extracted together in the same test tube. At least three sets of blank filters are extracted along with each group of samples.

8.3.1 Extraction

1. Remove the top portion of the filter holder. Hold the bottom portion containing the filters over a piece of weighing paper to catch any particulate material that may fall out. Remove the small plug from the bottom portion of the filter holder and insert an applicator stick through the hole. Gently raise the filters and grasp the unexposed edge with tweezers. Fold the filters in half and then lengthwise so that they can be inserted into a test tube. Push the folded filters to the bottom of the test tube with the applicator stick. Add to the test tube any particulate material remaining in the filter holder or collected on the weighing paper.

2. Pipette 5.0 mℓ of benzene into the test tube. This amount of solvent should completely cover the filters. Be careful during the subsequent extraction and filtering procedures to avoid significant loss of benzene by evaporation.

3. Place the test tubes in the test tube support in the ultrasonic bath so that the level of water in the bath is above the level of the solvent. Turn on the ultrasonic bath and sonicate the sample for 5 min.

4. Decant the solvent into an Allihn tube and collect the filtrate in a small vial. Force the solvent through the Allihn filter under positive nitrogen pressure. This may be accomplished by connecting the nitrogen cylinder to a glass "T," leaving one arm of the "T" open and connecting the other arm to a No. 4 one-hole rubber stopper, which is pressed against the top of the Allihn tube; pressure is controlled with a finger placed against the open arm of the "T." When the filtration has been completed, close the vial tightly with a Teflon-lined screw cap.

8.3.2 Gravimetric Determination

1. Transfer 1.0 mℓ of the benzene solution to a tared Teflon cup. Evaporate the solvent in a preheated vacuum oven at 40°C and 200 torr and continue drying for a total of 2 hr. The residue should be visibly dry in about 1 hr and in 2 hr all of the benzene will have been removed.

2. Equilibrate the sample for at least 30 min in a weighing room where the temperature and humidity are kept constant. Determine the weight of the residue to the nearest microgram. Because of the small weight difference being measured, it is critical that the temperature and humidity be the same when the residue is weighed as when the empty Teflon cup is weighed.

9. Calibration and Standards

No calibration or standards are required for this method.

10. Calculations

The result of this analysis is expressed as the weight (in mg) of benzene-soluble material in a filter sample. The weight of the residue from a 1.0-mℓ aliquot of sample extract is corrected by subtracting the average weight of the residue from aliquots of the blank-filter extracts. This corrected weight is multiplied by 5 to obtain the total amount of benzene-soluble material in the sample.

11. References

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11.5 Richards, R.T., D.T. Donovan, and J.R. Hall, Am. Ind. Hyg. Ass. J., 28, 590 (1967).

PHOSGENE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	Phosgene ($COCl_2$)	**Method No.:**	P&CAM 219
Matrix:	Air	**Range:**	0.005 to 5 ppm (0.02 to 20 mg/m³)
Procedure:	Midget impinger/ nitrobenzylpyridine, colorimetric	**Precision:**	Unknown
Date Issued:	6/1/75		
Date Revised:		**Classification:**	E (Proposed)

1. **Principle of the Method**

   4,4'-Nitrobenzyl pyridine in diethyl phthalate reacts with traces of phosgene to produce a brilliant red color. The addition of an acid acceptor such as N-phenylbenzylamine stabilizes the color and increases the sensitivity. The absorbance is determined at 475 nm (11.1).

2. **Range and Sensitivity**

   Sampling efficiency is 99% or better. Five micrograms of phosgene can be detected; the minimum sample size is 25 liters. Therefore, 0.02 mg/m³ phosgene should be detectable with a 250-liter sample. 20 mg/m³ upper limit should be within the range of most laboratory photometers for a 25-liter air sample.

3. **Interferences**

   3.1 Other acid chlorides, alkyl and aryl derivatives which are substituted by active halogen atoms, and sulfate esters are known to produce color with this reagent. However, most of these interferences can be removed in a prescrubber containing an inert solvent such as "Freon-113" cooled by an ice bath.

   3.2 This method is not subject to interference from likely concentrations of chloride, hydrogen chloride, chlorine dioxide or simple chlorinated hydrocarbons such as carbon tetrachloride, chloroform, and tetrachlorethylene. A slight depression of color density has been noted under high humidity conditions.

4. **Precision and Accuracy**

   The accuracy and precision of this method have not been determined. Calibration is a major problem because of the reactivity of phosgene. Flow dilution systems can be used but require anhydrous air for dilution. Permeation-type calibration standards may be employed, if available.

5. **Advantages and Disadvantages of the Method**

   5.1 Advantages include high sensitivity, standard laboratory equipment, and relative simplicity.

   5.2 Disadvantages include potential interferences, relative changes in color formation with various lots of reagents and the need for frequent calibration checks.

6. **Apparatus**

6.1 Midget impingers

6.2 Vacuum source, aspirator or pump

6.3 Flow meter (wet test meter)

6.4 Polyethylene bottles and beakers

6.5 Bausch and Lomb Spec 20, or equivalent photometer

6.6 Associated laboratory glassware and tubing. No plasticized tubing (such as Tygon) should be used.

7. **Reagents**

7.1 4,4'-nitrobenzyl pyridine (Aromil Chemical Co., Baltimore, Md., or Aldrich Chemical Co., Milwaukee, Wis.).

7.2 N-phenylbenzylamine (ACS reagent grade or better).

7.3 Diethylphthalate (special selection may be required since some lots of the phthalate produce unstable color).

7.4 Color Reagent. Reagent is made up of a solution of 2.5 g 4,4'-nitrobenzyl pyridine, 5 g N-phenylbenzylamine and 992.5 g diethyl phthalate. The concentration of color forming reagents in diethyl phthalate is critical as less color is developed with either a decreased or increased concentration.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware and plastic ware are to be doubly rinsed with isopropanol (IPA) and dried. If IPA rinse(s) does not thoroughly clean apparatus, use standard glassware cleaning procedures before IPA rinses.

8.2 **Collection of Samples**

8.2.1 Atmospheric samples are collected in midget impingers loaded with 10 ml of reagent prepared as described in Section 7.4 above.

8.2.2 A calibrated sample rate of 1 liter per minute is used.

8.2.3 A known volume of about 50 liters is drawn through the impingers for a range of 0.04 to 1 ppm phosgene. (For ranges above 1 ppm, 25 liters should be drawn.)

8.2.4 The final solution volume is recorded and the sample transferred to a small polyethylene or glass bottle or directly to the cuvette.

8.2.5 The red color formed is stable for at least 4 hr, but should be measured within 9 hr. A 10%-15% loss in color density may occur after 8 hr.

8.3 **Analysis of Samples**

8.3.1 The sample is transferred to a cuvette for the photometric measurement at 475 nm.

8.3.2 The photometer should be "zeroed" (at 100% T or 0 absorbance) with a matched cuvette containing unreacted reagent at the 475 nm wavelength.

8.3.3 The absorbance (or transmittance) of the sample is measured with the photometer at the 475 nm wavelength.

9. **Calibration and Standards**

Phosgene is highly reactive, and standards are difficult to prepare. However, dynamic standards may be prepared from a flow dilution system using a standard cylinder of 1000 ppm phosgene in nitrogen. (Lower concentrations of phosgene in cylinders are generally unstable. In any case, the "standard" cylinder concentration should be checked periodically by mass spectrometric or other methods.) A double dilution system with $COCl_2$-free air to dilute the phosgene about 1000/1 should be used. Permeation-type calibration technique can be employed if phosgene standard permeation tubes are available.

10. **Calculations**

10.1 A calibration curve should be prepared of absorbance at 475 nm versus the phosgene concentration in air with 50 liters of gas sample.

10.2 For a gas sample of volume X, the $COCl_2$ concentration as determined by the calibration curve should be multiplied by $\frac{50}{X}$ for the corrected value.

11. **References**

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11.2 Linch, A. L., S. S. Ford, K. A. Kubitz, and M. R. DeBrunner, "Phosgene in Air — Development of Improved Detector Procedures", Am Ind Hyg Assoc J 26:465, 1965.

11.3 Noweir, M. H. and E. A. Pfitzer, "The Determination of Phosgene in Air", Unpublished paper presented at the American Industrial Hygiene Conference in Philadelphia, Pennsylvania on April 28, 1964.

CHLOROMETHYL METHYL ETHER (CMME) AND BIS(CHLOROMETHYL) ETHER (BCME) IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	CMME and BCME	**Method No.:**	P&CAM 220
Matrix:	Air	**Range:**	0.5—7.5 ppb (CCME or BCME)
Procedure:	Collection via impinger, GC-electron capture detector	**Precision:**	10% RSD
Date Issued:	6/27/75		
Date Revised:		**Classification:**	E (Proposed)

1. **Principle of the Method**

   1.1 A known volume of air is drawn through glass impingers containing a methanolic solution of the sodium salt of 2,4,6-trichlorophenol.

   1.2 CMME and BCME react with the derivatizing reagent to produce stable derivatives.

   1.3 Sample is heated on a steam bath for 5 min, cooled, diluted with an equal volume of distilled water and 2 ml of hexane for extraction.

   1.4 An aliquot of the hexane is analyzed by electron capture gas chromatography.

   1.5 The peak heights of the CMME and BCME derivatives are measured and the concentration determined from standard curves.

2. **Range and Sensitivity**

   2.1 A relative standard deviation of 10% can be expected within the concentration range of 0.02-0.3 ng/μl of both CMME and BCME.

   2.2 The sensitivity of the method is 0.5 ppb (v/v) when a 10-liter air sample is used.

3. **Interferences**

   3.1 The known components used in chloromethylation processes do not interfere with the determination of CMME or BCME.

   3.2 Interferences can be expected from highly halogenated organic compounds or compounds that may produce same derivatives.

   3.3 The quality of 2,4,6-trichlorophenol is important since impurities can be extracted with hexane and seriously interfere with the chromatographic analysis.

4. **Precision and Accuracy**

   4.1 The precision of the sampling technique with an accurate, calibrated air sampling pump and analysis of the derivative samples is 10% relative standard deviation.

4.2 The accuracy of the method is typically affected by the efficiency in sampling, extraction, calibration and data handling.

5. Advantages and Disadvantages of the Method

5.1 The major advantage of this method is the sensitivity and the simultaneous analysis of both CMME and BCME.

5.2 The derivative stabilizes the CMME and BCME while significantly increasing the sensitivity.

5.3 Preparation of the derivatives eliminates further hazardous handling of both CMME and BCME during the analysis.

5.4 Disadvantages of the method are the handling of liquids, extractions and the dilution of the samples by the hexane extraction.

6. Apparatus

6.1 Sampling Equipment

The sampling unit for the impinger collection method consists of the following components:

6.1.1 Two standard air impinger assemblies, with fritted glass inlets.

6.1.2 Calibrated battery-powered pump, capable of drawing an accurate and reproducible volume of air through the impingers at a flow rate of 0.5 l/min is required.

6.1.3 Rotameter, calibrated for air. Parts should include only the sapphire ball and small Teflon tubing, inside at both ends.

6.1.4 Thermometer.

6.1.5 Stopwatch.

6.2 Steam bath. Any bath capable of maintaining a temperature of 65-90°C is adequate.

6.3 Gas chromatograph, equipped with a ^{63}Ni electron capture detector.

6.4 Gas chromatograph column. A 6-foot (1.83 m) × ¼-inch (6.35 mm) glass column is packed with 100/120 mesh textured glass beads (GLC-100) coated with a two component stationary phase consisting of 0.1% by weight QF-1 and 0.1% by weight OV-17. The column is equipped for on-column injection. The packed column is preconditioned at 160°C overnight with nitrogen carrier at a flow rate of 30-50 cm^3/min.

6.5 Strip chart recorder, 1.0 mv full scale range.

6.6 Hamilton microsyringes.

6.7 Assorted laboratory glassware, pipettes, graduated cylinders, etc.

7. Reagents

All reagents are analytical reagent grade. An analytical grade of distilled water must be used.

7.1 Derivatizing reagent, prepared in the laboratory as described in the procedure.

7.2 Sodium methoxide, AR grade.

7.3 2,4,6-Trichlorophenol, M.P. 67-68°C.

7.4 Methanol and hexane, distilled in glass.

7.5 Chloromethyl methyl ether, B.P. 55-58°C.

7.6 Bis(chloromethyl) ether, B.P. 100-102°C.

7.7 Sodium hydroxide.

7.8 Preparation of derivatizing reagent. Twenty-five grams of sodium methoxide is weighed into a beaker and dissolved in one liter of methanol. Five grams of 2,4,6-trichlorophenol is weighed into the beaker and allowed to dissolve in the methanol-sodium methoxide solution. The reagent is stable for three or four weeks when stored in a dark brown bottle. The sodium methoxide should be added slowly to the methanol since the reaction is very exothermic.

7.9 The sensitivity of BCME can be increased 6-8 fold by using stoichiometric quantities of sodium methoxide and 2,4,6-trichlorophenol (16.0 g of 2,4,6-trichlorophenol and 4.4 g of sodium methoxide in one liter of methanol). When using this formulation, 2.0 N sodium hydroxide is used in place of distilled water prior to the hexane extraction of the derivative (see Section 8.3).

8. Procedure

8.1 **Cleaning of Equipment.** All glassware used for the analysis must be thoroughly washed, rinsed with distilled water and dried. The impinger assemblies can be rinsed with reagent grade methanol for repeated use.

8.2 **Collection of Samples**

8.2.1 CMME and BCME in air are sampled at a rate of 0.5 l/min (up to 2 hr, if necessary) through the two impinger assemblies each containing 10 ml of the derivatizing solution.

8.2.2 Teflon connections should be used for the attachment of the two impinger assemblies in series and the rotameter to the first impinger. Rubber tubing may be used for the connection of the second impinger to the intake of the pump.

8.2.3 A bypass flow of air can be established in case the pump does not operate at low flow rates. An appropriate syringe needle injected into the rubber tubing between the impinger and the intake of the pump should provide satisfactory flow rates. Also a needle valve connected through a T should produce adjustable flow rates.

8.3 **Analysis of Samples**

8.3.1 After sampling, the solutions are transferred to a vial that is capped loosely and placed on a steam bath for five minutes (any bath capable of maintaining a temperature of 65-90°C is suitable). The samples are allowed to cool and an equal amount of distilled water and 2 ml of hexane are pipetted into the vial. Then the sample is shaken for 5 min. The mixture is allowed to stand for a few minutes to allow the phases to separate.

8.3.2 **GC conditions.** The following are the recommended starting instrumental conditions. A gas chromatograph with a ^{63}Ni electron capture detector is equipped with a 6-foot long (1.83 m) by ¼ inch (6.35 mm) glass column packed with 100-120 mesh textured glass beads (GLC-100) coated with a two component stationary phase consisting of 0.1% by weight QF-1 and 0.1% by weight OV-17. The column is equipped for on-column injection. The flow rate of the prepurified nitrogen carrier gas is set at 30 cm^3/minute. The temperature of the sample injection zone is adjusted at 175°C and that of the detector at 250°C. The column oven is operated isothermally at 140°C. An oxygen filter is required on the carrier gas.

8.3.3 **Injection.** A 2-μl aliquot of the hexane extract is injected into the GC. A complete chromatogram should be obtained in about 6 min. Duplicate injections of each sample and a standard should be made.

9. Calibration and Standards

9.1 Preparation of Standard Curve

9.1.1 Two microliters of CMME and BCME are added to 50 ml of hexane. The weights of the components are obtained by using their respective specific gravities, 1.03 g/ml for CMME and 1.33 g/ml for BCME. This concentrated standard is then used for preparing a standard curve. Both of these compounds should be handled in a good hood only!!

9.1.2 Ten ml of the derivatizing reagent (Section 8.3) is pipetted into five 20-dram Kimble screw cap vials. Ten, five, two, one and zero microliters of the concentrated standard are added. These volumes are equivalent to 0.50, 0.25, 0.10, and 0.05 μg of bis-CME and 0.40, 0.20, 0.08 and 0.04 μg of CMME.

9.1.3 The vials are capped loosely and placed on a steam bath for 5 min. The standard is cooled and 10 ml of distilled water and 2 ml of hexane are pipetted into the vials. Then the standards are shaken for 5 min.

9.2 Standard curves are established by plotting the peak height of recorder response versus concentration in nanograms.

10. Calculations

10.1 Determine from the calibration curve the concentration of the desired component in nanograms.

$$\text{Component (ng)} = \frac{A \times B}{C}$$

where:

A = response for the component of interest in the sample
B = weight of CMME or BCME in the standard expressed in nanograms
C = response for the component of interest in the standard

10.2 The concentration of CMME and BCME in the sampled atmosphere can be calculated in ppb (v/v).

$$\text{ppb (v/v)} = \frac{D \times 24.45}{V_s \times MW}$$

where:

D = total ng concentration as determined in Section 10.1
24.45 = molar volume of an ideal gas at 25°C and 760 Torr
V_s = volume of air sampled in liters at 25°C and 760 Torr
MW = molecular weight of CMME and BCME, 80.5 and 115, respectively.

11. Reference

11.1 Solomon, R. A. and G. J. Kallos, Anal Chem 47:955, 1975.

ALIPHATIC AMINES IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Aliphatic Amines	**Method No.:** P&CAM 221
Matrix: Air	**Range:** 1 to 2400 mg/m³ in a 10-liter sample of air
Procedure: Adsorption on silica gel; elution with acid; GC analysis	**Precision** (CV_T): 0.03 for methylamine at 300 mg/m³
Date Issued: 1/29/76	**Classification:** E (Proposed)
Date Revised:	

1. **Principle of the Method**

1.1 A known volume of air is drawn through a tube containing silica gel to trap the aliphatic amines. The silica gel is transferred to glass-stoppered tubes and treated with sulfuric acid. A portion of the resulting acid solution is made alkaline with an excess of sodium hydroxide and an aliquot of the alkaline solution is analyzed by gas chromatography with a flame ionization detector.

1.2 The method may be used to determine a single aliphatic amine or to determine two or more amines in a single sample. The method has been applied to the following individual compounds:

Methylamine
Ethylamine
Dimethylamine
Isopropylamine
Butylamine
Diethylamine
Diisopropylamine
Triethylamine
Cyclohexylamine

2. **Range and Sensitivity**

2.1 The limit of detection of the method is 0.01 mg of amine (0.02 mg of methylamine) per sample when the analyte is desorbed from 150 mg of silica gel with 2 mℓ of sulfuric acid and a 3-$\mu\ell$ aliquot of the alkaline mixture is analyzed. This limit corresponds approximately to 1 mg/m³ (2 mg/m³ for methylamine) in a 10-liter sample of air.

2.2 The upper limit is at least 24 mg of amine per sample. This amount of methylamine is retained by the 500-mg section of silica gel in the sorbent tube before breakthrough occurs. Larger amounts of other amines of higher molecular weight are retained. This limit for methylamine corresponds approximately to 2400 mg/m^3 in a 10-liter sample of air.

3. Interferences

3.1 The most common possible sampling interference is water vapor. With the sorbent tube described, 96 liters of air of 100% relative humidity can be sampled over an 8-hr period at 200 mℓ/min without displacement of the collected aliphatic amines by water vapor.

3.2 Any compound which has nearly the same retention time on the GC column as one of the aliphatic amines is an interferent. This type of interference can often be overcome by changing the GC operating conditions or by selecting another column. Retention time data on a single column, or even on a number of columns, cannot be considered as conclusive proof of chemical identity. It is important, therefore, that a sample of the bulk amine or mixture of amines be submitted at the same time as the sample tubes so that chemcial identification of possible interferents can be made. (The bulk sample must *not* be shipped in the same shipping container as the sample tubes.)

4. Accuracy and Precision

4.1 The volume of air sample can be measured to within ±1% if a pump with a calibrated volume indicator is used. Volumes calculated from initally set flow rates may be less accurate (± 5%) because of changes in flow rate during sampling.

4.2 The collection efficiency for aliphatic amines is esentially 100% under most conditions, as demonstrated by the negligible amount of amine collected in the backup sections of the sorbent tube.

4.3 The higher molecular weight amines and small amounts of all amines are incompletely desorbed. When a correction is made for desorption efficiency as described in Section 8.5, the accuracy approaches 100%.

4.4 Storage losses of 16-25% have been observed for primary amines on silica gel in sealed tubes stored for 1 month. Storage losses of 6-7% have been observed for secondary amines over 1 month. Triethylamine was stable for this period. Refrigeration of samples decreases such losses and is recommended if analysis is not to be performed within a few days.

4.5 The precision of the analysis is dependent upon the precision and sensitivity of the technique used to quantitate the gas chromatographic peaks of samples and standards. Electronic digital integrators with baseline correction capabilities will maximize analytical precision, particularly at lower concentrations. For the lower concentrations (0.01 to 0.2 mg/sample) the analytical precision may be ±20%; at higher concentrations it approaches ±3%.

4.6 A coefficient of variation of 0.03 has been determined for the analysis of 10 samples of 1.2 mg of methylamine collected from 4 liters of air (300 mg/m^3) with a personal sampling pump. The recovery was 99%.

5. Advantages and Disadvantages

5.1 The sampling method uses a small, portable device involving no liquids. The sorbent tube can be used for at least 8 hr to measure an average workday concentration, or for only 15 min to measure an excursion concentration. Desorption of the sample and preparation for analysis are simple. The analysis is accomplished by a rapid instrumental method.

5.2 Most analytical interferences can be eliminated by altering the GC conditions.

5.3 Several aliphatic amines can be collected and determined simultaneously. This is especially useful when the identity and composition of the amine vapors are not known.

5.4 The major disadvantage of the method is the necessity for measuring desorption efficiencies. When the desorption is less than complete, as for higher molecular weight amines, the effect of the amount of compound absorbed must also be determined.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 **Sorbent tubes.** The sorbent tubes consist of Pyrex glass tubes 125 mm long and 8 mm i.d., packed with three separate sections of 45/60-mesh activated silica gel. The weights of the three sections of silica gel are, in order, 600, 150, and 150 mg; these tubes are designed for sample flow in either direction. Plugs of 100-mesh stainless steel screen are used to contain the silica gel sections. These plugs of negligible pressure drop are prepared from 7-mm discs of screen held in place by Teflon rings. Pieces of Pyrex tubing 12 mm long and 7 mm in o.d. are located between the sorbent sections to inhibit migration of the amines. The ends of the tubes are flamesealed after packing to prevent contamination before use for sampling. The pressure drop across the tubes does not exceed 6 cm of water at a flow rate of 200 mℓ/min. The silica gel should be the equivalent of Silica Gel D-08, Chromatographic Grade, Activated and Fines Free, 45/60-mesh, as supplied by Applied Science Laboratories, Inc., State College, Pa. Polyethylene caps should be provided to seal the tubes after sampling has been completed.

6.1.2 **Personal Sampling Pump.** The personal sampling pump should be capable of operation at a constant flow rate or 200 mℓ/min for up to 8 hr. A sample volume indicator is desirable. The pump should be calibrated with a representative sorbent tube in the line. A wet or dry test meter or a glass rotameter capable of measuring a flow rate of 200 mℓ/min to within ±5% may be used in the calibration.

6.2 Gas chromatograph with a flame ionization detector and linear temperature programming.

6.3 A 1.8-m by 4-mm silanized glass column packed with 60/80-mesh Chromosorb 103 (Johns-Manville), or equivalent.

6.4 A 15-cm by 4-mm silanized glass precolumn packed with 10 cm of 8/20-mesh Ascarite (Arthur H. Thomas Company), or equivalent, and fitted into the injection port. The precolumn should be replaced when the precision of the analysis becomes poor.

6.5 Recorder with an electronic digital integrator.

6.6 Glass-stoppered test tubes or flasks.

6.7 Glass syringes, 10 μℓ.

6.8 Pipettes and volumetric flasks for preparation of standard solutions.

7. **Reagents**

7.1 Sulfuric acid, 1.0 *N*.

7.2 Sodium hydroxide, 1.1 *N*.

7.3 Aliphatic amines, highest purity available (for use as standards).

7.4 Helium, Bureau of Mines Grade A.

7.5 Hydrogen, prepurified.

7.6 Air, compressed and filtered.

8. **Procedure**

8.1 **Cleaning of Equipment.** Wash all glassware with detergent solution and rinse with tap and distilled water. Dry in an oven.

8.2 Collection and Shipping of Samples

8.2.1 Immediately before beginning the collection of a sample, break each end of the sorbent tube so as to provide openings at least half the inside diameter of the tube.

8.2.2 Choose the direction desired for sample flow, mark the inlet end of the tube with a permanent marker, and attach the other end to the sampling pump. For low concentrations of amines, low humidity conditions, or short sampling periods, pump the sample air through the 150-mg sorbent section first; for high concentrations, high humidity, or long sampling periods, pump the sample air through the 600-mg sorbent section first. Sample air must not pass through any hose or tubing before entering the sorbent tube.

8.2.3 With the sorbent tube in a vertical position, sample the air at 200 mℓ/min for the desired period of time (0.25 to 8 hr). The flow rate and sampling time, or the volume, must be measured as accurately as possible. The temperature and pressure of the air being sampled should be measured and recorded.

8.2.4 Immediately after sampling is completed, cap the sorbent tubes with the polyethylene caps provided. Rubber caps must not be used.

8.2.5 One tube should be handled in the same manner as the sample tubes (break, seal, and ship) except that no air is pumped through it. Label this tube as a blank.

8.2.6 Pack the tubes tightly before shipping to minimize breakage in transit.

8.2.7 After the sample is collected the tubes should not be subjected to extremes of high temperature or low pressure. Refrigeration will reduce sample loss.

8.3 Analysis of Samples

8.3.1 **Preparation of Samples.** Remove and discard the stainless steel plugs and glass spacers and transfer each section of silica gel to a separate glass-stoppered test tube or flask. Label the samples and analyze each section separately.

8.3.2 **Desorption.** Desorb the amines from the silica gel by adding 2 mℓ of 1.0 *N* sulfuric acid to the 150-mg sections and 8 mℓ to the 600-mg section. Shake the sample mixtures occasionally over a period of 1 hr. Tests have indicated that desorption reaches a maximum in an hour.

8.3.3 **Neutralization.** Transfer a 0.5-mℓ aliquot to another container and add 0.5 mℓ of 1.1 *N* sodium hydroxide to make the solution alkaline and regenerate the free amines. An aliquot of this solution is then analyzed by gas chromatography.

8.3.4 **Gas Chromatographic Conditions.** Typical operating conditions for the gas chromatograph are as follows:

Helium carrier gas flow rate, 120 mℓ/min.

Hydrogen gas flow rate to detector, 40 mℓ/min.

Air flow rate to detector, 540 mℓ/min.

Injection port temperature, 160°C.

Detector temperature, 190°C.

Column temperature, 115 to 180°C at 10°C/min; hold for 10 min.

For a sample containing only one amine or a sample containing two closely eluting amines, the column temperature may be kept isothermal at an appropriate temperature.

8.3.5 **Injection of Sample.** To eliminate difficulties arising from blowback or distillation within the syringe, use the solvent flush injection technique. Flush the 10-μℓ syringe several times with distilled water to set the barrel and plunger. Draw in 3 μℓ of water. With the needle removed from the water, pull the plunger back about 0.2 μℓ to draw in a pocket of air. Then immerse the needle in the sample solution and withdraw a 3-μℓ aliquot, taking into consideration the volume in the needle. After the needle is removed from the sample solution and prior to injection, pull the plunger back a short distance to minimize evaporation at the tip of the needle. Inject duplicate 3-μℓ aliquots of each sample and standard.

8.3.6 **Measurement of Peak Area.** Measure the area of each amine peak with an electronic digital integrator or other suitable means of area measurement. Estimate the amount of each individual amine from calibration curves prepared as described in Section 9.

8.4 **Determination of Desorption Efficiency**

8.4.1 **Importance of Determination.** Desorption efficiency for a particular compound can vary from one lot of silica gel to another, and also from one laboratory to another. Therefore, it is necessary to determine at least once the desorption efficiency for each amine with each lot of silica gel used. Among the aliphatic amines listed in Section 1.2 desorption efficiency decreases with increasing molecular weight. Desorption efficiency increases with increasing ratio of sulfuric acid to silica gel; however, an increase in this ratio results in a reduction in analytical sensitivity. A compromise ratio of 75 mg of silica gel per milliliter of sulfuric acid is recommended. Desorption efficiency may also vary with the amount of amine present on the silica gel. Measurements of efficiency should, therefore, be made for at least two amounts within the normal range of sample size.

8.4.2 **Procedure for Determination of Desorption Efficiency.** Place 150 mg of silica gel in a 2-mℓ glass-stoppered tube. The silica gel must be from the same lot as that used in collecting the sample; it can be obtained from unused sorbent tubes. With a microliter syringe, inject a known amount of the amine, either pure or in water solution, directly onto the silica gel. Close the tube with the glass stopper and allow it to stand at least overnight to insure complete adsorption of the amine. Prepare at least three tubes for each of at least two different amounts of amine. These tubes are referred to as samples. Prepare a blank in the same manner, omitting the amine. Analyze the samples and blank as described in Section 8.3. Also analyze three standards prepared by adding identical amounts of the amine to 2.0 mℓ of 0.1 *N* sulfuric acid. Determine the concentrations of the amine in the blank, samples, and standards using calibration curves prepared as described in Section 9. The desorption efficiency is calculated by dividing the concentration of amine found in the sample by the concentration obtained for the corresponding standard.

9. Standards and Calibration

9.1 For accuracy in the preparation of standards, it is recommended that an initial standard be prepared in a relatively large volume and at a high concentration. Aliquots of this standard can then be diluted to prepare other standards. Prepare the initial standard by pipetting an appropriate volume of amine into a 100-mℓ volumetric flask and adding distilled water to the mark. Check the concentration of this solution by titrating with standard sulfuric acid. Make the first dilution by adding an equal volume of 2.0 *N* sulfuric acid to an aliquot of the initial standard. Make additional dilutions with 1.0 *N* sulfuric acid so that the final concentration of sulfuric acid will be the same in standards and samples.

9.2 For example, to prepare butylamine standards add 1.0 mℓ of butylamine to a 100-mℓ volumetric flask and dilute to the mark with water. The concentration of this initial standard is 7.39 mg/mℓ or 0.101 *M*. This concentration can be easily checked by titration with 0.100 *N* sulfuric acid. Dilute a 25-mℓ aliquot of the initial standard with 25 mℓ of 2.0 *N* sulfuric acid to give a second standard of 3.695 mg/mℓ in 1.0 *N* sulfuric acid. Dilute 1.0 mℓ of this second standard to 25 mℓ with 1.0 *N* sulfuric acid to give a concentration of 0.148 mg/mℓ. This concentration in 1.0 *N* sulfuric acid is comparable to that obtained by sampling butylamine in air at its TLV concentration (15 mg/m^3) on 600 mg of silica gel for 6 hr at 200 mℓ/min and desorbing into 8 mℓ of 1.0 *N* sulfuric acid; if the desorption efficiency is 100%, the resulting concentration of butylamine is 0.135 mg/mℓ. Various volumes of the second standard (3.695 mg/mℓ) should be diluted to 25 mℓ to give a series of working standards covering the range of interest.

9.3 The standards prepared as described above should be analyzed under the same gas chromatographic conditions and during the same time period as the unknown samples. This will minimize the effect of day-to-day variations in the response of the flame ionization detector.

9.4 A calibration curve is prepared for each amine by plotting average peak area against concentration of the standard solution in mg/mℓ (mg of amine per mℓ of 1.0 *N* sulfuric acid).

10. Calculations

10.1 From the calibration curve read the concentration of the amine corresponding to the average peak height measured. Multiply this concentration value by the volume of 1.0 *N* sulfuric acid used to desorb the amine from the silica gel (8 mℓ for 600 mg of silica gel, or 2 mℓ for 150 mg). The result is the weight of amine (in mg) in the silica gel section. Correct each value for the amount of amine found in the corresponding blank. Also, correct each value for the desorption efficiency as determined in Section 8.4.2. Add the corrected amounts of amine found in the front and backup sections of the same sample tube to obtain the total weight of amine in the air sample.

10.2 The concentration of the amine in air may be expressed in mg/m^3:

$$\text{mg/m}^3 = \frac{\text{corrected weight (mg)}}{\text{volume of air sampled (ℓ)}} \times 1000\ \text{ℓ/m}^3$$

10.3 The concentration may also be expressed in terms of parts per million (ppm) by volume:

$$ppm = mg/m^3 \times \frac{24.45}{M.W.} \times \frac{760}{P} \times \frac{T+273}{298}$$

where: 24.45 = molar volume (ℓ/mole) at 25° C and 760 mmHg.

M.W. = molecular weight.

P = pressure (mmHg) of air sampled.

T = temperature (°C) of air sampled.

11. References

11.1 Campbell, Evan E., G.O. Wood, and R.G. Anderson, "Development of Air Sampling Techniques," Los Alamos Scientific Laboratory, Progress Reports LA-5634-PR (June 1974), LA-5973-PR (July 1975), and LA-6057-PR (September 1975).

11.2 Andre, C.E., and A.R. Mosier, "Precolumn Inlet System for the Gas Chromatographic Analysis of Trace Quantities of Short-Chain Aliphatic Amines," Anal. Chem., 45, 1971 (1973).

11.3 Wood, G. O., and R. G. Anderson, "Development of Air Monitoring Techniques Using Solid Sorbents," Los Alamos Scientific Laboratory, Progress Reports LA-6216-PR (February 1976) and LA-6513-PR (September 1976).

ZINC OXIDE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Zinc oxide	**Method No.:** P&CAM 222
Matrix: Air	**Range:** 1 to 80 mg/m^3 in a 25-liter sample of air
Procedure: X-Ray diffraction	**Precision** (CV): 0.15 (analytical) at 1 mg/m^3; 0.05 at > 2 mg/m^3
Date Issued: 1/29/76	**Classification:** E (Proposed)
Date Revised:	

1. **Principle of the Method**

Particulate matter is collected from air on a membrane filter and analyzed directly for crystalline zinc oxide by X-ray diffraction (XRD). An XRD scan over a wide angular range detects interferences as well as zinc oxide peaks. The integrated count from XRD step scanning over one of the intense zinc oxide peaks is compared to those of external standards for quantitative determination.

2. **Range and Sensitivity**

2.1 The range of the method is from 5 to 500 μg of zinc oxide per square centimeter of filter surface. This corresponds to 25 to 2000 μg of zinc oxide on a 25-mm filter, or 1 to 80 mg/m^3 in a 25-liter sample of air.

2.2 The detection limit has not been established.

3. **Interferences**

The following compounds may interfere with the measurement of the indicated zinc oxide peak:

0.2476 nm (Cu Kα, 2θ= 36.26°)	0.2816 nm (Cu Kα, 2θ= 31.75°)
α-Fe_2O_3	$Zn(NH_3)_2Cl_2$
$(NH_4)_3ZnCl_5$	$(NH_4)_2ZnCl_4$
$(NH_4)_2Zn(SO_4)_2 \cdot 6H_2O$	$(NH_4)_2Zn(SO_4)_2 \cdot 6H_2O$
Zn	$(NH_4)_3ZnCl_5$

Many other compounds may interfere; their presence must be determined for each sample.

4. Precision and Accuracy

4.1 The coefficient of variation for the analytical measurement is about 0.15 for a filter loading of 6 $\mu g/cm^2$ (1 mg/m^3 in air), and is less than 0.05 for a filter loading of 10 $\mu g/cm^2$ or more (2 mg/m^3 or more in air). These values are based on measurements with pure zinc oxide.

4.2 In a comparison of this method with atomic absorption spectrometry, zinc was determined by both methods in 15 filters containing added zinc oxide in the range of 250 to 1000 μg. The average percent difference in 15 pairs of results was 2.7%.

5. Advantages and Disadvantages of the Method

5.1 The method is straightforward and simple to execute. It is sensitive, precise, and nondestructive.

5.2 The method has certain disadvantages that may affect its accuracy. Since it uses an external standard rather than an internal standard, the mass absorption coefficient of the sample is not easily measured and is necessarily ignored. This presents no difficulty if filter loadings are kept below 500 $\mu g/cm^2$ and Cu Kα radiation is used.The problem is less critical with more penetrating radiation. If the zinc oxide particles are very small, they may penetrate well into the filter before being trapped. The filter material will absorb some of the radiation, causing an error for which a correction cannot be made.

5.3 An additional disadvantage is that the equipment is relatively expensive.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Acrylonitrile-polyvinyl chloride membrane filters, 0.8-μm pore size, 25 mm, Gelman Instrument Company Type DM800, or equivalent. Almost any membrane filter can be used for X-ray diffraction analysis provided that the pore size is small enough to trap the smaller particles and consideration is given to suitability for sampling and inertness to isopropanol. The filter must have a low X-ray background at the analytical wavelengths if very low levels of zinc oxide are to be determined. The Gelman Type DM filters are transparent to infrared radiation and thus permit the use of infrared absorption methods for the determinations of zinc oxide.

6.1.2 Filter holder (or cassette) for 25-mm filters, Gelman No. 1107, or equivalent.

6.1.3 Personal sampling pump, calibrated with a representative filter unit in the line. A wet or dry test meter or a glass rotameter capable of measuring a flow rate of 1.5 ℓ/min to within ± 5% may be used in the calibration.

6.1.4 Thermometer, manometer, and stopwatch.

6.2 An X-ray diffraction unit with a Cu tube, post-beam monochromator, and appropriate sample holders.

6.3 Vacuum filtration apparatus, suitable for depositing standard suspensions of zinc oxide on 25-mm membrane filters. A side-arm vacuum flask fitted with a filter funnel (Gelman Product No. 1112, or equivalent) may be used.

6.4 Ultrasonic bath or probe.

6.5 Small forceps for handling filters.

6.6 Glass bottles with ground-glass stoppers and short necks, 1 liter.

6.7 Pipette, serological, various sizes.

6.8 Pipetting bulb.

7. **Reagents**

7.1 Zinc oxide, ACS reagent grade. The average particle size should be greater than 0.5 μm and less than 10 μm.

7.2 Isopropanol, reagent grade.

8. **Procedure**

8.1 **Cleaning of Equipment.** Wash all glassware and plastic ware in detergent solution, rinse with tap water and distilled water, and air-dry.

8.2 **Collection and Shipping of Samples.** With a filter in place, connect the filter holder to the sampling pump and sample the air at 1.5 ℓ/min for a period of time estimated to yield a deposit of 25 to 2000 μg of zinc oxide on the filter. Record the volume of air sampled. It is necessary that the sample be collected on the same type and size of filter that will be used for depositing standards. The filters should be shipped in the filter holders.

8.3 Analysis of Samples

8.3.1 **Operating Conditions.** The following operating conditions are recommended for the X-ray diffractometer: Cu tube, 40 kV, 20 mA, 1° divergence slit, 4° receiving slit, postbeam monochromator.

8.3.2 **Qualitative Scan.** To identify possible interfering peaks, scan each sample over a wide angular range, for example, 2θ = 5° to 70° for Cu·Kα. Choose an interference-free peak of zinc oxide for step scanning.

8.3.3 **Step Scan.** Establish the calibration curves and perform the analyses of the samples by step scanning over the 101 (0.2476 nm) or 100 (0.2816 nm) peaks. The 2θ angles of these peaks for Cu Kα radiation are 32.26° and 31.75° respectively. The angular range to be scanned should be determined by the operator. For the operating conditions given in Section 8.3.1, a scan range of 2° centered on the peak has been used for the larger peak (101) and 1.5° for the smaller peak (100). For narrower optics or for other conditions, the scan ranges will have to be chosen accordingly. The step scanning procedure should be the same for the standards and samples.

Determine the integrated count accumulated during the step scan and subtract the background count. The method of computing the integrated count minus background will depend upon the data system available. Determine the quantity of zinc oxide in the sample from the appropriate calibration curve.

9. Calibration and Standards

9.1 Preparation of Standards

9.1.1 Place approximately 10 mg of zinc oxide on a sheet of weighed glassine paper and determine its exact weight. Transfer the zinc oxide to a 1-liter glass-stoppered bottle. Measure 1 liter of isopropanol in a volumetric flask and use a portion of it to wash the last traces of zinc oxide from the weighing paper into the bottle. Add the remainder of the isopropanol to the bottle. Label the bottle with the exact concentration of zinc oxide suspension.

9.1.2 In a similar manner, prepare another standard suspension containing approximately 50 mg of zinc oxide per liter.

9.1.3 Disperse the aggregates by the use of an ultrasonic probe or bath. The treatment time will depend on the power available but 5 to 10 min is usually sufficient.

9.1.4 Mount a filter on the vacuum filtration apparatus. Do not apply vacuum. Vigorously shake the standard zinc oxide suspension and place it on a magnetic stirrer. Withdraw an aliquot with a pipette, placing the tip of the pipette near the side of the bottle away from the vortex and approximately halfway to the bottom of the suspension. Draw up the suspension to the mark corresponding to the aliquot chosen. Do not adjust the volume by expelling part of the suspension back into the bottle. If more than the desired aliquot is drawn into the pipette, return all of the suspension to the bottle and begin again.

9.1.5 Transfer the aliquot from the pipette to the filter. Keep the tip of the pipette near the surface of the filter but not submerged in the suspension. Avoid leaving any zinc oxide high on the side walls of the filtering funnel. As soon as the pipette has drained, apply the vacuum and rapidly pull the alcohol through the filter. If the vacuum is left on for about a minute after the alcohol has been removed, the filter will be sufficiently dry. Do not wash down the sides of the funnel after the zinc oxide deposit is in place since this will rearrange the material on the filter. Transfer the filter to the sample mount that is to be used in the diffractometer.

9.1.6 Prepare a series of standards in the manner described above. Use the 10 mg/ℓ-suspension and 2.5-, 5.0-, 7.5-, 10.0-, 15.0-, and 20.0-mℓ aliquots to prepare 25-, 50-, 75-, 100-, 150-, and 200-μg standards. Use the 50 mg/ℓ-suspension and 5.0-, 10.0-, 20.0-, and 40.0-mℓ aliquots to prepare 250-, 500-, 1000-, and 2000-μg standards. Calculate the exact weights of zinc oxide deposited on each filter from the concentrations of the standard suspensions as determined in Sections 9.1.1 and 9.1.2.

9.2 **Calibration Curve.** Plot integrated count minus background against micrograms of zinc oxide on the filter to obtain the calibration curve.

10. Calculations

Determine the weight of zinc oxide in each filter sample from the calibration curve. Divide the weight of zinc oxide (mg) found by the volume of air sampled (m^3) to obtain the concentration of zinc oxide in air in mg/m^3.

11. Reference

Haartz, J.C., M.L. Bolyard, and M.T. Abell, "Quantitation of Zinc Chloride and Zinc Oxide in Airborne Samples," paper presented at the American Industrial Hygiene Conference, Minneapolis, Minnesota, June, 1975.

CADMIUM IN BLOOD

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Cadmium	**Method No.:** P&CAM 223
Matrix: Blood	**Range:** 0.002 to 1 μg/mℓ in 1 mℓ of blood
Procedure: Anodic stripping voltammetry	**Precision (CV) :** 0.05 (analytical) at 0.05 μg/mℓ
Date Issued: 4/15/73	**Classification:** E (Proposed)
Date Revised: 1/29/76	

1. **Principle of the Method**

A sample of whole blood is wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The residue is dissolved in an acetate buffer solution and the buffered sample solution is analyzed by anodic stripping voltammetry (ASV) with a composite mercury-graphite electrode (CMGE). In principle, a hanging mercury drop electrode (HMDE) can also be used. However, optimum analysis parameters may vary significantly and must be established for each electrode system. The method as presented is designed for use with the CMGE.

2. Range and Sensitivity

2.1 The sensitivity is limited by the reagent blank. A reasonable detection limit appears to be two times the standard deviation of the reagent blank. Statistically, the probability is 95% that cadmium is present in the sample if the signal is higher than this limit. The scatter in the reagent blank produces a standard deviation of 1 ng. The detection limit is therefore estimated to be 2 ng, which corresponds to 0.002 μg/mℓ in a 1-mℓ sample.

2.2 The linear range of the electrode response is estimated to extend from the detection limit to 1000 ng of cadmium, which corresponds to 1 μg/mℓ in a 1-mℓ sample. The range may be extended by analyzing a smaller blood sample or by diluting the sample digestate with acetate buffer and taking an aliquot of the diluted solution for the ASV determination.

3. Interferences

3.1 Thallium forms a shoulder on the anodic edge of the cadmium peak. However, the thallium peak does not affect the height of the cadmium peak until thallium and cadmium are present in roughly the same concentrations. If the presence of a

significant concentration of thallium is suspected, as in a sample from the blood of workers in certain ore smelting or roasting industries, the sample should be analyzed for thallium. Thallium is determined by making the digested sample solution 0.01 *M* in EDTA (ethylenediaminetetraacetic acid) and analyzing the resulting solution by ASV at the same instrument settings as those recommended for the cadmium determination. A typical response factor for thallium is 4 ng/μA when the sample plating time is 30 min. Since the presence of EDTA prevents the ASV response to cadmium but does not affect the response to thallium, the peak height obtained for thallium alone may be subtracted from the peak height obtained for both cadmium and thallium to estimate the response due to cadmium alone.

3.2 Incomplete digestion of a sample, indicated by a yellowish coloration of the sample, may lead to peak broadening and anomalous results.

4. Precision and Accuracy

4.1 Several processes determine the reproducibility of the overall analytical method: (1) sampling, (2) shipping, (3) preparation of samples, and (4) analysis. The variation introduced by sampling blood at industrial sites has not been evaluated. It has been found that precision is not significantly affected by the shipping of standard blood samples that have been ultrasonically mixed prior to shipping and are shipped in heparinized Vacutainer tubes (Becton, Dickinson, and Co.). The variations introduced by the preparation and analysis steps may be estimated for the entire analytical range from the following empirical relation:

$$CV = \frac{0.001}{[Cd]} + 0.03$$

where: CV = the estimated coefficient of variation of a measurement neglecting any contribution from sampling.

[Cd] = the measured concentration of cadmium (in μg/mℓ) in a 1-mℓ sample.

The factor 0.001 in the numerator of the first term of the right-hand side of this expression reflects the scatter in the reagent blank, which corresponds to a standard deviation of 1 ng in a 1-mℓ sample, or 0.001 μg/mℓ. The second term represents the normal statistical variation in the preparation and analysis steps. (See References 11.1 and 11.2.)

4.2 The accuracy of the overall sampling and analytical method has not been evaluated. However, it is estimated that the percent error in a measurement, neglecting any contribution from sampling and shipping, is less than about 10% in the determination of levels near 50 ng of cadmium in 1-mℓ blood samples, which corresponds to concentrations near 0.05 μg/mℓ.

5. **Advantages and Disadvantages of the Method**

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 One disadvantage is that the current-voltage peaks for thallium and cadmium are not resolved when their concentrations are comparable.

6. **Apparatus**

6.1 Vacutainer tubes (5 mℓ), heparinized, low-lead content; Becton, Dickinson, and Co., or equivalent. Care must be taken in the selection of Vacutainer tubes since the caps of many types contain significant amounts of cadmium.

6.2 Model W-140-E Sonifier, Heat Systems–Ultrasonics, Inc., or an equivalent ultrasonic mixer with a microprobe tip having a diameter no larger than 0.2 in.

6.3 Pyrex test tubes (20 x 100 mm) for digesting blood samples and subsequent use as analysis cells.

6.4 Digestion racks for the analysis cells.

6.5 Micro pipettes (2, 5, 10, 20, and 500 μℓ, and 1 mℓ), Eppendorf or equivalent.

6.6 Model SA 2011 Single-Cell Anodic Stripping Voltammeter with composite mercury-graphite electrode, Environmental Sciences Associates, Inc., Burlington, Mass., or equivalent. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations. Instructions for the preparation and reconditioning of the CMGE are included in the instruction manual.

6.7 Potentiometric recorder (100-mV range).

6.8 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric flasks, volumetric pipettes, and graduated pipettes of appropriate sizes.

7. **Reagents**

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in cadmium. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 Acid Ashing Solution. Prepare the acid ashing solution by adding 24 volumes of concentrated nitric acid to 24 volumes of perchloric acid (70%) and 1 volume of concentrated sulfuric acid.

7.5 **Acetate Buffer Solution (1 *M* NaAc, 0.2 *M* NaCl, pH 4.4).** To prepare 1 liter of this reagent, dissolve 82 g of sodium acetate, 58.5 g of sodium chloride, and 56 mℓ of 70% $HClO_4$ in water in a 1-liter volumetric flask and fill to the mark with water. Remove trace metals from the resulting solution electrolytically with a system such as the Model 2014 PM Reagent Cleaning System, Environmental Sciences Associates, Inc., or equivalent. The acetate buffer maintains the pH of the sample solution near 4.4, a pH favorable for cadmium determination. The chloride improves the resolution of the lead and cadmium peaks and reduces the effect of pH variations on the determination of cadmium.

7.6 **Cadmium (Cd(II)), Standard Solution "A" (10 mg/mℓ).** Transfer 2.0315 g of cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) to a clean 100-mℓ volumetric flask. Dissolve the salt in approximately 50 mℓ of water, add 2 mℓ of concentrated nitric acid, and dilute to the mark with water.

7.7 **Cadmium (Cd(II)), Standard Solution "B" (100 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "A" to a 100-mℓ volumetric flask containing 1 mℓ of concentrated nitric acid and dilute to the mark with water.

7.8 **Cadmium (Cd(II)), Standard Solution "C" (1 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "B" to a 100-mℓ volumetric flask containing 1 mℓ of nitric acid and dilute to the mark with water. Prepare fresh daily to avoid loss of cadmium or contamination.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this step is to remove any cadmium from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 220 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the hot plate for a period of 60 to 90 min, remove, and allow them to cool.

Complete decontamination requires four repetitions of this procedure. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom. Allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water.

8.2 **Collection and Shipping of Samples.** The samples are collected in Vacutainer tubes according to established procedures. The samples should be shipped to the laboratory in the sampling containers in a box with a minimum of 0.25 in. of foam packing to prevent breakage.

8.3 Analysis of Samples

8.3.1 Rinse the ultrasonic mixer tip thoroughly in distilled deionized water. Place the tip into a blood sample within 2 cm of the bottom of the Vacutainer tube, and mix at the maximum permissible power for 20 to 30 sec. If a sample in a Vacutainer tube sits for more than 10 min after it has been ultrasonically mixed, remix it before taking an aliquot for analysis. Pipette 1 mℓ of the blood into an analysis cell. Rinse the pipette once with distilled deionized water and add the rinse to the analysis cell. Duplicate 1-mℓ aliquots of the blood sample should be taken for analysis.

8.3.2 Into the analysis cell containing 1mℓ of blood, pipette 1.5 mℓ of the acid ashing solution.

8.3.3 Place the cell in a digestion rack on a hot plate operating at 220 to 240°C. Carry out the digestion in a hood. The sample will initially turn yellow but will clear in about 10 to 20 min. A reflux line will be established about halfway up the cell. At the end of 30 min, there will be approximately 100 to 200 μℓ of acid left in the bottom of the cell, with most of the nitric acid having evaporated. The digestion should be continued for an additional 70 to 100 min until the acid is almost completely evaporated.

8.3.4 Remove the cell from the hot plate, cool it, and add 5 mℓ of the acetate buffer solution.

8.3.5 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the buffered solution under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -1000 mV
- Auto-hold cutoff potential, 1050 mV
- Plating time, 15 to 30 min. (The plating time for samples and standard must be the same.)
- Sweep rate, +60 mV/sec
- Current range, 0.1 to 2 mA (full scale)

The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of cadmium plated on the electrode may occur. As the sample is stripped, the cadmium peak (-720 mV) will occur first, followed by a lead peak (-530 mV). A baseline is drawn by connecting with a straight line the flat portions of the curve before and after the cadmium peak. The peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions: (1) reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solutions of cadmium in acetate buffer; (4) blood standards prepared as described in Section 9.1. The sequence in which these four kinds of solutions are analyzed is as follows: (1) a set of standard solutions of cadmium in acetate buffer; (2) sample solutions, with reagent blanks and blood standards interspersed at the rate of one blank and one blood standard for every ten samples; and (3) a second set of standard solutions of cadmium in acetate buffer. The standard solutions of cadmium in acetate buffer are used for calibration; the results for the samples are corrected for the amount of cadmium found in the reagent blank; and the blood standards are used to determine the efficiency of recovery of known amounts of cadmium from blood. The procedure described is for use with a four-cell ASV unit.

9.1 Preparation of Blood Standards

9.1.1 A "normal" blood sample from an individual not suspected of exposure to cadmium is analyzed by the method of standard additions. One-milliliter samples of the blood are placed in each of five Vacutainer tubes. Known amounts of cadmium are added to four of the tubes (2-, 5-, 10-, and 20-μℓ aliquots of standard solution "C," containing 2, 5, 10, and 20 ng of cadmium, respectively). These samples are ultrasonically mixed, transferred to analysis cells, digested, and analyzed by ASV according to the procedure given in Section 8.3. The concentration of cadmium in the unspiked "normal" blood is then estimated.

9.1.2 Finally, an amount of standard cadmium solution is added to 1.0 mℓ of unspiked "normal" blood to make the total concentration of cadmium equal to 50 ng/mℓ. This standard blood solution is subsequently analyzed along with calibration standards and blanks (Section 9.3) to determine the percent recovery of cadmium.

9.2 **Initial Analysis of Four Standards.** Four standards of 50 ng of cadmium, prepared by adding 50 μℓ of standard solution "C" to 5.0 mℓ of acetate buffer solution, should be analyzed first. Each of the four standards should be analyzed with a different electrode. At the level of 50 ng, the contributions from blanks should be negligible, and a single point may be taken to describe the calibration curve for each electrode, which has been determined to be linear through 1 μg.

9.3 **Analysis of Samples and Controls.** With every ten samples, a blood standard (Section 9.1.2) and a reagent blank are analyzed by the ASV procedure given in Section 8.3. At least one reagent blank and one blood standard should be analyzed with each electrode that is used for analysis of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 50 ng of cadmium will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA. The overall response factor is the amount of cadmium in each standard (50 ng) divided by the average of the eight peak heights (in μA). However, it will be necessary to calculate an individual response factor for an electrode if the average of the two peak height values for an individual electrode differs from the overall average of the eight peak heights by more than 10%. For example, if the average for the eight peak heights is 90 μA, and the average of the two values for one of the electrodes is 79 μA, then the response factor to be used for that electrode is 50 ng/79 μA, or 0.64 ng/μA. The overall response factor in this example is 50 ng/90 μA, or 0.56 ng/μA.

10. Calculations

10.1 The amount of cadmium in a sample is calculated from the cadmium peak height (in μA), as measured according to Section 8.3.5, and the response factor in the following manner:

Amount (in ng) = peak height (in μA) x response factor (in ng/μA)

Any amount of cadmium found in the reagent blank should be subtracted from the sample value to give the correct amount (in ng) of cadmium in the sample. The average recovery of cadmium from blood standards is usually greater than 90%. However, it may be necessary to include a factor in the above equation to correct for incomplete recovery.

10.2 The concentration of cadmium in a blood sample may be expressed as $\mu g/m\ell$ of cadmium in blood:

$$\mu g/m\ell = \frac{\text{corrected weight (in ng) of cadmium in sample}}{\text{volume (in m}\ell\text{) of blood x 1000 (ng/}\mu g\text{)}}$$

11. References

11.1 Environmental Sciences Associates, Inc., Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Division of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

11.2 Matson, W.R., R.M. Griffin, and T.J. Sapienza, "A Total System and Quality Control Procedure for Micro Blood Lead Analysis by Anodic Stripping Voltammetry (ASV)," presented at the Laboratory Director's Conference, Department of Health, Education, and Welfare, Center for Disease Control, August 23, 1974.

CADMIUM IN URINE

Physical and Chemical Analysis Branch

Analytical Method

Analyte: Cadmium	**Method No.:** P&CAM 224
Matrix: Urine	**Range:** 1 to 1000 μg/ℓ in 1mℓ of urine
Procedure: Anodic stripping voltammetry	**Precision (CV):** 0.05 (analytical) at 200 μg/ℓ
Date Issued: 4/15/73	**Classification:** E (Proposed)
Date Revised: 1/29/76	

1. **Principle of the Method**

A sample of urine is wet-ashed with a mixture of nitric, perchloric, and sulfuric acids. The residue is dissolved in an acetate buffer solution and the buffered solution is analyzed by anodic stripping voltammetry (ASV) with a composite mercury-graphite electrode (CMGE). In principle, a hanging mercury drop electrode (HMDE) can also be used. However, optimum analysis parameters may vary significantly and must be established for each electrode system. The method as presented is designed for use with the CMGE.

2. **Range and Sensitivity**

2.1 The sensitivity is limited by the reagent blank. A reasonable detection limit appears to be two times the standard deviation of the reagent blank. Statistically, the probability is 95% that cadmium is present in the sample if the signal is higher than this limit. Reagent blanks for cadmium are usually 1 ± 0.5 ng. By the stated criterion, the detection limit is 1 ng, which corresponds to 1 μg/ℓ in a 1-mℓ sample of urine.

2.2 The linear range of the electrode response is estimated to extend from the detection limit to 1000 ng of cadmium, which corresponds to 1000 μg/ℓ in a 1-mℓ sample of urine. The range may be extended to 5000 μg/ℓ by taking a 200-μℓ aliquot of the original urine sample and digesting it in 300 μℓ instead of 1 mℓ of the acid ashing solution. (See Section 8.3.1.)

3. **Interferences**

3.1 Thallium forms a shoulder on the anodic edge of the cadmium peak. However, the thallium peak does not affect the height of the cadmium peak until thallium and cadmium are present in roughly the same concentration. If the presence of a significant concentration of thallium is suspected, as in a sample from the urine of workers in

certain ore smelting or roasting industries, the sample should be analyzed for thallium. Thallium is determined by making the digested sample solution 0.01 *M* in EDTA (ethylenediaminetetraacetic acid) and analyzing the resulting solution by ASV at the same instrument settings as those recommended for the cadmium determination. A typical response factor for thallium is 4 ng/μA when the plating time is 30 min. Since the presence of EDTA prevents the ASV response to cadmium but does not affect the response to thallium, the peak height obtained for thallium alone may be subtracted from the peak height obtained for both cadmium and thallium to estimate the response due to cadmium alone.

3.2 Incomplete digestion of a sample, indicated by a yellowish coloration, may lead to peak broadening and anomalous results.

4. Precision and Accuracy

4.1 Several processes determine the reproducibility of the overall analytical method: (1) sampling, (2) shipping, (3) preparation of samples, and (4) analysis. The variations introduced by sampling urine in industrial plants have not been evaluated. It has been determined that precision is not significantly affected by the shipping of urine in polyethylene bottles. The variations introduced by the preparation and analysis steps may be estimated for the entire analytical range from the following empirical relation:

$$CV = \frac{0.5}{[Cd]} \times 0.05$$

where: CV = the estimated coefficient of variation of a measurement neglecting any contribution from the sampling or shipping.

[Cd] = the measured cadmium concentration (in μg/ℓ) in a 1-mℓ sample.

The factor 0.5 in the numerator of the first term of this expression reflects the scatter in the reagent blank, which corresponds to a standard deviation of 0.5 ng in a 1 mℓ sample, or 0.5 μg/ℓ. The second term represents the normal statistical variations in the preparation and analysis steps. (See References 11.1 and 11.3.)

4.2 The accuracy of the overall sampling and analytical method has not been evaluated. However, it is estimated that the percent error in a measurement, neglecting any contribution from sampling and shipping, is less than about 10% in the determination of levels near 200 ng of cadmium in 1-mℓ urine samples, which corresponds to concentrations near 200 μg/ℓ.

5. Advantages and Disadvantages of the Method

5.1 The primary advantages of the method are its high sensitivity and wide range.

5.2 Another advantage is that the analysis is less expensive in terms of equipment than some other methods.

5.3 One disadvantage is that the current-voltage peaks for thallium and cadmium are not resolved when their concentrations are comparable.

6. Apparatus

6.1 Polyethylene bottles (180 mℓ) with screw caps.

6.2 A urinometer (or hydrometer capable of measuring specific gravities in the range of 1.000 to 1.040 with an uncertainty of only ± 0.001 units).

6.3 Pyrex test tubes (20 x 100 mm) for digesting urine samples and subsequent use as analysis cells.

6.4 Digestion racks for the analysis cells.

6.5 Micro pipettes (10, 50, 100, 200, and 500 μℓ, and 1 mℓ), Eppendorf or equivalent.

6.6 Model SA 2011 Single-Cell Anodic Stripping Voltammeter with composite mercury-graphite electrode, Environmental Sciences Associates, Inc., Burlington, Mass., or equivalent. Instruments with more than one analysis cell are available and are recommended for large numbers of routine determinations. Instructions for the preparation and reconditioning of the CMGE are included in the instruction manual.

6.7 Potentiometric recorder (100-mV range).

6.8 Assorted laboratory glassware, including beakers, graduated cylinders, volumetric flasks, volumetric pipettes, and graduated pipettes of appropriate sizes.

7. Reagents

All reagents must be at least analytical reagent grade. The acids used in digestion must be of even greater purity to provide a reagent blank low in cadmium. Solutions should be prepared with doubly distilled or distilled deionized water.

7.1 Nitric acid, concentrated (doubly distilled).

7.2 Perchloric acid, 70% (doubly distilled).

7.3 Sulfuric acid, concentrated (doubly distilled from Vycor).

7.4 Acid Ashing Solution. Prepare the acid ashing solution by adding 327 volumes of concentrated nitric acid to 653 volumes of perchloric acid (70%) and 20 volumes of concentrated sulfuric acid.

7.5 Acetate Buffer Solution (1 *M* NaAc, 0.2 *M* NaCl, pH 4.4). To prepare 1 liter of this reagent, dissolve 82 g of sodium acetate, 58.5 g of sodium chloride, and 56 mℓ of 70%

$HClO_4$ in water in a 1-liter volumetric flask and fill to the mark with water. Remove trace metals from the resulting solution electrolytically with a system such as the Model 2014 PM Reagent Cleaning System, Environmental Sciences Associates, Inc., or equivalent. The acetate buffer maintains the pH of the resulting sample solution near 4.4, a pH favorable for cadmium determination. The chloride improves the resolution of lead and cadmium peaks in the analysis procedure and reduces the effect of pH variations on the determination of cadmium.

7.6 **Cadmium (Cd(II)), Standard Solution "A" (10 mg/mℓ).** Transfer 2.0315 g of cadmium chloride ($CdCl_2.2.5H_2O$) to a clean, 100-mℓ volumetric flask. Dissolve the salt in approximately 50 mℓ of water, add 2 mℓ of concentrated nitric acid, and dilute to the mark with water.

7.7 **Cadmium (Cd(II)), Standard Solution "B" (100 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "A" to a 100-mℓ volumetric flask containing 1 mℓ of concentrated nitric acid and dilute to the mark with water.

7.8 **Cadmium (Cd(II)), Standard Solution "C" (1 μg/mℓ).** With a 1-mℓ volumetric pipette, transfer 1 mℓ of standard solution "B" to a 100-mℓ volumetric flask containing 1 mℓ of concentrated nitric acid and dilute to the mark with water.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 All unused analysis cells should be cleaned by the following procedure. The purpose of this procedure is to remove any cadmium from the surface and immediate subsurface of the cell. Pipette 300 to 500 μℓ of the acid ashing solution into each of the cells placed in digestion racks. Place the racks on a hot plate with a surface temperature of 220 to 240°C so that the acid refluxes about halfway up the side of each cell. Leave the cells on the plate for a period of 60 to 90 min, remove, and allow them to cool. Complete decontamination requires four repetitions of this procedure. Rinse the cells four times with distilled deionized water and invert them in a plastic rack with toweling on the bottom and allow them to air-dry. After use for an analysis, a cell does not have to be cleaned again by the acid cleaning procedure. It is sufficient to rinse it four times with distilled deionized water and store it inverted in a plastic rack with toweling on the bottom.

8.1.2 All other glassware should be acid-washed and rinsed with distilled deionized water. Polyethylene bottles used in sample collection are cleaned by rinsing in dilute nitric acid and distilled deionized water. The bottles should not be rinsed with concentrated acid as this may cause sample loss.

8.2 **Collection and Shipping of Samples.** Collect the samples in precleaned 180-mℓ polyethylene bottles. Ship the samples in a box with a minimum of 0.25 in. of foam packing to prevent shock that may lead to opening of the screw caps and resultant leakage.

8.3 **Analysis of Samples**

8.3.1 Shake the urine sample thoroughly and allow the foam to settle. Measure the specific gravity of the sample with a urinometer or suitable hydrometer. (If the specific gravity is less than 1.010, discard the sample. Obtain another sample from the subject if desired.) Pipette 1 mℓ of sample into an analysis cell and then add 1 mℓ of the acid ashing solution to the cell. Duplicate 1-mℓ aliquots of the urine sample should be taken for analysis.

8.3.2 Place the cell in a digestion rack on a hot plate operating at 100 to 120°C. Carry out the digestion in a hood. The sample will initially turn brown and will evaporate to about half-volume in 10 min. Adjust the temperature of the hot plate to 220 to 240°C. A reflux line will be established about halfway up the cell. At the end of 20 min, there will be approximately 100 to 200 μℓ of acid left in the bottom of the cell, with most of the nitric acid having evaporated. The digestion should be continued for an additional 20 to 30 min until the acid is almost completely evaporated.

8.3.3 Remove the cell from the hot plate, cool it, and add 4.5 mℓ of the acetate buffer solution.

8.3.4 Following the manufacturer's instructions for operation of the Anodic Stripping Voltammeter, analyze the buffered solution under the following conditions (all voltages are given with respect to a Ag/AgCl reference electrode):

- Plating potential, -1000 mV
- Auto-hold cutoff potential, 1050 mV
- Plating time, 15 to 30 min. (The plating time for samples and standards must be the same.)
- Sweep rate, + 60 mV/sec
- Current range, 0.1 to 2 mA (full scale)

The stirring rate for the sample solution during plating should not be lower than 170 mℓ/min since at lower rates significant variations in the amount of cadmium plated on the electrode may occur. As the sample is stripped, the cadmium peak (-720mV) will occur first, followed by a lead peak (-530 mV). A baseline is drawn by connecting with a straight line the flat portions of the curve before and after the cadmium peak. The peak height is measured by dropping a perpendicular from the apex to the baseline.

9. Calibration and Standards

The sequence of steps outlined in this section should be followed for the analyses of the following four kinds of solutions: (1) reagent blanks carried through the entire digestion process; (2) sample solutions prepared as described in Section 8.3; (3) standard solutions of cadmium in acetate buffer; (4) urine standards prepared as described below in Section 9.1. The sequence in which these four kinds of solutions are analyzed is as follows: (1) a set of standard solutions of cadmium in acetate buffer; (2) sample solutions, with reagent blanks and urine standards interspersed at the rate of one blank and one urine standard for every ten samples; and (3) a second set of standard solutions of cadmium in acetate buffer. The standard solutions of cadmium in acetate buffer are used for calibration; the results for the samples are corrected for the amount of cadmium found in the reagent blank; and the urine standards are used to determine the efficiency of recovery of known amounts of cadmium from urine. The procedure described is for use with a four-cell ASV unit.

9.1 Preparation of Urine Standards

9.1.1 A "normal" urine sample from an individual not suspected of exposure to cadmium is analyzed by the method of standard additions. One-milliliter samples of the urine are placed in each of six analysis cells. Five of these samples are spiked with known amounts of cadmium (10-, 50-, 100-, 200-, and 500-μℓ aliquots of standard solution "C," containing 10, 50, 100, 200, and 500 ng of cadmium, respectively). These samples are digested and analyzed by ASV according to the procedure given in Section 8.3 and the concentration of cadmium in the "normal" urine is estimated.

9.1.2 Finally, an amount of standard cadmium is added to 1 mℓ of unspiked "normal" urine to make the total amount of cadmium equal to 200 ng. This standard urine solution is subsequently analyzed along with calibration standards and blanks (Section 9.3) to determine the percent recovery of cadmium.

9.2 **Initial Analysis of Four Standards.** Four standards of 200 ng of cadmium, prepared by adding 200 $\mu\ell$ of standard solution "C" to 4.5 mℓ of acetate buffer solution, should be analyzed first. Each of the four standards should be analyzed with a different electrode. At the level of 200 ng, the contributions from blanks should be negligible, and a single point may be taken to describe the calibration curve for each electrode, which has been determined to be linear through 1000 ng.

9.3 **Analysis of Samples and Controls.** With every ten samples, a urine standard (Section 9.1.2) and a reagent blank are analyzed by the ASV procedure given in Section 8.3. At least one reagent blank and one urine standard should be analyzed with each electrode that is used for analysis of samples.

9.4 **Final Analysis of Standards.** The final analysis should include four more standards prepared in the manner described in Section 9.2 and analyzed with the same four electrodes. Thus, two peak height values for 200 ng of cadmium will be obtained for each of the four electrodes. These values are used to calculate a response factor in ng/μA. The overall response factor is the amount of cadmium in the standard (200 ng) divided by the average of the eight peak heights (in μA). However, it will be necessary to calculate an individual response factor for an electrode if the average of the two peak values for an individual electrode differs from the overall average of the eight peak heights by more than 10%. For example, if the average for the eight peak heights is 360 μA, and the average of the two values for one of the electrodes is 320 μA, then the response factor to be used for that electrode is 200 ng/320 μA, or 0.63 ng/μA. The overall response factor in this example is 200 ng/360 μA, or 0.56 ng/μA.

10. **Calculations**

10.1 The amount of cadmium in a sample is calculated from the peak height (in μA), as measured according to Section 8.3.4, and the response factor in the following manner:

$$\text{Amount (in ng)} = \text{peak height (in } \mu\text{A)} \times \text{response factor (in ng/}\mu\text{A)}$$

Any amount of cadmium found in the reagent blank should be subtracted from the sample value to give the correct amount (in ng) for cadmium in the sample. The average recovery of cadmium from urine standards is usually greater than 90%. However, it may be necessary to include a factor in the above equation to correct for incomplete recovery.

10.2 The concentration of cadmium in a urine sample may be expressed as μg/ℓ:

$$\mu g/\ell = \frac{\text{corrected weight (in ng) of cadmium in sample}}{\text{volume (in m}\ell\text{) of sample}}$$

10.3 The use of a specific gravity correction factor to normalize values to 1.024, the average specific gravity of urine, has been recommended. (See Reference 11.2.) The preferred method of normalization is to make a straightforward gravity correction as follows:

$$\text{Corrected } \mu g/\ell = \mu g/\ell \times \frac{0.024}{(\text{specific gravity} - 1.000)}$$

Report the uncorrected concentration, the corrected concentration, and the specific gravity of the sample.

11. References

11.1 Environmental Sciences Associates, Inc., Burlington, Mass., Final Report for Contract No. HSM-99-72-107 to National Institute for Occupational Safety and Health, Division of Laboratories and Criteria Development, Physical and Chemical Analysis Branch, Cincinnati, Ohio, 1973.

11.2 Elkins, H.B., "Concentration Adjustments in Urinalysis," Amer. Ind. Hyg. Ass. J., 35, 559 (1974).

11.3 Matson, W.R., R.M. Griffin, and T.J. Sapienza, "A Total System and Quality Control Procedure for Micro Blood Lead Analysis by Anodic Stripping Voltammetry (ASV)," presented at the Laboratory Director's Conference, Department of Health, Education, and Welfare, Center for Disease Control, August 23, 1974.

KEPONE® IN AIR

Measurement Research Branch

Analytical Method

Analyte:	Kepone*	**Method No.:**	P&CAM 225
Matrix:	Air	**Range:**	$< 2\ \mu g/m^3$ to $> 1\ mg/m^3$
Procedure:	Filter/impinger collection, GC analysis	**Precision:**	0.23 RSD at $2.5\ \mu g/m^3$
Date Issued:	2/15/76		
Date Revised:		**Classification:**	E (Proposed)

1. **Principle of the Method**

   1.1 Kepone from the air is collected on a membrane filter and in a backup impinger containing 0.1 N sodium hydroxide solution.

   1.2 The Kepone on the filter is dissolved in 99:1 benzene-methanol (v/v).

   1.3 The sodium hydroxide solution is acidified with 1 N sulfuric acid and the Kepone present is extracted with benzene.

   1.4 Gas chromatographic analysis of each solution is performed using an electron-capture detector.

2. **Range and Sensitivity**

   2.1 The range of the electron-capture detector for the quantitation of Kepone is dependent upon the type of detector and chromatograph used. For a nonlinearized ^{63}Ni electron-capture detector in a Tracor MT 220 Gas Chromatograph, the range most useful for the quantitation was 25 to 250 pg of Kepone per aliquot injected. This range corresponds to 50 to 500 ng of Kepone per sample solution obtained in the recovery from the filter or impinger. For a 480-liter air sample, this method is applicable to air concentrations which range from levels below $2\ \mu g/m^3$ to levels above $1\ mg/m^3$. The lower limit depends upon the collection and recovery efficiencies pertaining to the filter and, perhaps, the collection and recovery efficiencies pertaining to the impinger. (See Section 4.3 on collection efficiency.) The upper limit depends upon the quantity of dust the filter can collect without becoming severely loaded.

   2.2 The detection limit was 5 pg per injection (10 ng per sample solution). It is estimated that Kepone at concentrations below $0.1\ \mu g/m^3$ could be detected in a 480-liter air sample.

3. **Interferences**

   3.1 Compounds which are detected by the electron-capture detector and have retention times approximating that of Kepone will interfere with the analysis.

   3.2 When air samples containing the pesticide Mirex are passed through the impinger of 0.1 N sodium hydroxide solution, some Kepone is formed.

*Also known as chlordecone and decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalen-2-one.

4. Precision and Accuracy

4.1 The relative standard deviation for the total sampling and analytical method at 2.5 $\mu g/m^3$ (750-1400 μg/filter sample) was 0.23.

4.2 The bias in the sampling and analytical method was not determined.

4.3 From accumulated data it was estimated that the average collection efficiency of the filter-impinger combination is $> 92\%$ at a level of 2 $\mu g/m^3$ and $> 98\%$ at levels of 10 $\mu g/m^3$ and above. Low levels of Kepone pass through the filter and are trapped in the impinger at an efficiency roughly estimated at 50%. The amount of Kepone present in the impinger after sampling may be below the quantitation limit of 50 ng.

5. Advantages and Disadvantages of the Method

5.1 The selectivity of the electron-capture detector minimizes the number of potential interferences.

5.2 The range of Kepone concentrations in solution useful for quantitation is limited to approximately one order of magnitude when the volume of sample injected into the chromatograph remains constant. Solutions having Kepone concentrations above this range must be diluted.

5.3 Since the detector is easily contaminated by impurities, the detector sensitivity can change with time. If a drift in response does occur, it becomes necessary to base the calculation of the amount of Kepone in the sample on the two standards bracketing the sample.

6. Apparatus

6.1 Sampling Equipment. The sampling unit consists of the following components:

6.1.1 Membrane filter, mixed cellulose ester, 37-mm diameter, 0.8-μm pore size. The filter is mounted on a support pad inside a filter holder.

6.1.2 Filter holder, three piece, 37-mm diameter, held together by tape or shrinkable band.

6.1.3 Midget impinger.

6.1.4 Personal sampling pump capable of maintaining the flow of air through the filter-impinger combination at a rate of 1 liter/min with an accuracy of $\pm$ 5%.

6.1.5 Midget impinger with the stem broken off. This piece of apparatus protects the sampling pump from splashover and water condensation and is inserted between the exit arm of the impinger containing sodium hydroxide solution and the pump.

6.1.6 Flexible tubing, such as Tygon, for connecting the filter holder, impingers, and pump.

6.1.7 Integrating volume meter, such as dry gas or wet test meter.

6.1.8 Thermometer.

6.1.9 Manometer.

6.1.10 Stopwatch.

6.1.11 Scintillation vials, 20-ml, and polyethylene caps equipped with cone-shaped plastic liners.

6.1.12 Vinyl electrical tape or shrinkable band.

6.2 Forceps, 2 pairs.

6.3 Separatory funnels, 125-ml, with Teflon stopcocks.

6.4 Beakers, 100-ml.

6.5 Volumetric flasks, 10-ml and 1-liter.

6.6 Disposable Pasteur pipettes with rubber bulbs.

6.7 Glass pipettes, 5-ml and 10-ml.

6.8 pH test paper.

6.9 Gas chromatograph, equipped with an electron-capture detector. A tube filled with activated charcoal should be attached to the exit port for the purpose of trapping Kepone in the effluent gas stream.

6.10 Gas chromatography column, 1.8 m x 4 mm I.D., constructed from borosilicate glass and packed with one of the following:

A. 3% OV-1 on 80/100 Chromosorb W HP.

B. 3% OV-210 on 80/100 Gas Chrom Q.

Columns A and B are conditioned by heating at 260°C for 12 hours while nitrogen is flowing at 30 ml/min.

6.11 Small spatula.

6.12 Syringes, 10-μl and 100-μl.

7. Reagents

All reagents must be analytical reagent grade or better

7.1 Sodium hydroxide solution, 0.1 N_f aqueous

7.2 Benzene, pesticide quality

7.3 Methanol, pesticide quality

7.4 99:1 Benzene-methanol (v/v)

7.5 Sulfuric acid, 1 N

7.6 Kepone

8. Procedure

8.1 Cleaning of Equipment

8.1.1 The syringes should be rinsed well, first with methanol and then with benzene.

8.1.2 All other glassware should be cleaned with soap and water, then rinsed with the following in sequence: deionized water, methanol, and benzene.

8.2 Collection and Shipping of Samples

8.2.1 Pour 15 ml of 0.1 N sodium hydroxide solution into each midget impinger.

8.2.2 Remove the small plugs from the filter holder and connect the filter holder to the impinger with a short piece of flexible tubing. The tubing should be short in order to minimize the exposure of Kepone to the tubing.

8.2.3 Connect both the exit arm of the impinger containing sodium hydroxide solution and the inlet of the pump to the empty impinger with the stem broken off. This empty impinger protects the pump from splashover.

8.2.4 Turn on the pump to begin sample collection. Care should be taken to measure the flow rate, time and volume as accurately as possible. The sample should be taken at a flow rate of 1.0 liter/min and should have a volume of at least 120 liters.

8.2.5 After sampling, place the plugs back into the openings of the cassette filter holder.

8.2.6 Remove and clean the impinger stem. Tap the stem gently against the inside wall of the impinger bottle to recover as much of the sampling solution as possible.

Transfer the solution in the impinger to a 20-ml scintillation vial. Wash the impinger and stem with a small amount (1-2 ml) of unused NaOH solution and add the wash to the vial. Tightly screw on the cap and seal with vinyl electrical tape or shrinkable band.

8.2.7 Handle a blank filter as the other filters (seal and transport). Do not draw air through this filter.

8.2.8 Handle a blank impinger as the other impingers (transfer the solution to a vial, seal and transport). Do not draw air through this impinger.

8.3 **Analysis of Samples**

8.3.1 Open the filter holder and, with the use of forceps, fold the membrane filter without the support pad into a configuration suitable for placement into a 10-ml volumetric flask. Place the filter into the flask.

8.3.2 Add 10.0 ml of 99:1 benzene-methanol (v/v) to the flask. Stopper the flask and shake vigorously for 1 minute. Allow the flask to stand for at least 2 hours and then shake briefly prior to gas chromatographic analysis.

8.3.3 Transfer the sodium hydroxide solution from the vial to a 125-ml separatory funnel. Wash the vial with two 15-ml portions of 0.1 N sodium hydroxide solution and add the washings to the separatory funnel. Add 1 N sulfuric acid to the solution until the pH is less than 7.

8.3.4 Extract the Kepone from the aqueous solution with two 5-ml portions of benzene. During each extraction process, shake the mixture vigorously for 2 minutes and then allow 5 minutes for the two phases to separate. Combine the benzene layers in a 10-ml volumetric flask. Add 100 μl of methanol and enough benzene to bring the total volume to 10.0 ml.

8.3.5 The ^{63}Ni detector is operated in the DC mode at 90% standing current. Other chromatographic conditions are:

Injection port temperature	225°C
Detector temperature	335°
Column temperature	
Column A (3% OV-1)	200° or 220°
Column B (3% OV-210)	200°
Carrier gas flow rate	70 ml/min

8.3.6 The approximate retention times of Kepone and a related pesticide, Mirex, are:

	Column A		Column B
Pesticide	(200°C)	(220°C)	(200°C)
Kepone	4.6 min	2.6 min	3.0 min
Mirex	9.2 min	4.6 min	4.0 min

8.3.7 By means of the solvent-flush injection technique, inject 5-μl aliquots of the sample solutions into the gas chromatograph. If a sample gives a peak which is larger than the largest standard peak, dilute an aliquot of the sample to 10 ml to bring the sample peak onto the calibration curve.

8.3.8 After each injection, rinse the syringe thoroughly, first with methanol and then with benzene. Otherwise, traces of Kepone will remain in the syringe and will contaminate the solvent flush solution [99:1 benzene-methanol (v/v)]. Periodically make an injection of pure solvent-flush solution to check the cleanliness of the syringe and the purity of the solvent-flush solution.

8.4 **Determination of Recovery**

8.4.1 Impregnate six filters with a known quantity of Kepone in 99:1 benzene-methanol (v/v) and allow the filters to dry in the air. The quantity of Kepone should be in the vicinity of the amounts found on the filters in the field samples.

662

8.4.2 Impregnate six impingers of 0.1 N sodium hydroxide solution with a known quantity of Kepone in methanol solution. The quantity of Kepone should be in the vicinity of the amounts found in the impingers in the field samples, unless this amount is too low for measurement.

8.4.3 Prepare two sets of standards, six standards for the filters and six standards for the impingers, by adding the same quantities of the same Kepone solutions used in preparing the samples to 10-ml volumes of 99:1 benzene-methanol (v/v).

8.4.4 Analyze the samples and standards in pairs according to Section 8.3.

8.4.5 Analyze a blank filter and a blank impinger of 0.1 N sodium hydroxide solution according to Section 8.3.

8.4.6 The recovery, R, of each sample equals the difference between the sample peak height, H_a, and the blank peak height, H_b, divided by the height of the corresponding standard peak, H_s, or

$$R = \frac{H_a - H_b}{H_s}$$

Calculate the average recovery from the six R values obtained for each lot of samples.

8.4.7 If the quantity of Kepone in the samples varies an order of magnitude or more, determine the recovery at two or three selected levels and construct a plot of recovery versus quantity of Kepone measured.

9. Calibration and Standards

9.1 Prepare a series of standard solutions of Kepone in 99:1 benzene-methanol (v/v). The concentrations should range from approximately 30 to 250 ng/10 ml. This range depends upon the useful range of the detector. The standard solutions may be stored for 1 week at room temperature.

It is convenient to express the concentration in ng of Kepone/10 ml of solution. In view of the low concentrations involved, the volume of a solution prepared from X number of ng of Kepone and 10 ml of solvent is very nearly 10 ml and can be assumed to be 10 ml for the purpose of this analytical method.

Caution: Kepone is a carcinogen (11.1). The pure compound and its dust formulations should be handled in such a manner as to avoid personnel exposure and area contamination.

9.2 Analyze the standard solutions according to Section 8.3. It is helpful to analyze the standards first, construct the standard curve, and then reanalyze them with the samples as a check on detector drift.

9.3 Establish a calibration curve by plotting peak height versus ng Kepone/10 ml.

10. Calculations

10.1 Determine the quantity of Kepone, W (in ng), in 10 ml of the sample from the calibration curve.

10.2 Determine the total quantity of Kepone in the air sample:

$$Q = \left(\frac{W \cdot F}{R}\right)_A + \left(\frac{W \cdot F}{R}\right)_B$$

where:

Q = The total quantity of Kepone (in ng) in the air sample.

W = Quantity of Kepone (in ng) in 10 ml of sample solution.

F = The factor by which the Kepone solution is diluted; e.g., a 1-ml sample diluted to 10 ml reflects a dilution factor of 10. If the solution is not diluted, F = 1.

R = The recovery (See Section 8.4).

A = Kepone on the filter.

B = Kepone in the impinger of NaOH solution.

10.3 Determine the total quantity of Kepone, Y, on the blank filter and in the blank impinger solution according to Section 10.2.

10.4 Determine the corrected total quantity of Kepone, Q' in the air sample:

$$Q' = Q - Y$$

10.5 Determine the concentration, C, of Kepone in air:

$$C = \frac{Q'}{V}$$

where:

C = The concentration of Kepone in $\mu g/m^3$

V = Volume of air sampled in liters

11. Reference

11.1 Cueto, C., Jr., N. P. Page, and U. Saffiotti, "Report on Carcinogenesis Bioassay of Technical Grade Chlordecone (Kepone)", Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Maryland, January 1976.

2,6-DI-*t*-BUTYL-*p*-CRESOL(DBPC)* IN AIR

Measurements Support Branch

Analytical Method

Analyte: 2,6-Di-*t*-Butyl-*p*-Cresol (DBPC)*	**Method No.:** P&CAM 226
Matrix: Air	**Range:** 1 to 500 mg/m³ in a 10-liter sample of air
Procedure: Silica gel adsorption; methanol-carbon disulfide desorption; GC	**Precision (CV):** 0.05 (estimated analytical) over the above range
Date Issued: 6/30/76	**Classification:** D(Operational)
Date Revised:	

1. **Principle of the Method**

A known volume of air is drawn through a tube containing silica gel to trap the DBPC. The silica gel is transferred to a small, graduated test tube and desorbed with a mixed solvent, 5% (v/v) methanol in carbon disulfide. An aliquot of the solution is analyzed by gas chromatography with flame ionization detection (FID). The area of the resulting peak is determined and compared with areas obtained from the injection of standards.

2. **Range and Sensitivity**

2.1 The calibration curve of peak area *versus* concentration is linear over the range of 0.02 to 10 mg/mℓ of DBPC in the mixed solvent. This range corresponds to 0.01 to 5 mg of DBPC in a sample, since the silica gel is extracted with 0.5 mℓ of the mixed solvent. A convenient working range for the sampling and analytical method is therefore 1 to 500 mg/m³ in a 10-liter sample of air.

2.2 The detection limit of the analytical method is 0.2 μg/mℓ of DBPC in the mixed solvent. This corresponds to 0.1 μg in a sample or 0.01 mg/m³ in a 10-liter sample of air.

3. **Interferences**

3.1 When the amount of water in the air is so great that condensation occurs in the tube, organic vapors will not be trapped efficiently. Preliminary experiments indicate that high humidity severely decreases the breakthrough volume.

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*Also called butylated hydroxy toluene (BHT).

3.2 Any compound that has the same GC retention time as DBPC under the operating conditions described in this method is an interference. Hence, retention time data on a single column, or even on a number of columns, cannot be considered as proof of chemical identity. For this reason it is important that a bulk sample of DBPC be submitted so that its composition can be established by other means. If the possibility of interference in the GC analysis exists, operating conditions (column packing, temperature, etc.) must be changed to circumvent the problem.

4. Precision and Accuracy

4.1 No precision or accuracy data for the overall sampling and analytical method are available.

4.2 Although the precision of the analytical method has not been explicitly determined, the correlation coefficient is greater than 0.99 for the calibration curve in the linear concentration range.

4.3 The efficiency of desorption of 0.05 and 0.2 mg/tube of DBPC has been found to be 0.90, or greater, with coefficients of variation of 0.05 and 0.04, respectively.

5. Advantages and Disadvantages of the Method

5.1 The sampling device is small, portable, and involves no liquids. Interferences are minimal, and most of those that do occur can be eliminated by altering chromatographic conditions. The tubes are analyzed by means of a rapid instrumental method.

5.2 One disadvantage of the method is that the amount of DBPC that can be collected on a tube without breakthrough is limited. When the procedure for the determination of desorption efficiency outlined in Section 8.4 was followed, at least 0.2 mg of DBPC was trapped in the front section without breakthrough. However, no detailed breakthrough study for this compound has been conducted. If the amount of DBPC found in the backup section exceeds 25% of that found in the front section, the possibility of sample loss exists.

5.3 Furthermore, the precision of the method is limited by the reproducibility of the pressure drop across the tubes. This drop will affect the flow rate and cause the volume to be imprecise, because the pump is usually calibrated for one tube only.

6. Apparatus

6.1 Sampling Pump. An approved and calibrated personal sampling pump is required for personal samples. Each pump should be calibrated with a representative sorbent tube in the sampling line. A dry or wet test meter or glass rotameter that will determine the flow rate to within 5% may be used for the calibration. For an area sample any vacuum pump whose flow can be determined accurately for flow rates of 1 ℓ/min or less can be used.

6.2 **Sorbent Tubes.** Glass tubes, 7 cm long, 6 mm o.d., and 4 mm i.d., are packed with two sections of 45/60-mesh silica gel separated by a 2-mm plug of urethane foam. The front section contains 100 to 150 mg of silica gel and the backup section contains 50 to 75 mg. A 3-mm plug of urethane foam is placed between the outlet end of the tube and the backup section. A plug of silylated glass wool is placed in the inlet end of the tube. The pressure drop across the tube must be less than 1 in. of Hg at a flow rate of 1 ℓ/min.

6.3 Gas chromatograph equipped with a flame ionization detector.

6.4 A stainless steel GC column (10 ft by 0.125 in.) packed with 5% SE-30 on 60/80-mesh Chromosorb W, or an equivalent column.

6.5 A mechanical or electronic integrator or a recorder with a means of determining peak area.

6.6 Graduated glass-stoppered micro centrifuge tubes or 2-mℓ sealed sample vials for desorbing samples.

6.7 Microsyringes, 10 μℓ, and other sizes convenient for preparing standards.

6.8 Volumetric pipettes, 0.5 mℓ, or 1-mℓ pipettes graduated in 0.1 mℓ increments.

6.9 Volumetric flasks, 10 mℓ, and other sizes convenient for preparing standard solutions.

7. **Reagents**

7.1 Carbon disulfide and methanol, spectroquality. Prepare a solution containing 5% of methanol in carbon disulfide.

7.2 2,6-Di-*t*-butyl-*p*-cresol(DBPC), reagent grade.

7.3 Helium, Bureau of Mines Grade A, or prepurified nitrogen.

7.4 Hydrogen, prepurified.

7.5 Compressed air, filtered.

8. **Procedure**

8.1 **Cleaning of Equipment.** Wash all glassware with detergent solution and rinse thoroughly with tap water and distilled water.

8.2 **Collection and Shipping of Samples.**

8.2.1 Immediately before beginning the collection of a sample, break each end of the sorbent tube so as to provide openings at least half the inside diameter of the tubes.

8.2.2 Connect the backup end (smaller section of silica gel) of the sorbent tube to the pump and mount the tube vertically to prevent channeling, with the larger section of silica gel on top. Sample air must not pass through any hose or tubing before entering the sorbent tube.

8.2.3 Sample the air at 200 mℓ/min. A maximum sample volume of 10 liters is recommended. If very high concentrations of DBPC are known to be present, take smaller samples to minimize the possibility of breakthrough.

8.2.4 Cap the sorbent tubes with the plastic caps supplied immediately after sampling is completed. Do not use rubber caps.

8.2.5 One tube should be handled in the same manner as the sample tube (break, seal, and ship) except that no air is pumped through it. Label this tube as a blank.

8.2.6 Pack the capped tubes tightly in a suitable container to minimize breakage during transport to the laboratory.

8.2.7 Submit a sample of the bulk DBPC to the laboratory. Do not ship this bulk sample in the same container as the sample or blank tubes. If possible, ship a bulk air sample for qualitative identification purposes.

8.3 Analysis of Samples

8.3.1 **Preparation of Samples.** In preparation for analysis, score each sorbent tube with a file in front of the first section of silica gel and break it open. Transfer both the front glass fiber plug and the larger initial section of silica gel to a small stoppered test tube or sample vial. Tests indicate that the front glass fiber plug does trap some of the DBPC so it must be extracted with this first sorbent section. Remove and discard the separating section of foam; transfer the second smaller section of silica gel to another test tube. Analyze these two sections separately.

8.3.2 Desorption of Samples. Prior to analysis, pipette 0.5 mℓ of the mixed solvent into each test tube. (All work with carbon disulfide should be performed in a hood because of its toxicity.) Tests indicate that desorption is time dependent and requires at least 60 min with periodic agitation. The use of graduated, glass-stoppered micro centrifuge tubes is recommended so that one can observe any apparent change in volume during the desorption process. Carbon disulfide is a very volatile solvent; thus volume changes can occur during the desorption process depending on the surrounding temperature. Note the initial volume occupied by the silica gel plus the 0.5 mℓ of mixed solvent and make corresponding volume adjustments whenever necessary prior to GC analysis.

8.3.3 GC Conditions. Typical operating conditions for the gas chromatograph are:

- Helium carrier gas flow rate, 85 mℓ/min.
- Hydrogen gas flow rate to detector, 65 mℓ/min.
- Air flow rate to detector, 500 mℓ/min.
- Injector temperature, 200°C.
- Column temperature, 160°C.

8.3.4 Injection. The first step in the analysis is the injection of the sample into the gas chromatograph. To eliminate difficulties arising from blowback or distillation within the syringe needle, one should employ the solvent flush injection technique. First flush the 10-μℓ syringe with solvent several times to wet the barrel and plunger. Draw 3 μℓ of mixed solvent into the syringe to increase the accuracy and reproducibility of the injected sample volume. Remove the needle from the solvent, and pull back the plunger about 0.2 μℓ to separate the solvent flush from the sample with a pocket of air to be used as a marker. Then immerse the needle into the sample, and withdraw a 5-μℓ aliquot, taking into consideration the volume of the needle, since the sample in the needle will be completely injected. Remove the needle from the sample and pull back the plunger a short distance to minimize evaporation of the sample from the tip of the needle prior to injection. Make duplicate injections of each sample and standard. No more than a 3% difference in area is to be expected.

8.3.5 Measurement of Area. Measure the area of the sample peak with an electronic integrator or by some other suitable method of area measurement, and read preliminary results from a standard curve prepared as discussed below in Section 9.

8.4 **Determination of Desorption Efficiency**

8.4.1 **Importance of Determination.** The desorption efficiency of a particular compound can vary from one laboratory to another and possibly from one batch of silica gel to another. Thus, it is necessary to determine at least once the fraction of the specific compound that is removed in the desorption process. The Measurements Support Branch of NIOSH has found that the desorption efficiency for DBPC is 0.90 or greater down to levels of 0.05 mg.

8.4.2 **Procedure for Determining Desorption Efficiency.** Transfer an amount of silica gel equivalent to the amount in the first section of a sorbent tube (100 to 150 mg) into a 20-in., 4-mm i.d. glass tube, flame-sealed at one end. (This silica gel must be from the same batch as that used in obtaining the samples and can be obtained from unused tubes.) Cap the open end of the tube with Parafilm. With a microliter syringe, inject directly into the silica gel a known amount of DBPC standard prepared in carbon disulfide. Cap the tube with more Parafilm. The amount injected is usually equivalent to that present in a 10-liter sample of air at a concentration equal to the OSHA standard. Prepare at least six tubes in this manner and allow them to stand at least overnight to assure complete absorption of the DBPC by the silica gel. Treat a parallel blank tube in a similar manner, but add no DBPC standard to it. Desorb and analyze these standard and blank tubes as described in Section 8.3. Prepare two or three solution standards by injecting the same volume of DBPC into 0.5 mℓ of the mixed solvent with the same syringe used in the preparation of the silica gel-tube samples. Analyze these solution standards along with the tube samples. The desorption efficiency (D.E.) equals the difference between the average peak area for the tube samples and the peak area for the blank divided by the average peak area for the solution standards, or

$$\text{D.E.} = \frac{\text{tube sample area - blank area}}{\text{solution standard area}}$$

9. **Calibration and Standards**

9.1 Prepare a series of standards, varying in concentration over the range of interest. It is convenient to express the concentrations of standards in terms of milligrams of DBPC in 0.5 mℓ of desorbing solution. To minimize error caused by evaporation of the carbon disulfide, one can measure into 10 mℓ of the mixed solvent 20 times the weight of DBPC that would have been present in 0.5 mℓ. Analyze these standards under the same GC conditions and during the same time period as the unknown samples. Establish a calibration curve by plotting concentration (in mg/0.5 mℓ) versus peak area.

9.2 If no internal standard is used in the method, the standard solutions must be analyzed at the same time the sample analyses are done. This will minimize the effect of day-to-day variations and variations of FID response during the same day. However, it is recommended that a suitable internal standard be employed.

10. Calculations

10.1 From the standard curve, read the weight of DBPC (in mg) corresponding to the peak area for the particular sample. No volume corrections are needed, because the standard curve is based on mg/0.5 mℓ of extract, and the volume of sample injected into the GC is identical to the volume of the standards injected.

10.2 Make a correction for the blank for each sample.

$$\text{Corrected mg} = mg_s - mg_b$$

where: mg_s = mg found in front section of sample tube.

mg_b = mg found in front section of blank tube.

A similar procedure is followed for the backup section.

10.3 Add the corrected amounts present in the front and backup sections of the same sample tube to determine the total measured amount of DBPC in the sample.

10.4 Divide this total weight by the desorption efficiency (D.E.) to obtain the total amount, W (in mg), in each sample.

10.5 The concentration of DBPC in the air samples can be expressed in mg/m^3, which is numerically equal to $\mu g/\ell$:

$$mg/m^3 = \frac{W\ (mg) \times 1000\ \ (\mu g/mg)}{V\ (\ell)}$$

where: W = the total amount of DBPC collected in the sample.

V = volume of air sampled.

11. References

11.1 White, L. D., D. G. Taylor, P. A. Mauer, and R. E. Kupel, "A Convenient Optimized Method for the Analysis of Selected Solvent Vapors in the Industrial Atmosphere," Am. Ind. Hyg. Assoc. J., 31, 225 (1970).

11.2 Choy, T. K., J. J. Quattrone, Jr., and N. J. Alicino, "A Gas Chromatographic Method for the Determination of the Antioxidants BHA, BHT, and Ethoxyquin in Aqueous and in Hydrocarbon-Soluble Samples," J. Chromotogr., 12, 171 (1963).

11.3 Grote, A. A., "Sampling and Analysis of 2,6-Di-*t*-Butyl-*p*-Cresol (DBPC) in an Industrial Atmosphere," presented at the American Industrial Hygiene Conference, Atlanta, Ga., May 1976.

POLYMETHYLSILOXANE MIST IN AIR

Measurements Support Branch

Analytical Method

Analyte: Polymethylsiloxane (as silicon)	Method No.: P&CAM 227
Matrix: Air	Range: 0.01 to 0.1 mg/m³ polymethylsiloxane in a 400-liter sample of air
Procedure: Filter collection; solvent extraction; flameless atomic absorption analysis	Precision(CV): 0.15 (estimated analytical) over the above range
Date Issued: 6/30/76	
Date Revised:	Classification: E (Proposed)

1. **Principle of the Method**

A known volume of air is drawn through a cellulose membrane filter to trap the polymethylsiloxane mist. The filter is extracted with chloroform, and the amount of polymethylsiloxane is determined as total silicon by flameless atomic absorption spectrophotometry.

2. **Range and Sensitivity**

2.1 The calibration curve of absorbance *versus* concentration is linear in the range of 0.4 to 4 μg/mℓ of polymethylsiloxane in chloroform. Since the chloroform extract of an exposed filter is diluted to 10 mℓ prior to analysis, this range will correspond to 4 to 40 μg in a sample, or 0.01 to 0.1 mg/m³ of polymethylsiloxane in a 400-liter sample of air.

2.2 The detection limit of the analytical method is approximately 0.04 μg/mℓ of polymethylsiloxane in chloroform, which corresponds to 0.001 mg/m³ in a 400-liter sample of air.

3. **Interferences**

There are no known interferences; however, other chloroform-soluble silicon compounds presumably would interfere.

4. **Precision and Accuracy**

The precision and accuracy of the method have not been completely established. Preliminary data suggest a coefficient of variation of 0.15 for the analytical method.

5. Advantages and Disadvantages of the Method

5.1 The graphite furnace method is an order of magnitude more sensitive than conventional atomic absorption spectrophotometry.

5.2 One disadvantage is that many laboratories are not equipped with a graphite furnace accessory.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Cellulose ester membrane filters, 0.8-μm pore size, 37-mm diameter, Millipore Type AA, or equivalent.

6.1.2 Filter holder for 37-mm filters, Millipore MAWP 037 AO, or equivalent.

6.1.3 Personal sampling pump, calibrated with a representative filter unit in the line. A wet or dry test meter or a glass rotameter capable of measuring a flow rate of 1.7 ℓ/min to within ±5% may be used in the calibration.

6.1.4 Thermometer, manometer, and stopwatch.

6.2 Atomic Absorption Spectrophotometer

6.2.1 Atomic absorption spectrophotometer with graphite furnace and tube or rod atomizer. Reproducible control of times and temperatures during the drying, ashing, and atomizing cycles is essential. A deuterium background corrector is desirable.

6.2.2 Hollow cathode lamp for silicon.

6.2.3 Purge gas, high purity argon, in a cylinder equipped with a two-stage, two-gauge pressure regulator and appropriate hose connections. The pressure on the instrument side of the regulator should be 30 psig.

6.3 Glassware(borosilicate)

6.3.1 Phillips beakers with watchglass covers, 125 mℓ.

6.3.2 Volumetric flasks, 10 mℓ.

6.3.3 Micropipettes, 10 to 500 μℓ.

7. **Reagents**

7.1 Chloroform, distilled in glass.

7.2 **Polymethylsiloxane Stock Solution** (5 mg/mℓ). Dissolve 500 mg of polymethylsiloxane in chloroform and dilute the solution with chloroform to 100 mℓ in a volumetric flask.

8. **Procedure**

8.1 **Cleaning of Equipment**

8.1.1 Soak new glassware in chromic acid cleaning solution (saturated solution of sodium dichromate in concentrated sulfuric acid) and rinse thoroughly, first with warm tap water, then with concentrated nitric acid, and finally with double-distilled or deionized water.

8.1.2 Clean used glassware by soaking in detergent solution and rinsing with warm tap water, distilled water, 5% nitric acid, and finally with double-distilled water.

8.2 **Collection and Shipping of Samples**

8.2.1 With a filter in place, connect the filter holder to the sampling pump. Sample the air at 1.7 ℓ/min for 4 hr. This sampling period provides an amount of polymethylsiloxane suitable for the determination of 0.01 mg/m^3 in air.

8.2.2 Place the filters in clean individual filter holders for shipment to the laboratory.

8.3 **Analysis of Samples**

8.3.1 Remove the filter from the cassette filter holder and place it in a 125-mℓ Phillips beaker.

8.3.2 Add 3 to 5 mℓ of chloroform to the beaker and swirl vigorously. Allow the sample to stand for a minimum of 30 min with occasional swirling.

8.3.3 Quantitatively transfer the chloroform extract to a 10-mℓ volumetric flask and wash the filter and beaker with several portions of chloroform. Combine the extract and washings and dilute to 10 mℓ with chloroform.

8.3.4 Measure the absorption of 25 μℓ of each sample by the procedure described in Section 8.3.5. If the sample is too concentrated, make the appropriate dilution with chloroform and repeat the measurement.

8.3.5 Prepare the spectrophotometer according to the manufacturer's recommendations. Set the wavelength at 251.6 nm and the slit at 0.2 mm. The following program is recommended for the graphite furnace; however, because of differences in instruments, this program may require some modification.

Cycle	Temperature, °C	Time, sec
Drying	60	20
Charring	1000	60
Atomization	2700	2

Place a 25-µℓ aliquot of a sample or standard in the atomizer with a micropipette. (The use of a syringe fitted with a metal needle should be avoided.) Read and record the absorbance. Analyze a minimum of three aliquots for each standard sample. If a deuterium background corrector is not available, the background absorbance should be measured and subtracted from that of the standards and samples.

8.3.6 Analyze a blank filter with each group of samples according to the procedure described in Sections 8.3.1 to 8.3.5.

9. Calibration and Standards

9.1 From the polymethylsiloxane stock solution prepare several standard solutions in the concentration range of 0.5 to 5 µg/mℓ by diluting appropriate aliquots with chloroform.

9.2 Analyze the standards along with the samples and blanks according to the procedure described in Section 8.3.

9.3 Prepare a calibration graph by plotting absorbance *versus* concentration (in µg/mℓ).

10. Calculations

10.1 From the standard curve, read the concentration, C (in µg/mℓ), corresponding to the absorbance of the sample solution. Calculate the amount of polymethylsiloxane in the sample as follows:

$$W_s = C \times 10 \text{ mℓ} \times \text{dilution factor}$$

where: W_s = amount (in μg) of polymethylsiloxane in the extract of the sample filter.

C = concentration (in $\mu g/m\ell$) in the 10 $m\ell$ extract.

10.2 Correct the sample weight for the weight found on the blank filter to give the actual amount collected in the sample, as follows:

$$W = W_s - W_b$$

where: W = corrected amount (in μg) collected in the sample.

W_b = amount (in μg) found in the extract of the blank filter.

10.3 The concentration of polymethylsiloxane in the air sample is calculated as follows:

$$mg/m^3 = \frac{W\ (\mu g)}{V\ (\ell)}$$

where: V = volume of air sampled.

11. **Reference**

Solomons, E. T., and J. K. Jones, J. Forensic Sciences, 20, 191 (1975).

THIRAM IN AIR

Measurements Support Branch

Analytical Method

Analyte: Thiram (tetramethyl-thiuram disulfide)	Method No.: P&CAM 228
Matrix: Air	Range: 0.1 to 5.0 mg/m³ in a 50-liter sample
Procedure: Filter collection; chloroform elution; spectrophotometric determination	Precision(CV): 0.23 over the range 5 to 20 μg/mℓ
Date Issued: 6/30/76	Classification: D(Operational)
Date Revised:	

1. Principle of the Method

Thiram in air is collected on a membrane filter. The filter is extracted with chloroform. If acrylamide polymers are present, they are removed by precipitation with acetone. Cuprous iodide is added to the chloroform solution of thiram to form cupric dimethyldithiocarbamate. The absorbance of the solution at 440 nm is measured spectrophotometrically and the concentration of thiram is determined from a calibration curve.

2. Range and Sensitivity

2.1 The linear range of the calibration curve extends from about 5.0 μg/mℓ to 25 μg/mℓ of final solution. Taking into account the volume of chloroform (10 mℓ) used to extract the filter, this range is calculated to correspond to 1.0 to 5.0 mg/m³ of thiram in a 50-liter sample of air. The upper limit of the range can be increased by dilution of the extract prior to the development of color; however. the variability of results also is increased.

2.2 The detection limit of the analytical method is about 2.5 μg/mℓ, which corresponds to 0.5 mg/m³ in a 50-liter sample of air. However, for thiram dissolved in latex paint, the detection limit is only about 5.0 μg/mℓ because the extraction efficiency is lower.

3. Interferences

3.1 Acrylamide polymers interfere with the extraction and determination of thiram.

3.2 Other dithiocarbamates such as ferbam and ziram intensify the color and cause erroneously high values.

3.3 The presence of metallic chelates and iron and other metal compounds will interfere with the spectrophotometric determination of thiram.

3.4 The collection on a filter of airborne particles of latex may reduce the amount of thiram that can be collected before the filter becomes clogged.

3.5 The precision and accuracy of the method are particularly sensitive to the type and amount of latex paint collected on the filter. A sample of the bulk paint should be submitted at the same time as the filter samples to allow determination of the extraction efficiency and the reproducibility of the determination of thiram in the presence of the paint.

4. Precision and Accuracy

4.1 The precision of the total sampling and analytical method has not been determined. However, the coefficient of variation for the analytical procedure has been found to be 0.23 over the range of 5 to 20 μg/mℓ.

4.2 The accuracy of the procedure has not been determined. The recovery of thiram by this method ranged from 84 to 98% in twenty spiked filter samples.

5. Advantages and Disadvantages

5.1 The nonspecificity of the reaction of cuprous iodide with dithiocarbamates and other sulfur compounds is a disadvantage.

5.2 The deterioration of the standard solutions of thiram in chloroform is a disadvantage. (Refrigeration retards deterioration.)

5.3 Thiram is sensitive to heat and light, especially in the presence of latex paint. Samples should be stored in a cool, dry, and dark place and analyzed as soon as possible.

5.4 In the absence of interferences the method is simple, rapid, and sensitive.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Cellulose ester membrane filters, 0.8-μm pore size, 37-mm diameter, Millipore Type AA, or equivalent. Plain white, white-gridded, or black-gridded filters may be used.

6.1.2 Personal sampling pump capable of operation at 1.5 to 2.0 ℓ/min for extended periods of time. The pump should be calibrated with a representative filter unit in the sampling line. A wet or dry test meter or a glass rotameter capable of measuring a flow rate of 1.5 to 2.0 ℓ/min to within 5% may be used in the calibration.

6.2 Spectrophotometer, for use at 440 nm, with matched glass cells or cuvettes, 1-cm path length.

6.3 Vortex mixer.

6.4 Centrifuge capable of attaining 2500 rpm.

6.5 Centrifuge tubes, glass-stoppered, 15 mℓ.

6.6 Water bath, 60 ± 5°C.

6.7 Filter paper, Whatman No. 42, or equivalent, and long-stem filter funnels.

6.8 Volumetric flasks, 10, 25, and 100 mℓ.

6.9 Volumetric pipettes, assorted sizes.

6.10 Burette, 25 mℓ.

7. **Reagents**

7.1 Chloroform, spectro grade.

7.2 Acetone, spectro grade.

7.3 **Thiram Standard Solutions**

7.3.1 **Stock Solution (500 μg/mℓ).** Dissolve 50 mg of thiram in chloroform and dilute the solution with chloroform to 100 mℓ in a volumetric flask.

7.3.2 **Working Solution (25 μg/mℓ).** Dilute 5 mℓ of the stock solution to 100 mℓ with chloroform in a volumetric flask.

7.4 Cuprous iodide, purified.

7.5 Nitrogen, prepurified, water-pumped.

681

8. **Procedure**

8.1 **Cleaning of Equipment.** Rinse all glassware with acid, deionized water, and chloroform, and allow to dry. Store the cleaned glassware in such a manner as to prevent contamination.

8.2 **Collection and Shipping of Samples**

8.2.1 Connect the filter unit to the pump and sample at a rate of 1.7 ℓ/min for a period of time anticipated to provide sufficient thiram for analysis. Measure as accurately as possible the flow rate and time, or volume.

8.2.2 After the sampling is completed, tape in place the top and bottom plugs for the inlet and outlet of the filter unit.

8.2.3 Wrap the filter unit in toweling or packaging material and place it in a suitable container for shipment to the laboratory.

8.2.4 Handle one filter unit in the same manner as the filter units exposed to thiram, but sample no air through it. Label this unit as a blank and ship it to the laboratory with the unknown samples.

8.2.5 Ship to the laboratory in a separate container samples of the bulk substances contaminating the workplace atmosphere. These substances may include thiram, latex paint, and thiram in latex paint.

8.2.6 After their receipt in the laboratory, store the samples in a cool, dry, and dark area to prevent oxidative and photocatalyzed degradation.

8.3 **Analysis of Samples**

8.3.1 Disassemble the filter unit. Remove the membrane filter with tweezers and place it in a 15-mℓ centrifuge tube.

8.3.2 Add 8 mℓ of chloroform to the centrifuge tube and agitate with a vortex mixer for 2 min. Then centrifuge the tube at 2500 rpm for 4 to 6 min. If the solution is cloudy, proceed to Section 8.3.3 If no cloudiness is apparent, transfer the solution quantitatively to a 10-mℓ volumetric flask and dilute to the mark with chloroform; proceed to Section 8.3.4.

8.3.3 Cloudiness indicates the presence of acrylamide polymers. Decant the supernatant and add acetone to it dropwise until the polymers precipitate out of solution. Recentrifuge and decant again. Evaporate the solution to dryness under nitrogen on a water bath at 60°C. Add 8 mℓ of chloroform to the dry residue. (If no precipitate forms on the addition of a few drops of acetone, the solution should be recentrifuged, but need not be evaporated to dryness.) Quantitatively transfer the solution to a 10-mℓ volumetric flask and bring to volume with chloroform. Proceed to Section 8.3.4.

8.3.4 Add 30 ± 1 mg of cuprous iodide to the 10-mℓ volumetric flask. Allow the color to develop for 60 ± 5 min. Shake the flask at least five times during this time span. The color is stable for about 30 min after it is developed. Filter the sample solution through Whatman No. 42 filter paper into another 10-mℓ volumetric flask. Measure the absorbance of the sample solution at 440 nm with a reagent blank as the reference.

8.4 **Determination of Extraction Efficiency**

8.4.1 Place a blank membrane filter into each of five centrifuge tubes. From a 25-mℓ burette add 0.8, 2, 4, 6, and 10 mℓ of the thiram working standard (25 μg/mℓ) to the respective tubes. Without removing the filters evaporate the chloroform under nitrogen at room temperature. Then add 8 mℓ of chloroform to each tube and proceed as described in Sections 8.3.2 and 8.3.4.

8.4.2 The extraction efficiency is found by comparing the results of the analyses of these "spiked" filters to the results of the analyses of the standard solutions of thiram as described in Section 9.1.

8.4.3 If the unknown samples contain latex paint, a thiram and latex paint mixture should be spiked on the filter to ascertain the effect on extraction efficiency. The analysis procedure for filters spiked with the thiram in latex paint must include the precipitation of acrylamide polymers with acetone.

9. **Calibration and Standards**

9.1 Prepare standards by adding 0.8, 2, 4, 6, and 10 mℓ of the thiram working solution to separate volumetric flasks and diluting to the mark with chloroform. Respectively, the standards contain 20, 50, 100, 150, and 250 μg of thiram. For their analysis follow the procedure described in Section 8.3.4. Plot absorbance versus the amount (in μg) of thiram in the standard.

9.2 Prepare a calibration curve daily; the curve should cover the anticipated range of the sample unknowns. Maintain a quality control chart for the replicate determinations of standards. (See Reference 11.3.)

10. **Calculations**

10.1 From the calibration curve, read the amount (in μg) of thiram found in the sample solutions. Correct this amount for any dilution of the chloroform extract beyond that called for in Section 8.3.4. Also correct for any thiram found on the blank filter and for the extraction efficiency. These calculations are summarized in the following equation.

1983

$$W = \frac{(W_S \times D) - W_b}{E}$$

where:
- W = the net amount of thiram found on the sample filter.
- W_S = the amount of thiram found in the sample solution.
- D = the dilution factor
- W_b = the amount of thiram found on the blank filter
- E = the average extraction efficiency.

10.2 The concentration of thiram in air may be expressed in mg/m^3:

$$mg/m^3 = \frac{W\ (\mu g)}{V\ (\ell)}$$

where: V = volume of air sampled.

11. References

11.1 Horwitz, W., Ed., *"Methods of Analysis of the Association of Official Analytical Chemists,"* 12th ed, A.O.A.C., Washington, D.C., 1975, pp 551-552.

11.2 Linch, A. L., *"Evaluation of Ambient Air Quality by Personnel Monitoring,"* CRC Press, Cleveland, Ohio, 1974, pp 127-171.

11.3 *"Handbook for Analytical Control in Water and Wastewater Laboratories,"* Analytical Quality Control Laboratory, National Environmental Research Center, Cincinnati, Ohio, 1972.

11.4 *"Pesticide Analytical Manual,"* Vol. II U. S. Dept. of Health, Education and Welfare, Federal Drug Administration, Rockville, Maryland.

11.5 Sunshine, I., Ed., *"Handbook of Analytical Toxicity,"* CRC Press, 1969.

APPENDIX

Analysis of Tree Samples for Thiram

Weigh each tree, cutting into pieces when necessary. Place the tree in a centrifuge tube. Wash with 20 mℓ of chloroform, centrifuge, and filter into a 25-mℓ volumetric flask. Add 30 mg of cuprous iodide. Allow the color to develop for 60 ± 5 min, shaking the flask every 10 min. Filter and measure the absorbance at 440 nm. If the concentration of thiram is high, further dilution may be necessary. Calculate the total micrograms of thiram and the micrograms of thiram per tree.

PENTACHLOROPHENOL IN URINE

Measurements Support Branch

Analytical Method

Analyte: Pentachlorophenol (PCP)	Method No.: P&CAM 230
Matrix: Urine	Range: 20 to 180 μg/ℓ in 2 mℓ of urine
Procedure: Benzene extraction; gas chromatography	Precision (CV): 0.15 (estimated analytical)
Date Issued: 6/30/76	Classification: E (Proposed)
Date Revised:	

1. **Principle of the Method**

   Urine is acidified with concentrated sulfuric acid, shaken with benzene, and centrifuged. An aliquot of the benzene layer is injected into a gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD). The peak heights for the PCP in the samples are compared with those of calibration standards.

2. **Range and Sensitivity**

   2.1 The linear range of the method is about 20 to 180 μg/ℓ of PCP in a 2-mℓ sample of urine. The upper limit can be extended by diluting the benzene extract and applying the dilution factor in the calculations.

   2.2 The detection limit is about 10 μg/ℓ in a 2-mℓ sample of urine.

3. **Interferences**

   Chlorinated or other electrophilic organic compounds having the same chromatographic retention time as pentachlorophenol will interfere in the analysis. This type of interference often can be eliminated by changing the operating conditions of the gas chromatograph.

4. **Precision and Accuracy**

   4.1 A coefficient of variation of 0.15 was estimated from the data obtained from the injection of standard solutions.

   4.2 The accuracy of the method has not been determined.

5. Advantages and Disadvantages of the Method

5.1 The method is highly sensitive and selective for PCP.

5.2 PCP is analyzed directly without the additional step of derivative formation. PCP is eluted from the gas chromatographic column within a reasonable time, while less polar compounds are eluted earlier.

5.3 If samples containing large amounts of PCP are injected into the gas chromatograph, the detector may be overloaded and may require considerable time to recover to the baseline response.

6. Apparatus

6.1 Glass bottles, 50 mℓ, with Teflon caps for the collection of samples.

6.2 Gas chromatograph equipped with a ^{63}Ni electron capture detector.

6.3 GC column, 0.25 in. o.d. by 0.08 in. i.d. by 6 ft long, silanized glass, packed with 1% DEGS-PS on 60/80-mesh Supelcoport with phosphoric acid-treated glass wool at the column ends, or an equivalent column.

6.4 An oxygen scrubber and a molecular sieve drying trap filled with 30/40-mesh Molecular Sieve 5A for the nitrogen carrier gas in the gas chromatographic procedure.

6.5 Strip chart recorder.

6.6 Mixer or shaker for vials.

6.7 Centrifuge.

6.8 Reaction vials, 7 mℓ, with aluminum caps containing self-sealing septa.

6.9 Volumetric flasks, 5, 10, 25, and 50 mℓ.

6.10 Pipettes, 1, 2, 4, and 5 mℓ.

6.11 Micropipettes, 5, 10, 25, 100, 250, and 500 μℓ.

6.12 Microsyringes, 10 μℓ.

6.13 Analytical balance capable of weighing to 0.1 mg.

6.14 A urinometer or suitable hydrometer.

7. **Reagents**

7.1 Benzene, distilled in glass, pesticide quality.

7.2 Sulfuric acid, ultra high purity.

7.3 Pentachlorophenol, reagent grade or better.

7.4 **PCP Stock Solution "A" (200 μg/mℓ).** In a 50-mℓ volumetric flask, dissolve 10.0 mg of PCP in about 25 mℓ of benzene and dilute to the mark with benzene.

7.5 **PCP Stock Solution "B" (1 μg/mℓ).** In a 25-mℓ volumetric flask, dilute 125 μℓ of Stock Solution "A" to the mark with benzene.

7.6 Nitrogen, prepurified grade.

8. **Procedure**

8.1 **Cleaning of Equipment**

8.1.1 Clean all glassware with detergent and rinse well with both tap and distilled water before beginning the analysis. Glassware should be baked in a muffle furnace, if one is available.

8.1.2 Clean the microsyringes with organic solvents.

8.2 **Collection and Shipping of Samples**

Collect the samples in glass bottles cleaned as described in Section 8.1.1. If the analysis is not to be performed immediately, refrigerate the samples.

8.3 **Analysis of Samples**

8.3.1 Pipette 2.0 mℓ of urine into a 7-mℓ vial and add two drops of concentrated sulfuric acid.

8.3.2 Pipette 2.0 mℓ of benzene into the same vial, seal the vial, and agitate it for 1 to 2 min with the shaker.

8.3.3 Centrifuge the samples at about 1500 rpm until the benzene and urine layers have completely separated. This step requires about 15 to 30 min depending on the consistency of the urine.

8.3.4 GC Operating Conditions.

- Inlet temperature, 195°C.
- Detector temperature, 285°C.
- Column temperature, 200°C.
- Purge flow rate, as required by the specific ECD system employed.

8.3.5 Using the solvent flush technique, inject 5.0 μℓ of the benzene layer into the gas chromatograph.

8.3.6 If the concentration of PCP is above the range of the calibration curve, dilute the extract until a response within the range is obtained.

8.3.7 Determine the peak area electronically or measure the peak height manually. Read preliminary results from the calibration curve prepared as directed in Section 9.

8.4 Determination of Extraction Efficiency

8.4.1 Prepare standard benzene solutions of PCP having concentrations of 2.00 and 200 μg/mℓ.

8.4.2 Into each of eight vials pipette 2.0 mℓ of urine uncontaminated with PCP.

8.4.3 "Spike" the urine samples with the standards to obtain the indicated concentrations of PCP in urine:

Concentration of PCP in benzene, μg/mℓ	Amount of solution used, μℓ	Concentration of PCP in urine, μg/ℓ
2.00	50	50
2.00	100	100
200	30	3000

Prepare two spikes at each concentration and also two blank urine samples.

8.4.4 Add two drops of concentrated sulfuric acid to each vial.

8.4.5 Pipette 2.0 mℓ of benzene into each vial, seal the vials, shake, and centrifuge as in Sections 8.3.2 and 8.3.3.

8.4.6 Into each of four more vials pipette 2.0 mℓ of benzene. Add the same amounts of the standard solution as in Section 8.4.3; however, prepare only one standard for each concentration. Use the fourth vial as a blank. Cap all four vials.

8.4.7 Dilute the standards and samples containing 3000 μg/ℓ to 100 mℓ.

8.4.8 Analyze the spiked urine samples and standards together as described in Sections 8.3.4 to 8.3.7. The extraction efficiency (EE) at a particular concentration is the average amount of PCP found in the spiked urine samples divided by the average amount found in the standards. These amounts should be corrected for any PCP found in the blanks.

$$EE = \frac{\mu g \text{ PCP recovered}}{\mu g \text{ PCP added}}$$

9. Calibration and Standards

9.1 Prepare a series of standards according to the table below. With a micropipette add the indicated volumes of Stock Solution A or B to a volumetric flask of the indicated size (third column). Fill each flask to the mark with benzene and mix.

Stock solution used	Volume, μℓ	Final volume, mℓ	Concentration, μg/ℓ
B	200	25	8
B	500	25	20
B	1000	25	40
B	800	10	80
A	30	50	120
A	40	50	160

9.2 Inject 5.0 μℓ-aliquots of these standards into the gas chromatograph.

9.3 Construct a calibration curve by plotting peak height *versus* PCP concentration (in μg/ℓ).

10. Calculations

10.1 From the calibration curve, read the concentration (in μg/ℓ) of PCP found in the benzene extract. If the extract was not diluted prior to GC analysis, the concentration read from the curve corresponds to the PCP concentration in the original urine sample since 2 mℓ of urine was extracted with 2 mℓ of benzene. If the benzene extract was diluted, then the concentration read from the curve should be corrected for this dilution. Obtain the dilution factor from Section 8.3.6.

689

10.2 If the extraction efficiency is found to be less than 1.0, divide the determined concentration by the extraction efficiency.

10.3 These calculations are summarized as follows:

$$\mu g/\ell = \frac{\mu g/\ell \text{ (from curve) x dilution factor}}{\text{extraction efficiency}}$$

10.4 The use of a specific gravity factor to normalize values to 1.024, the average specific gravity of urine, has been recommended. (See Reference 11.5.) The preferred method of normalization is to make a straightforward gravity correction as follows:

$$\text{Corrected mg/m}\ell = \text{mg/m}\ell \times \frac{0.024}{\text{specific gravity} - 1.000}$$

Report the uncorrected concentration, the corrected concentration, and the specific gravity of the sample.

11. References

11.1 Rivers, J. B., **Bull. Environ. Contam. Toxicol.**, 8, 294 (1972).

11.2 Bevenue, A., M. L. Emerson, L. J. Casarett, and W. L. Yauger, **J. Chromatogr.**, 38, 467 (1968).

11.3 Chau, A. S. Y., and J. A. Coburn, **J. Assoc. Offic. Anal. Chemists**, 57, 389 (1974).

11.4 Bevenue, A., J. R. Wilson, E. F. Potter, Moon Ki Song, H. Beckman, and G. Mallett, **Bull. Environ. Contam. Toxicol.**, 1, 257 (1966).

11.5 Elkins, H. B., "Concentration Adjustments in Urinalysis," **Am. Ind. Hyg. Assoc. J.**, 35, 559 (1974).

NITROGEN DIOXIDE AND NITRIC OXIDE IN AIR

Measurements Support Branch

Analytical Method

Analyte: Nitrogen Dioxide and Nitric Oxide	**Method No.:** P&CAM 231
Matrix: Air	**Range:** 0.8 to 30 ppm of NO_2 or NO in a 1-liter sample
Procedure: Solid sorbent collection; triethanolamine extraction; spectrophotometry	**Precision(CV_T):** NO_2, 0.07 at 0.5 to 5 ppm; NO, 0.06 at 12.5 to 50 ppm
Date Issued: 6/30/76	**Classification:** D (Operational)
Date Revised:	

1. **Principle of the Method**

Nitrogen dioxide (NO_2) and nitric oxide (NO) are collected from air in a three-section sorbent tube. The NO_2 is absorbed in the first section, which contains triethanolamine (TEA) impregnated on molecular sieve. The NO is converted to NO_2 by a proprietary oxidizer in the second section. The NO_2 thus formed from the NO is absorbed in the third section by another bed of TEA-impregnated molecular sieve. The first and third sections are desorbed with solutions of TEA in water and the nitrite in these solutions is determined spectrophotometrically by the Griess-Saltzman reaction. (Reference 11.1). The nitrite found in the first section is reported as NO_2 and the nitrite in the third section is reported as NO.

2. **Range and Sensitivity**

2.1 The linear range of the standard curve is from 0.5 to 18 μg of nitrite in 10 mℓ of desorbing solution, which corresponds in this method to a range of 0.8 to 30 ppm of NO_2 or NO in a 1-liter sample of air.

2.2 The sensitivity is 0.4 μg/10 mℓ for an absorbance of 0.04.

2.3 The upper limit of the range can be extended by taking smaller aliquots for analysis, or be diluting intensely colored solutions with water.

3. Interferences

3.1 Inorganic nitrites cause positive interference.

3.2 Nitric acid and nitrates do not interfere.

3.3 Ammonia does not interfere.

4. **Precision and Accuracy**

4.1 The average recovery for 22 samples in the range 0.5 to 5 ppm of NO_2 was greater than 96% and the coefficient of variation was 0.07.

4.2 For 18 samples the average recovery of NO varied with the amount of NO collected. The recovery was 100% at 12.5 ppm. At 25 ppm only 84% recovery was achieved, and at 50 ppm only 67%. However, the coefficient of variation over the range was only 0.06. The recovery may vary depending upon the sample flow rate and the properties of the particular lot of oxidizer used. Each laboratory should determine the efficiency of the sampling tubes employed.

4.3 The accuracy of the overall sampling and analytical method has not been determined.

5. **Advantages and Disadvantages of the Method**

5.1 Both nitrogen dioxide and nitric oxide are collected simultaneously.

5.2 This method is simple and convenient for field sampling.

5.3 Samples can be stored at ambient temperature for at least 10 days without any effect on the results.

5.4 At 50 ppm of NO the collection efficiency is poor (about 67%) because the oxidizer is consumed.

5.5 If high humidity or water mist is present, the breakthrough volume can be severely reduced. If water condenses in the tube, NO_2 and NO may not be collected quantitatively.

6. **Apparatus**

6.1 **Sampling Equipment**

6.1.1 Solid sorbent tubes are made in the following manner. Using a gas-oxygen torch, heat a section of 5-mm i.d., 7-mm o.d. Pyrex glass tubing and pull it

apart to form a tube approximately 15 cm long with a taper 2 cm long. Seal the tapered end of the tube in the flame. Allow it to cool, then insert a small plug of glass wool through the open end of the tube; push the glass wool through the open end of the tube with a thin wooden stick and pack gently. Weigh 400 mg of TEA sorbent and pour the material into the tube. (See Section 7.2) Gently tap the tube on the table top several times to ensure uniform packing. Insert another small plug of glass wool to keep the TEA sorbent in place. For the next section, pour 800 mg of oxidizer into the tube. (See Section 7.1.) Again tap the tube and insert a plug of glass wool; pack lightly. Insert another plug of glass wool, maintaining an air gap of 12 mm between these two plugs. Weigh 400 mg of TEA sorbent and pour the material into the tube. Carefully tap the tube and gently pack another glass wool plug without closing the 12-mm air gap. Seal the open end of the tube with the torch. See the figure on page 231-9.

6.1.2 A personal sampling pump that can provide a flow rate of 50 mℓ/min within 5% accuracy is required. The pump should be calibrated with a representative sorbent tube in the sampling line. A dry or wet test meter or glass rotameter that will determine the flow rate to within 5% may be used for the calibration.

6.2 Spectrophotometer capable of measurements at 540 nm.

6.3 Matched glass cells or cuvettes, 1-cm path length.

6.4 Assorted laboratory glassware: pipettes, glass-stoppered graduated cylinders, and volumetric flasks of appropriate sizes.

7. **Reagents**

7.1 **Oxidizer.** Proprietary material Number 1900277 from the Drägerwerk Company of West Germany, supplied through its U.S. distributor, National Mine Safety Company, or the equivalent.

7.2 **TEA Sorbent.** Place 25 g of triethanolamine in a 250-mℓ beaker; add 4 g of glycerol, 50 mℓ of acetone and sufficient distilled water to bring the volume up to 100 mℓ. To the mixture add about 50 mℓ of Type 13X, 30/40-mesh Molecular Sieve. Stir and let stand in a covered beaker for about 30 min. Decant the excess liquid, and transfer the molecular sieve to a porcelain pan. Place the pan under a heating lamp until most of the moisture has evaporated. Complete the drying in an oven at 110°C for 1 hr. The sorbent should be free flowing. Store it in a closed glass container.

7.3 **Desorbing Solution.** Dissolve 15.0 g of triethanolamine in approximately 500 mℓ of distilled water, add 0.5 mℓ of *n*-butanol, and dilute to 1 liter.

7.4 **Hydrogen Peroxide, 0.02%(v/v).** Dilute 0.2 mℓ of 30% hydrogen peroxide to 250 mℓ with distilled water.

7.5 **Sulfanilamide Solution.** Dissolve 10 g of sulfanilamide in 400 mℓ of distilled water. Add 25 mℓ of concentrated phosphoric acid, mix well, and dilute to 500 mℓ.

7.6 **NEDA Solution.** Dissolve 0.5 gm of N-(1-naphthyl)ethylenediamine dihydrochloride in 500 mℓ of distilled water.

7.7 **Nitrite Stock Standard Solution (100 μg/mℓ).** Dissolve 0.1500 g of reagent grade sodium nitrite in distilled water and dilute to 1 liter.

8. **Procedure**

8.1 **Cleaning of Equipment.** Wash all glassware with detergent solution, soak in nitric acid, rinse in tap water and distilled water, and then rinse thoroughly with double distilled water.

8.2 **Collection and Shipping of Samples**

8.2.1 Before sampling, break open the ends of the sorbent tube to provide an opening that is approximately one-half the internal diameter of the tube.

8.2.2 The air must flow through the 12-mm air space before it flows through the oxidizer. Therefore attach the end of the tube without the air gap between the oxidizer section and TEA sorbent section to the pump with a length of small diameter Tygon® tubing.

8.2.3 Mount the tube in a vertical position to avoid channeling.

8.2.4 The air being sampled should not pass through any hose or tubing before it enters the sorbent tube.

8.2.5 Turn on the pump to begin sample collection. Sample at a flow rate of 50 mℓ/min or less to obtain a maximum sample volume of 1 liter. Measure the flow rate and time, or volume, as accurately as possible. If a low flow rate pump is used, set the rate to an approximate value and record the initial and final stroke counter readings. Obtain the sample volume by multiplying the number of strokes by the stroke volume.

8.2.6 Measure and record the temperature and pressure of the atmosphere being sampled.

8.2.7 Cap the sorbent tubes with 7-mm i.d. plastic caps immediately after sampling. (Masking tape can be substituted for the plastic caps.)

8.2.8 With each batch of samples, submit one blank sorbent tube. This tube is handled in the same manner as the other tubes (break, seal, and transport) except that no air is drawn through it. When more than ten samples are submitted, include an additional blank for every ten samples.

8.2.9 Pack the capped sorbent tubes tightly and pad them to minimize breakage during shipping.

8.3 **Analysis of Samples**

8.3.1 With tweezers remove and discard the glass wool plugs from an exposed sorbent tube and transfer each TEA sorbent bed to separate, 25-mℓ glass-stoppered graduated cylinders. Label the graduated cylinder as to the location of the TEA sorbent with respect to the oxidizer section.

8.3.2 To each graduated cylinder add enough of the desorbing solution to make the volume up to 20 mℓ, and shake the mixture vigorously for about 30 sec.

8.3.3 Allow a few minutes for the solids to settle, and then transfer 10 mℓ to another 25-mℓ glass-stoppered graduated cylinder.

8.3.4 Develop the color of the solution for 10 min in the same manner as described for the preparation of the standard curve (Sections 9.4 to 9.6). From the standard curve determine the amount of nitrite in the 10-mℓ aliquot.

8.4 **Determination of Collection and Desorption Efficiencies**

8.4.1 **Importance of Determination.** The collection and desorption efficiencies of a given compound can vary from one laboratory to another and also from one batch of sorbent tubes to another. Thus, it is necessary to determine at least once the percentages of sample collected and then removed in the desorption process. Results indicate that the recovery of NO varies with the amount of NO collected, particularly at higher concentrations (for example, at 50 ppm).

8.4.2 **Procedure for Determining Collection and Desorption Efficiencies.** Sorbent tubes from the same batch as that used in obtaining samples are used in this determination. Known volumes of NO_2 and NO are injected into a bag containing a known volume of air. The bag is made of Tedlar (or another material that will not absorb NO_2 or NO) and should have a gas sampling valve and a septum injection port. The concentrations of NO_2 and NO in the bag may be calculated at room temperature and pressure. A measured volume is then sampled through a sorbent tube with a calibrated sampling pump. At least five tubes are prepared in this manner. These tubes are desorbed and analyzed in the same manner as the samples (Section 8.3).

8.4.3 **Calculation of Desorption Efficiency.** The desorption efficiency (D.E.) is the average concentration (corrected for the blank) of NO_2 or NO found by analysis of the sorbent tubes divided by the concentration of NO_2 or NO in the bag.

9. **Calibration and Standards**

9.1 Dilute 2 mℓ of the nitrite stock standard (100 μg/mℓ) to 100 mℓ with the desorbing solution to prepare a solution with a nitrite concentration of 2 μg/mℓ.

9.2 To a series of 25-mℓ glass-stoppered graduated cylinders add 1, 3, 5, 7, and 9 mℓ of the dilute standard solution.

9.3 Add enough of the absorbing solution to bring the volume in each cylinder up to 10 mℓ to prepare working standards with nitrite concentrations of 2, 6, 10, 14, and 18 μg/10 mℓ.

9.4 To each graduated cylinder, add 1 mℓ of the 0.02% hydrogen peroxide solution, 10 mℓ of the sulfanilamide solution, and 1.4 mℓ of the NEDA solution, with thorough mixing after the addition of each reagent.

9.5 Allow 10 min for complete color development.

9.6 Measure the absorbance of the solutions at 540 nm, using a reagent blank in the reference cell.

9.7 Prepare a standard curve by plotting absorbance *versus* weight of nitrite (in μg) in 10 mℓ of the desorbing solution.

10. Calculations

10.1 From the standard curve, read the weight of nitrite (in μg) in 10 mℓ of the desorbing solution corresponding to the absorbance of the sample solution. Multiply this weight by 2 to determine the total amount (in μg) of nitrite extracted with 20 mℓ of desorbing solution from the sorbent section being analyzed. The calibration procedure is based upon the empirical observation that 0.63 mole of sodium nitrite produces the same absorbance in the color-developed solution as 1 mole of NO_2. (See Reference 11.2.) Divide the amount of nitrite desorbed from the sorbent material by 0.63 to determine the apparent amount of NO_2 collected in the sorbent section. These calculations are summarized in the following equation:

$$W = \frac{\mu g\ NO_2^- \times 2}{0.63}$$

where: W = weight (in μg) of NO_2 found.

10.2 Correct the amount of NO_2 calculated in Section 10.1 for the amount of NO_2, if any, found on the corresponding sorbent section of a blank tube to obtain the amount of NO_2 in the sample, as follows:

$$W_s = W - W_b$$

where: W_s = corrected weight (in μg) of NO_2 in sample.

W_b = weight (in μg) of NO_2 in the corresponding section of a blank tube.

10.3 The concentration of NO_2 in parts per million (ppm) by volume in the air sample is calculated as follows:

$$ppm = \frac{W_s}{V} \times \frac{24.45}{M.W.} \times \frac{760}{P} \times \frac{T+273}{298}$$

where: V = volume (liters) of air sampled.
M.W.= molecular weight.
24.45= molar volume (liter/mole) at 25°C and 760 mmHg.
P = pressure (mmHg) of air sampled.
T = temperature (C°) of air sampled.

10.4 The ppm of NO_2 found in the third section (downstream from the oxidizer) is reported as ppm of NO.

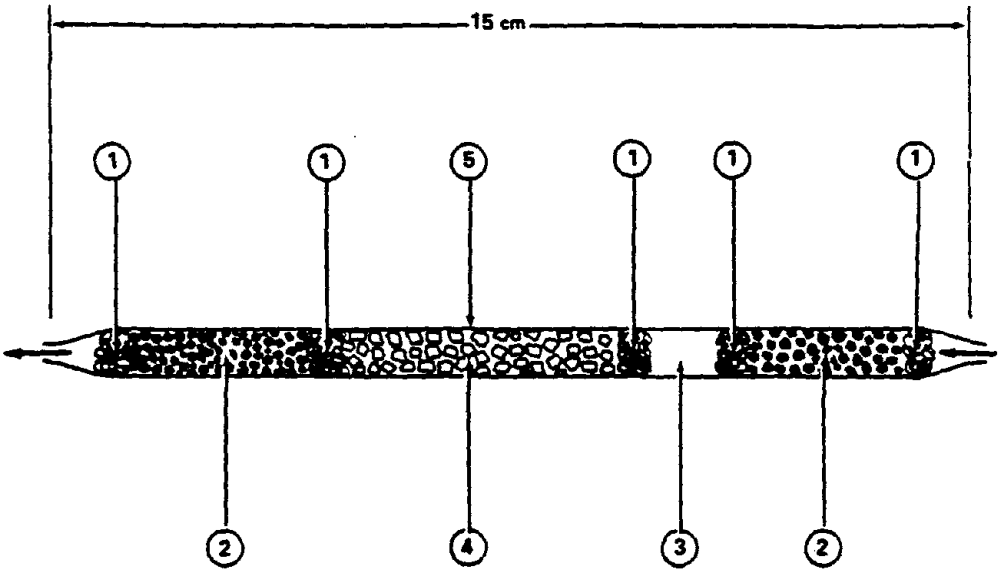
11. References

11.1 Saltzman, B.E. "Colorimetric Microdetermination of Nitrogen Dioxide in the Atmosphere," Anal. Chem., 26, 1949 (1954).

11.2 Blacker, J. H., "Triethanolamine for Collecting Nitrogen Dioxide in the TLV Range," Am. Ind. Hyg. Assoc. J., 34, 390 (1973).

11.3 NIOSH Sampling Data Sheet No. 32.01, "NIOSH Manual of Sampling Data Sheets," Measurements Research Branch, Division of Physical Sciences and Engineering, National Institute for Occupational Safety and Health, December 22, 1975.

11.4 Willey, M.A., C. S. McCammon, Jr., and L. J. Doemeny, "A Solid Sorbent Personal Sampling Method for the Simultaneous Collection of Nitrogen Dioxide and Nitric Oxide in Air," presented at the American Industrial Hygiene Association Conference, Atlanta, Georgia, May 1976.



1. GLASS WOOL PLUGS
2. TEA SORBENT, 400 mg
3. AIR GAP, 12 mm
4. OXIDIZER, 800 mg
5. GLASS TUBE, 5 mm i.d.

SORBENT TUBE FOR NO_2 and NO

FORMIC ACID IN AIR

Measurements Support Branch

Analytical Method

Analyte: Formic Acid	**Method No.:** P&CAM 232
Matrix: Air	**Range:** 3.8 to 75 mg/m³ in a 10-liter sample of air
Procedure: Impinger collection; gas chromatography	**Precision(CV):** 0.11 (analytical) over the above range
Date Issued: 6/30/76	**Classification:** D(Operational)
Date Revised:	

1. **Principle of the Method**

Formic acid in air is trapped in a solution of 0.1 *N* NaOH in a midget impinger. A derivatizing solution consisting of ethanol and sulfuric acid is added to an aliquot of the absorbing solution. The formic acid is converted to ethyl formate by heating to 55°C for 2 hr. A partitioning of the volatile ethyl formate takes place between the solution and the headspace gas above it. The headspace gas is analyzed for ethyl formate by gas chromatography. The amount of ethyl formate found is proportional to the amount of formic acid in the sample.

2. **Range and Sensitivity**

2.1 For 15 mℓ of absorbing solution, the useful range extends from 38 to 750 μg of formic acid, which corresponds to 3.8 to 75 mg/m³ in a 10-liter sample of air.

2.2 The range may be extended to lower amounts by using a smaller volume of absorbing solution.

2.3 Recovery data indicate that the linear range may extend from the detection limit of 7.5 μg/15 mℓ to 750 μg/15 mℓ.

3. **Interferences**

3.1 Low molecular weight acids and alcohols may interfere in the derivatization step if present in large amounts. These interferences can be reduced by following the analysis procedure carefully.

3.2 Since water is a product of the derivatization reaction, the ester will not be produced quantitatively in the aqueous reaction mixture. However, the amount of ethyl formate found has been shown to be proportional to the amount of formic acid collected and, also, has been shown to be reproducible. See Reference 11.1.

3.3 Any compound having the same retention time as ethyl formate in the GC procedure will interfere with the analysis. This type of interference can often be eliminated by changing the operating conditions of the chromatograph.

4. **Precision and Accuracy**

4.1 The average coefficient of variation was 0.11 for the analysis of 24 spiked samples in the range of 38 to 750 μg per sample.

4.2 An average recovery of 105% was obtained in the analyses of eight 15-mℓ samples of 0.1 *N* NaOH spiked with 15 to 600 μg of formic acid.

4.3 The accuracy of the method has not been determined.

5. **Advantages and Disadvantages of the Method**

5.1 The method is a straightforward technique with three steps. It requires no separation or distillation steps.

5.2 Derivatization is required.

6. **Apparatus**

6.1 A gas chromatograph equipped with dual flame ionization detectors.

6.2 A stainless steel column (20 ft by 0.125 in.) packed with 10% Carbowax 20 M on 80/100-mesh Chromosorb W, or an equivalent column.

6.3 Hamilton gas syringe, 1 mℓ, or equivalent.

6.4 Reaction vials, 10 mℓ.

6.5 Teflon-coated septums with tear-away seals for the reaction vials.

6.6 Volumetric pipettes, 2 mℓ; Eppendorf pipettes, 5, 10, 25, 50, and 100 μℓ.

6.7 Midget impinger, 15 mℓ.

6.8 A properly calibrated personal sampling pump capable of sampling at a flow rate of 2.5 ℓ/min for periods of up to 40 min. The pump should be calibrated with a midget impinger containing 15 mℓ of the absorbing solution in the sampling train. A dry or wet test meter or a glass rotameter that will measure the appropriate flow rate within 5% may be used for the calibration.

6.9 Constant temperature water bath capable of maintaining 55°C.

7. **Reagents**

7.1 **Absorbing Solution, 0.1 *N* NaOH.** Dissolve 40 g of sodium hydroxide pellets, ACS reagent grade, in distilled water, and dilute the solution to 1 liter with distilled water.

7.2 Absolute ethanol (anhydrous), uv spectrophotometric grade.

7.3 Sulfuric acid, concentrated, ACS reagent grade.

7.4 Ethyl formate, 99%.

7.5 **Derivatizing Solution.** To a 100-mℓ graduated cylinder add 70 mℓ of absolute ethanol. To the ethanol slowly add 20 mℓ of concentrated sulfuric acid. Allow the solution to cool to room temperature. Add ethanol until the volume is 100 mℓ and mix.

7.6 **Sodium Formate Solution.** In a 100-mℓ volumetric flask, dissolve 0.1478 g of sodium formate, ACS reagent grade, in 0.1 *N* NaOH and fill to the mark with 0.1 *N* NaOH. This solution is equivalent to a formic acid concentration of 1000 μg/mℓ.

7.7 Helium, Bureau of Mines Grade A, or equivalent.

7.8 Prepurified hydrogen.

7.9 Filtered, compressed air.

8. **Procedure**

8.1 **Cleaning of Equipment**

Wash all glassware in detergent solution and rinse with tap water. Soak the glassware in chromic acid cleaning solution (saturated solution of sodium dichromate in concentrated sulfuric acid) and rinse with tap water and distilled water.

8.2 **Collection and Shipping of Samples**

8.2.1 Pour 15 mℓ of 0.1 *N* NaOH into a midget impinger and connect the impinger to the vacuum pump with a short piece of flexible tubing.

8.2.2 Turn on the pump to begin sample collection. Measure the flow rate and time, or volume, as accurately as possible and record the temperature and pressure of the atmosphere being sampled. Sample at a flow rate of 2.5 ℓ/min. Collect a sample of 45 to 100 liters. A sample of 100 liters of air will allow measurement of at least 0.9 mg/m^3, one tenth the OSHA standard.

8.2.3 After sampling, remove the impinger stem. Tap the stem gently against the inside wall of the impinger to recover as much of the solution as possible. Then seal the impinger with a nonreactive stopper.

8.2.4 Care should be taken to minimize spillage or loss by evaporation.

8.2.5 Whenever possible, hand deliver the samples to the laboratory. Otherwise, ship the samples in the special impinger shipping cases designed by NIOSH.

8.2.6 Handle at least one impinger in the same manner as the other samples (fill, seal, and transport), but sample no air through this impinger. Label this sample as a blank.

8.3 Analysis of Samples

8.3.1 Withdraw a 2-mℓ aliquot from the impinger and place it in a 10-mℓ reaction vial.

8.3.2 Add 2 mℓ of derivatizing solution to the vial and seal it immediately with a Teflon-coated septum.

8.3.3 Place the sealed vial in a constant temperature water bath at 55°C for 2 hr.

8.3.4 After 2 hr, pierce the septum with the needle of the gas syringe.

8.3.5 Flush the syringe three times with the headspace gas.

8.3.6 Inject 1 mℓ of the headspace gas into the gas chromatograph. The operating conditions for the gas chromatographic analysis are as follows:

- Helium carrier gas flow rate, 30 mℓ/min.
- Hydrogen gas flow rate to the detector, 65 mℓ/min..
- Air flow rate to the detector, 500 mℓ/min.
- Injector temperature, 100°C.
- Detector temperature, 150°C.
- Column temperature, 70°C.

8.3.7 Measure the height of the ethyl formate peak. The retention time of ethyl formate is determined by injecting ethyl formate vapor into the gas chromatograph before the sample is injected.

9. Calibration and Standards

9.1 To 2 mℓ of distilled water in each of five 10-mℓ reaction vials add the amounts of sodium formate solution specified in the following table to prepare the five standards necessary for the calibration curve.

Vial No.	Volume of sodium formate solution, mℓ	Equivalent amount of formic acid per vial, μg
1	0.100	100
2	0.050	50
3	0.025	25
4	0.010	10
5	0.005	5

Standards of higher concentration may also be prepared.

9.2 Analyze the standards by the procedure described in Sections 8.3.2 to 8.3.7. Plot the height of the ethyl formate peak as a function of the equivalent amount (in μg) of formic acid in each vial.

10. Calculations

10.1 From the standard curve, read the weight (in μg) of formic acid corresponding to the peak height for the headspace sample. If the blank impinger solution produces a peak with the same GC retention time as the ethyl formate peak, subtract the calculated amount of formic acid from the sample value. To calculate the total amount of formic acid collected in the sample, use the following equation:

$$W = \frac{W_c \times I}{A}$$

where: W = amount of formic acid (μg) collected in the impinger.
W_c = amount of formic acid (μg) read from the calibration curve and corrected for the blank.
I = volume of the impinger sample (15 mℓ).
A = volume of the aliquot taken (2 mℓ).

10.2 The concentration of formic acid in air may be expressed in mg/m^3:

$$mg/m^3 = \frac{W\ (\mu g)}{V_s\ (\ell)}$$

where: V_s = volume of air sampled.

10.3 The concentration may also be expressed in terms of parts per million (ppm) by volume:

$$ppm = mg/m^3 \times \frac{24.45}{M.W.} \times \frac{760}{P} \times \frac{T+273}{298}$$

where: 24.45 = molar volume (ℓ/mole) at 25°C and 760 mmHg.

M.W. = molecular weight
P = pressure (mmHg) of air sampled.
T = temperature (°C) of air sampled.

11. References

11.1 Mraz, M., and V. Sedwec, "Determination of Toxic Substances and Their Metabolites in Biological Fluids by Gas Chromatography. VIII. Formic Acid in Urine," Collect. Czech. Chem. Commun., 38, 3426 (1973).

11.2 Smallwood, A. W., "Analysis of Formic Acid in Air Samples," presented at American Industrial Hygiene Conference, Atlanta, Georgia, May 1976.

DIAZONIUM SALTS AND AZO DYES IN AIR

Measurements Support Branch

Analytical Method

Analyte: Diazonium Salts and Azo Dyes	**Method No.:** P&CAM 234
Matrix: Air	**Range:** 0.01 to 0.4 mg/m³ in a 500-liter sample of air
Procedure: Filter collection; UV-VIS spectrophotometry	**Precision (CV):** 0.12 (analytical)
Date Issued: 6/30/76	**Classification:** D(Operational)
Date Revised:	

1. Principle of the Method

Duplicate samples of airborne particulate material are collected on cellulose acetate membrane filters. One filter is extracted with dilute hydrochloric acid and analyzed for diazonium salts spectrophotometrically at 375 nm. The second filter is extracted with an appropriate solvent and a spectrophotometric scan of the solution is made in the 400- to 700-nm range. The absorbance maxima are compared to the absorbance maxima of standard solutions prepared from bulk samples of the azo dyes.

2. Range and Sensitivity

2.1 Diazonium salts and azo dyes follow a Beer's law relationship in the range of 5 to 200 μg in 40 mℓ of solvent, the volume used to extract the filters. This corresponds to 0.01 to 0.4 mg/m³ in a 500-liter air sample.

2.2 Dilution of the sample solution with the appropriate solvent may be used to extend the upper limit of the range.

2.3 The lower limit of the range can be extended by decreasing the volume of the extracting solvent or by using an absorption cell of longer length.

2.4 The sensitivity is determined by the absorptivities of the individual salts and dyes.

3. Interferences

Any compound that absorbs radiation at the wavelengths corresponding to the absorbance maxima of the diazonium salts and azo dyes is a positive interference.

4. Precision and Accuracy

4.1 An average coefficient of variation of 0.12 has been established for the analytical procedure over the concentration range of the method.

4.2 The recovery of salts and dyes from filters with the appropriate solvent yielded an efficiency of 95 ± 5% in the range of 5 to 200 μg/40 mℓ of solvent.

4.3 The accuracy of the overall sampling and analytical method has not been determined.

5. Advantages and Disadvantages

5.1 The sampling procedure is rapid, simple, and involves no liquids.

5.2 The procedure can be used to distinguish between diazonium salts and azo dyes but not between two salts or two dyes absorbing at the same wavelength.

5.3 Since diazonium salts exhibit maximum absorption at approximately 375 nm, the method cannot be used for qualitative identification of individual salts. Thus the total diazonium salt concentration must be reported if more than one salt is being processed in the testing area on the day of sampling. Since each salt has a characteristic absorptivity, the concentration of total diazonium salts must be given as a range. The salt having the highest absorptivity determines the upper limit of the range and the salt having the lowest absorptivity determines the lower limit.

5.4 Diazonium salts deteriorate in the presence of light and moisture. Care must be taken to store them in a dry environment and to minimize exposure to light.

5.5 Bulk samples of all of the diazonium salts processed in the testing area on the day of sampling must be collected and a calibration curve must be established for each.

5.6 The solubilities of the various classes of azo dyes differ widely. Therefore, an appropriate solvent for each azo dye must be determined. Azo dyes that contain one or more sulfonic or carboxylic acid groups are usually soluble in dilute acid. Azo dyes with sulfonated naphthol groups are usually soluble in water or dilute base. Azo dyes with no solubilizing groups are usually insoluble in water but are soluble in polar or nonpolar organic solvents.

5.7 Absorption curves must be established for all azo dyes processed in the plant on the day of sampling. Azo dyes exhibit maxima in the 400- to 700-nm range, the exact wavelength being dependent on the structure of the dye molecule.

5.8 If more than one azo dye is being processed on the day of sampling and those dyes absorb at a common wavelength, the total azo dye concentration must be reported. The concentration of total azo dye must be given as a range with the dye exhibiting the highest absorptivity determining the upper limit and the dye exhibiting the lowest absorptivity determining the lower limit at a specific wavelength.

6. **Apparatus**

6.1 **Air Sampling Equipment**

6.1.1 Cellulose ester membrane filters, 0.8-μm pore size, 37-mm diameter, Millipore Type AA, or equivalent.

6.1.2 Filter holder for 37-mm filters, Millipore MAWP 037 A0, or equivalent.

6.1.3 Personal sampling pump, calibrated with a representative filter unit in the line. A wet or dry test meter or a glass rotameter capable of measuring a flow rate of 2 ℓ/min to within 5% may be used in the calibration.

6.2 Beckman Model 25 Scanning Spectrophotometer, or equivalent.

6.3 Matched fused silica cells, 1, 5, and 10 cm.

6.4 Balance capable of weighing accurately to one milligram.

6.5 Pipettes, various sizes.

6.6 Beakers, 100 mℓ.

7. **Reagents**

7.1 Hydrochloric acid, 0.1 *N* (pH = 0.9).

7.2 Bulk samples of all diazonium salts and azo dyes processed in the testing area on the day of sampling.

7.3 Appropriate solvents for dissolving the azo dyes.

7.3.1 Toluene, ACS reagent grade.

7.3.2 Distilled water.

7.3.3 **pH 5 Buffer Solution**. Prepare by adding potassium hydrogen phthalate to distilled water and adjusting the pH to 5.0 ± 0.2 with a calibrated pH meter.

7.3.4 **pH 9 Buffer Solution**. Prepare as above with ammonium acetate, adjusting the pH to 9.0 ± 0.2.

8. **Procedure**

8.1 **Cleaning of equipment**. Wash all glassware in hot detergent solution and rinse well with hot tap water, then several times with double distilled water.

8.2 Collection and Shipping of Samples

8.2.1 Place a clean filter and back-up pads into the cassette filter holder.

8.2.2 Connect the cassette to the vacuum pump. No tubing should be placed in front of the filter.

8.2.3 Turn on the pump to begin sample collection. Measure the flow rate and time, or volume, as accurately as possible. Sample 500 liters or more at 1.5 to 2 ℓ/min.

8.2.4 Take duplicate samples at each sampling site. One is for the diazonium salt analysis; the other for the azo dye analysis. Protect the filters from light to prevent photodecomposition of the diazonium salts.

8.2.5 Ship the filters in a suitable container designed by NIOSH to minimize contamination and to prevent damage in transit. Include two or more blank filters, which are handled in the same manner as the sample filters except that no air is sampled through them.

8.3 Analysis of Samples

8.3.1 Diazonium Salts

Extract one of the duplicate sample filters from each site with 40 mℓ of 0.1 *N* HCl in a 100-mℓ beaker. Agitate the mixture in an ultrasonic bath for 5 min. Allow the filter and any suspended solids to settle. Extract a blank filter in the same manner. Perform the extractions with minimum exposure to light. Transfer a portion of each solution to a 1-, 5-, or 10-cm fused silica cell, depending on the anticipated diazonium salt concentration. Determine the absorbance of the blank and the sample at 375 nm. Use 0.1 *N* HCl as the reference solution.

8.3.2 Azo Dyes

Select an appropriate solvent by experimentation with the bulk samples. (See Section 5.6.) Extract one of the duplicate sample filters and a blank filter with the solvent using the extraction procedure outlined in Section 8.3.1. Transfer a portion of each solution to a 1-, 5-, or 10-cm fused silica cell, depending on the anticipated concentration. Using the solvent as a reference, scan the 400- to 700-nm range to locate the absorbance maxima. Measure the absorbance at each wavelength where the bulk azo dyes absorb. (See Section 9.2)

9. Calibration and Standards

9.1 Diazonium Salts

9.1.1 Prepare a set of five standards for each diazonium salt that may be present in the sample. Each set should consist of standards containing 5, 25, 50, 100 and 200 μg of one diazonium salt in 40 mℓ of 0.1 *N* HCl.

9.1.2 Transfer a portion of each solution to a 1-, 5-, or 10-cm fused silica cell.

9.1.3 Using the 0.1 *N* HCl solution as the reference solution, determine the absorbance of each standard at 375 nm.

9.1.4 Construct a standard curve for each diazonium salt by plotting the absorbance against the amount (in μg) of the individual diazonium salt in the corresponding standard.

9.2 Azo Dyes

9.2.1 Prepare a set of standards for each azo dye as in Section 9.1.1 using the appropriate solvent.

9.2.2 Transfer each solution to a 1-, 5-, or 10-cm fused silica cell.

9.2.3 Scan the spectrum of one standard solution for each different dye to determine the wavelength of maximum absorption. Use the solvent as the reference solution.

9.2.4 Determine the absorbance of each standard at the wavelength of its maximum.

9.2.5 Construct a standard curve for each azo dye by plotting the absorbance against the amount (in μg) of the individual azo dye in the corresponding standard.

10. Calculations

10.1 Diazonium Salts

10.1.1 If only one diazonium salt is present in the sample, read from the appropriate calibration curve the amount (in μg) corresponding to the absorbance of the sample.

10.1.2 If more than one diazonium salt is present, the results must be given as a range. (See Section 5.3.) From the calibration curve for each diazonium salt, read the amount (in μg) corresponding to the absorbance of the sample. The upper limit of the range is taken from the curve yielding the largest value. The lower limit of the range is taken from the curve yielding the lowest value.

10.2 Azo Dyes

10.2.1 If only one azo dye is present in the sample, read from the appropriate calibration curve the amount (in μg) corresponding to the absorbance of the sample at the wavelength of maximum absorption.

10.2.2 If more than one azo dye is present, the results must be given as a range. (See Section 5.8) From the calibration curve for each azo dye absorbing at the same wavelength as the sample, read the amount (in μg) of azo dye corresponding to the absorbance of the sample. The upper limit of the range is taken from the curve yielding the largest value. The lower limit of the range is taken from the curve yielding the lowest value.

10.3 Correct the calculated amount or range of amounts of the appropriate diazonium salt(s) or azo dye(s) for any corresponding value found by the analysis of blank filters.

10.4 The concentration or range of concentrations of diazonium salts and azo dyes may be expressed in mg/m^3:

$$mg/m^3 = \frac{\text{Amount } (\mu g)}{V_s\,(\ell)}$$

where: V_s = volume (liters) of air sampled.

11. **Reference**

Neumeister, C.E., and H.J. Brass, "A Colorimetric Determination of Total Diazonium Salt and Azo Dye Concentrations in the Workplace Environment," presented at the American Industrial Hygiene Association Conference, Atlanta, Ga., May 1976.

FORMALDEHYDE IN AIR

Measurements Research Branch

Analytical Method

Analyte: Formaldehyde	**Method No.:** P&CAM 235
Matrix: Air	**Range:** 0.4 to 52 mg/m³ in a 6-liter sample
Procedure: Alumina collection; methanol-water elution; spectrophotometric measurement	**Precision** (CV_T) : 0.06 at 3.5 or 3.9 mg/m³
Date Issued: 6/30/76	**Classification:** E(Proposed)
Date Revised:	

1. **Principle of the Method**

   Formaldehyde is collected from air on alumina. The sample is stabilized immediately after collection by desorption into a 1% solution of methanol in water. The formaldehyde in this solution is then determined by the chromotropic acid - sulfuric acid spectrophotometric method. (References 11.1, 11.2, and 11.3)

2. **Range and Sensitivity**

   2.1 The linear range of the spectrophotometric analysis extends from a detection limit of about 1 μg to an amount near 20 μg, or about 0.1 to 2 μg/mℓ in the final solution. This corresponds to approximately 0.4 to 0.9 mg/m³ (0.3 to 0.7 ppm) of formaldehyde in a 6-liter sample of air.

   2.2 The absorbance of the sample solution at the detection limit is about 0.05 units greater than that of the reagent blank.

   2.3 The capacity of the sorbent tube at high humidity is at least 312 μg. This corresponds to a concentration of 52 mg/m³ when 6 liters of air are sampled. At lower humidities the capacity of the sorbent tube is higher.

3. **Interferences**

   3.1 The interferences discussed below have been evaluated for the chromotropic acid procedure described in Reference 11.3, which involves the collection of formaldehyde in an impinger-bubbler containing 0.1% chromotropic acid in concentrated sulfuric acid. These interferences have not been evaluated for the overall sorbent collection and analysis procedure described here.

713

3.2 The chromotropic acid procedure for formaldehyde suffers very little interference from other aldehydes. Saturated aldehydes give less than 0.01% positive interference. Ethanol and higher molecular weight alcohols and olefins in mixtures with formaldehyde are negative interferences. However, concentrations of alcohols in air are usually much lower than formaldehyde concentrations; therefore, alcohols are not serious interferents.

3.3 Phenols cause 10 to 20% negative interference when present at an 8:1 excess over formaldehyde. They are, however, ordinarily present at lower concentrations than formaldehyde and, therefore, are not serious interferents.

3.4 Ethylene and propylene in a 10:1 excess over formaldehyde in air cause 5 to 10% negative interference and 2-methyl-1,3-butadiene in a 15:1 excess over formaldehyde in air causes a 15% negative interference. Aromatic hydrocarbons also constitute a negative interference (Reference 11.3). It has recently been found that cyclohexanone causes a bleaching of the final color.

4. Precision and Accuracy

4.1 The precision of the overall sampling and analytical procedure has been determined at two concentrations of formaldehyde in air, 3.5 and 3.9 mg/m^3. The analysis of two sets of seven consecutive samples, one set exposed at each concentration, indicated a coefficient of variation for all results of 0.06.

4.2 The accuracy of the sorbent tube sampling method relative to impinger sampling has been measured to be 101% at 2.7 mg/m^3 and 107% at 6.6 mg/m^3.

5. Advantages and Disadvantages of the Method

5.1 Advantages

5.1.1 The sampling device contains a solid sorbent rather than a liquid absorbing solution. This results in easier sample handling without danger of spillage. The sorbent tube is smaller and less subject to accidental breakage than impingers.

5.1.2 Eluted alumina sections are transported to the laboratory in small, easily packed containers.

5.1.3 Formaldehyde is stabilized in the 1% methanol eluent. Eluted samples can be shipped and stored for at least one month without loss.

5.2 Disadvantages

5.2.1 A significant loss of formaldehyde absorbed on alumina occurs after an hour. Therefore, the sampling period is limited to 30 min and immediate elution is required.

5.2.2 The absorbance of the solution increases slowly on standing. An increase of 3% in absorbance was noted after the solution stood for one day and an increase of 10% was noted after the solution stood for eight days (Reference 11.3).

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Sorbent tubes. (See the figure on page 235-10.) The sorbent tubes consist of Pyrex glass tubes (8 mm i.d.) packed with two separate sections of 8/14-mesh activated alumina (Fisher Scientific Co., or equivalent). The weight of alumina in each section is 1.65 g. The sections are enclosed and separated by 100-mesh stainless steel screens held in place by Teflon rings. Other nonreactive plugs of negligible pressure drop may be used. The ends of each tube are flame-sealed after packing to prevent contamination prior to sampling. Polyethylene caps should be provided to seal the tubes after sampling has been completed.

6.1.2 Personal Sampling Pump. The personal sampling pump should be capable of operation at a constant flow rate of 200 mℓ/min for 30 min. A sample volume indicator is desirable. The pump should be calibrated with a representative sorbent tube in the line. A wet or dry test meter or a glass rotameter capable of measuring a flow rate of 200 mℓ/min to within 5% may be used in the calibration.

6.1.3 Thermometer, manometer, and stopwatch.

6.1.4 Small glass bottles or vials, with tightly fitted caps, that are sufficiently large to accommodate 1.65 g (2 mℓ) of 8/14-mesh alumina and 10 mℓ of the 1% methanol solution.

6.2 Spectrophotometer or Colorimeter. An instrument capable of measuring absorbance at 580 nm.

6.3 Assorted laboratory glassware.

6.4 Laboratory centrifuge capable of handling tubes containing 10.1 mℓ of solution.

915

7. Reagents

7.1 **Chromotropic Acid Reagent.** Dissolve in distilled water 0.10 g of 4,5-dihydroxy-2,7-naphthalenedisulfonic acid, disodium salt (Eastman Kodak Company, Rochester, New York, Cat. No. P230, or equivalent). Dilute the solution to 10 mℓ with water. Filter, if necessary, and store in a brown bottle. Prepare a fresh solution weekly. If the solution darkens, discard it.

7.2 Concentrated sulfuric acid.

7.3 **Formaldehyde Standard Solution "A" (1 mg/mℓ).** Dilute about 3 mℓ of 37% formalin solution to 1 liter with distilled water. This solution must be standardized as described in Section 9.1. The solution is stable for at least 3 months. Alternatively, sodium formaldehyde bisulfite (Eastman Kodak Company Cat. No. P6450, or equivalent) can be used as a primary standard (Reference 11.4). Dissolve 4.190 g in distilled water and dilute to 1 liter.

7.4 **Formaldehyde Standard Solution "B" (10 μg/mℓ).** Dilute 1 mℓ of standard solution "A" to 100 mℓ with distilled water. Make up fresh daily.

7.5 **Iodine, 0.1 *N* (approximate).** Dissolve 25 g of potassium iodide in about 25 mℓ of water, add 12.7 g of iodine, and dilute to 1 liter.

7.6 **Iodine, 0.01 *N*.** Dilute 100 mℓ of the 0.1 *N* iodine solution to 1 liter. Standardize against sodium thiosulfate.

7.7 **Starch Solution, 1%.** Make a paste of 1 g of soluble starch and 2 mℓ of water and slowly add the paste to 100 mℓ of boiling water. Cool, add several milliliters of chloroform as a preservative, and store in a stoppered bottle. Discard when a mold growth is noticeable.

7.8 **Buffer Solution.** Dissolve 80 g of anhydrous sodium carbonate in about 500 mℓ of water. Slowly add 20 mℓ of glacial acetic acid and dilute to 1 liter.

7.9 **Sodium Bisulfite, 1%.** Dissolve 1 g of sodium bisulfite in 100 mℓ of water. It is best to prepare a fresh solution weekly.

7.10 **Methanol, 1% (v/v).** Dilute 10 mℓ of reagent grade methanol to 1 liter with doubly distilled water.

8. Procedure

8.1 **Cleaning of Equipment.** Care must be exercised to ensure the absence of contaminants such as organic materials that can be charred by concentrated sulfuric acid. Soak glassware for 1 hr in a 1:1 mixture of nitric and sulfuric acids and follow by thorough rinsing with deionized water to remove all possible organic contaminants.

8.2 Collection and Shipping of Samples

8.2.1 Immediately before sampling. break the ends of a tube to provide openings of at least 2 mm diameter.

8.2.2 Connect either end of the tube to the sampling pump with flexible tubing and mark this end as "B" (for backup) with a permanent marker. Do not sample air through any hose or tubing before it enters the sorbent tube.

8.2.3 Attach the sampling tube vertically to the clothing of the test subject. Also attach the pump in a convenient position.

8.2.4 Record the time and the volume indicator reading before sampling. Sample at a flow rate of 200 mℓ/min for exactly 30 min. Record the final volume indicator reading and the time.

8.2.5 Measure and record the temperature and pressure of the atmosphere being sampled.

8.2.6 Immediately after sampling, break the tube open and transfer each section of alumina without loss to small bottles containing 10 mℓ of the 1% methanol solution. Immediate elution is required because of losses of formaldehyde that occur if the sorbent is stored dry. The methanol prevents polymerization of formaldehyde in solution.

8.2.7 Tightly seal and label the bottles. Pack all samples tightly in a suitable container to avoid breakage and transport them to the laboratory for analysis.

8.2.8 Handle at least one unexposed tube in the same manner as the sample tubes (break open, transfer both alumina sections to a bottle containing 10 mℓ of 1% methanol, seal and transport), but sample no air through this tube. Label this bottle as a blank.

8.3 Analysis of Samples

8.3.1 If the analysis is to be conducted soon after sampling. allow at least 1 hr for desorption of formaldehyde from the alumina. Gently agitate the mixtures several times.

8.3.2 Pipette a 4-mℓ aliquot from each of the sample solutions into glass-stoppered test tubes. A reagent blank containing 4 mℓ of the 1% methanol solution must also be run. If the formaldehyde content of the aliquot is anticipated to exceed the limit of the method, dilute a smaller aliquot to 4 mℓ with the 1% methanol solution.

911

8.3.3 Add 0.1 mℓ of the 1% chromotropic acid reagent to the solution and mix.

8.3.4 Pipette slowly and cautiously 6 mℓ of concentrated sulfuric acid into the solution. The solution becomes extremely hot during the addition of the sulfuric acid. If the acid is not added slowly, some loss of sample could occur because of spattering.

8.3.5 Allow the sample solution to cool to room temperature. Separate the alumina from the solution by centrifugation prior to making the absorbance measurement. Determine the absorbance of the solution at 580 nm in a spectrophotometer with a 1-cm cell. No change in absorbance has been noted over a 3-hr period after color development. Subtract the absorbance of the reagent blank. Determine the formaldehyde content of the solution from the calibration curve (Section 9.2).

8.4 Determination of Desorption Efficiency

8.4.1 Add 1.65 g of alumina (from the same batch as that used in the sampling tubes) to a 2-mℓ glass-stoppered tube. With a microliter syringe evenly distribute 18 μℓ of Standard Solution "A" (1 μg/μℓ) in the alumina in the tube. Note the exact time. Seal the tube and gently shake the alumina. This amount of formaldehyde (18 μg) corresponds to what would be collected in a 6-liter air sample at a concentration of 3 mg/m³ (2 ppm), the OSHA standard. Smaller or larger amounts may be used as desired.

8.4.2 Prepare at least three samples in this manner for each amount of formaldehyde tested. *After exactly 30 min* from the time of addition of the standard transfer each portion of alumina to 10 mℓ of the 1% methanol solution and extract for at least 1 hr. Prepare a "desorption" blank in the same manner, omitting the formaldehyde.

8.4.3 For each amount of formaldehyde tested prepare three standards by injecting with the same syringe the same volume of Standard Solution "A" into 10 mℓ of the 1% methanol solution.

8.4.4 Analyze these samples, blanks, and standards as described in Section 8.3. The desorption efficiency DE is the amount of formaldehyde found in a sample (after correction for the desorption blank) divided by the amount found for the corresponding standards:

$$DE = \frac{\mu g \text{ in sample} - \mu g \text{ in blank}}{\mu g \text{ in standard}}$$

An average desorption efficiency of 0.85 has been found for amounts of formaldehyde added to alumina in the range of 10 to 37 μg.

9. Calibration and Standards

9.1 Standardization of Formaldehyde Solution

9.1.1 Pipette 1 mℓ of formaldehyde standard solution "A" into an iodine flask. Into another flask pipette 1 mℓ of distilled water; this is the blank.

9.1.2 Add 10 mℓ of 1% sodium bisulfite and 1 mℓ of 1% starch solution to each flask.

9.1.3 Titrate with 0.1 *N* iodine to a dark blue color.

9.1.4 Destroy the excess iodine with 0.05 *N* sodium thiosulfate.

9.1.5 Add 0.01 *N* iodine until a faint blue end point is reached. The excess inorganic bisulfite is now completely oxidized to sulfate, and the solution is ready for the assay of the formaldehyde-bisulfite addition product.

9.1.6 Chill the flask in an ice bath and add 25 mℓ of chilled buffer solution. Using a microburette, titrate the liberated sulfite with 0.01 *N* iodine to a faint blue end point. The amount of iodine added in this step must be accurately measured and recorded.

9.1.7 Since 1 mℓ of 0.01 *N* I_2 solution is equivalent to 0.15 mg of formaldehyde, the concentrationon C_S of the standard formaldehyde solution (in mg/mℓ) is given by the equation:

$$C_S = V_{I_2} \times N_{I_2} \times 15$$

where:

V_{I_2} = volume (in mℓ) of 0.01 *N* I_2 solution used to titrate the liberated sulfite, corrected for the blank.

N_{I_2} = the exact normality of the 0.01 *N* I_2 solution.

9.2 Preparation of Calibration Curve

9.2.1 Pipette 0, 0.1, 0.3, 0.5, 0.7, 1.0, and 2.0 mℓ of formaldehyde standard solution "B" into glass-stoppered test tubes.

719.

9.2.2 Dilute each standard to 4 mℓ with distilled water.

9.2.3 Develop the color as described in the analysis procedure (Section 8.3).

9.2.4 Plot absorbance against micrograms of formaldehyde in the solution. The amount of formaldehyde in each standard is based on the standardization value of solution A.

10. Calculations

10.1 From the calibration curve, read the amount (in μg) of formaldehyde corresponding to the absorbance of the sample solution. This is the amount of formaldehyde in the 4-mℓ sample aliquot (Section 8.3.2). Multiply this value by 2.5. The result is the weight of formaldehyde desorbed from the alumina. Correct this value for the desorption efficiency as determined in Section 8.4. Add the corrected amounts of formaldehyde found in the front and backup sections of the same sorbent tube. Correct this sum for the amount of formaldehyde found in the corresponding unexposed, or "blank" sorbent tube to obtain the total weight of formaldehyde in the air sample. These calculations are summarized in the following equation:

$$W = \frac{2.5}{D.E.} \times (W_1 + W_2 - W_b)$$

where:

W = the total amount (in μg) of formaldehyde in the sample.
D.E. = desorption efficiency.
W_1 = the amount (in μg) of formaldehyde found in the extract of the first sorbent section.
W_2 = the amount (in μg) found in the extract of the backup sorbent section.
W_b = the total amount (in μg) found in the blank tube.

10.2 The concentration C of formaldehyde in air may be expressed in mg/m³, which is numerically equivalent to μg/ℓ:

$$C\ (mg/m^3) = \frac{W\ (\mu g)}{V\ (\ell)}$$

where: V = volume of air sampled.

10.3 The concentration may also be expressed in terms of parts per million by volume at standard conditions of 25°C and 760 mmHg:

$$C\ (ppm) = \frac{W}{V} \times \frac{24.45}{M.W.} \times \frac{760}{P} \times \frac{T+273}{298}$$

where:

24.45 = the molar volume at standard conditions of 25°C and 760 mmHg.
M.W. = the molecular weight of formaldehyde (30.03).
P = pressure in mmHg of air sampled.
T = temperature in °C of air sampled.

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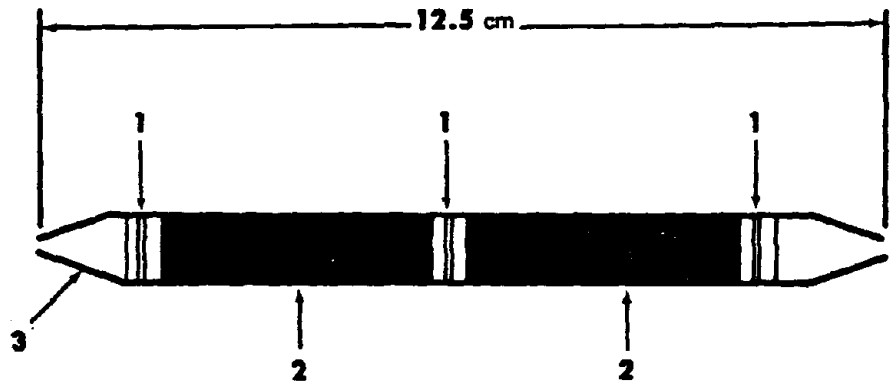
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11.11 Wood, G.O., and R.G. Anderson, "Air Sampling of Formaldehyde with a Solid Sorbent Tube," presented at the American Industrial Hygiene Conference, Minneapolis, Minnesota, June 1975; to be published in the Am. Ind. Hyg. Assoc. J.

791.



1. Stainless steel-screen plugs, 100 mesh, and Teflon supporting rings
2. Alumina section, 1.64g (2ml), 8/14 mesh
3. Glass tube (8mm i.d. and 12.5 cm long) with tapered ends.

Alumina Sampling Tube For Formaldehyde

732

4,4'-METHYLENEBIS (2-CHLOROANILINE) IN AIR

Measurements Research Branch

Analytical Method

Analyte: 4,4'-Methylenebis-(2-chloroaniline), MOCA®*	Method No.: P&CAM 236
Matrix: Air	Range: 3 to 200 $\mu g/m^3$ in a 50-liter sample
Procedure: Filter-sorbent collection; methanol elution; HPLC.	Precision (CV_T): 0.10 at 49 $\mu g/m^3$
Date Issued: 6/30/76	Classification: E (Proposed)
Date Revised:	

1. Principle of the Method

A known volume of air is drawn through a two-stage sampler consisting of a high-efficiency glass-fiber filter followed by a bed of silica gel sorbent to collect MOCA aerosol and vapor. The exposed filter and sorbent are transferred to stoppered tubes, and the MOCA is desorbed with methanol. An aliquot of this solution is injected into a high performance liquid chromatograph (HPLC). Peak areas are determined and compared with calibration curves obtained from injections of standard solutions.

2. Range and Sensitivity

2.1 The detection limit of this method is 0.15 μg when MOCA is desorbed from the filter and sorbent with 0.5 mℓ of methanol and a 10-$\mu\ell$ aliquot is injected into the HPLC. This amount of MOCA corresponds to a concentration of 3 $\mu g/m^3$ in a 50-liter sample of air.

2.2 The range of the method extends from the detection limit to an upper limit of 10 μg, which corresponds to a concentration of 200 $\mu g/m^3$ in a 50-liter sample of air. However, the method is capable of handling samples of at least 100 μg by an increase in the volume of extracting solvent, a decrease in the sensitivity of the HPLC detector, or by a reduction in the size of the aliquot injected into the HPLC.

*Registered trademark of E.I. du Pont de Nemours & Co., Inc.

123

3. Interferences

3.1 In the chromatographic procedure, the following amines were found to have retention times close enough to that of the analyte to cause interference: α- and β-naphthylamine, *o*-tolidine, N-methylaniline, 3,3'-dichlorobenzidine, 2-chloro-4-methylaniline, 3-chloromethylaniline, and *p*-toluidine.

3.2 In methanol solutions containing a MOCA concentration of 5 μg/mℓ, the following amines were found not to interfere when their concentrations were no greater than 2.5 μg/mℓ: aniline, *o*- and *m*-chloraniline, 4,4'-methylenedianiline, and benzidine.

3.3 Any compound collected with MOCA and having the same chromatographic retention time as MOCA will interfere with the analysis. This type of interference often can be eliminated by changing the operating conditions of the chromatograph, *e.g.*, by changing the composition of the mobile phase.

3.4 If interfering compounds are known or suspected to be present in the atmosphere sampled, information on their identities should be transmitted with the samples.

3.5 If the relative humidity of the atmosphere sampled is high enough to cause water to condense in the silica gel sorbent tube during sampling, the collection efficiency for MOCA vapor may be reduced.

4. Accuracy and Precision

4.1 The collection efficiencies for MOCA as particulate material and as vapor are essentially 100%. It has been demonstrated that the Gelman Type A glass fiber filter exhibits collection efficiencies greater than 99.95% for a polydisperse sodium chloride aerosol with a mass median aerodynamic diameter (MMAD) of 0.6 μm and a geometric standard deviation (σ_g) of 2.0. This efficiency is observed over a range of linear face velocities corresponding to sample flow rates between 0.2 and 1.0 ℓ/min through a 13-mm filter. MOCA has been generated in test atmospheres as a polydisperse aerosol with an MMAD of 1.7 μm and a σ_g of 1.7; the collection efficiency of the sampler for these particles would be expected to be greater than for the smaller sodium chloride particles. Tests have indicated that MOCA vapor does not break through the silica gel section of the sampler at temperatures up to 150°C with sample volumes up to 250 liters.

4.2 Sample volumes can be measured with a coefficient of variation of 0.05 at a flow rate of 1 ℓ/min. Factors contributing to variability in flow rate are: (1) variation in pressure drop among samplers, and (2) variability in the pumping rate of a pump. These factors can be minimized by judicious selection and calibration of the pump to be used.

4.3 The coefficient of variation for the analysis procedure including the sorbent extraction step is 0.07 for a 0.05-μg sample and 0.02 for a 1.5-μg sample.

4.4 The precision of the overall sampling and analytical method has been estimated by pooling the results for 37 samples collected from a test atmosphere with a MOCA concentration of 49 $\mu g/m^3$ at temperatures ranging from 25° to 43°C and at relative humidities ranging from 0 to 90%. The sampling rate was 0.82 ℓ/min and the sample volumes ranged from 12 to 49 liters. Samples of approximately 1.5 μg were collected. The coefficient of variation for all results was estimated to be 0.10.

4.5 The accuracy of the method has not been determined.

5. Advantages and Disadvantages

5.1 The sampler is small and portable and contains no liquids. It can be used for 15 min to 8 hr to collect either grab samples or integrated work-day samples. Environmental conditions of temperature and relative humidity usually encountered do not significantly affect the sampling method.

5.2 Extraction of the collected sample is simple and the analysis is accomplished by a rapid instrumental method.

5.3 Possible interferences can be obviated by changing the composition of the mobile phase or by solvent programming to provide gradient elution.

5.4 The inlet (4 mm i.d.) of the sampling device produces a face velocity of 26.5 cm/sec at a flow rate of 0.2 ℓ/min. This ensures that all particles of significance will be sampled.

5.5 The major limitation of this method is that probable contaminants present in an environment containing MOCA have not been tested to determine if they will interfere.

5.6 Another disadvantage is that the high resistance of the sampling train places a heavy load on the sampling pump.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 **Sampler.** The sampling system consists of a filter in a filter holder coupled to a sorbent tube. (See Figure 1.) The components are described below:

- Glass fiber filter, 13-mm diameter, Gelman Type A, or equivalent.
- Filter holder for 13-mm filters, Millipore SX00-013-00, or equivalent.

725

- Sorbent tube. A 3-cm section of Pyrex glass tubing (6.4 mm o.d. and 4.0 mm i.d.) is flared at one end to fit over the outlet of the filter holder. The tube contains 50 mg of 30/60-mesh silica gel (Silica Gel D-08, Applied Sciences Laboratory, or equivalent) enclosed by two 100-mesh stainless steel screens (3.5 mm diameter) held in place by Teflon rings (4 mm diameter).

To connect the two stages, the filter holder is pressed into the flared end of the sorbent tube. Plastic caps (6 mm i.d.) are used to seal the ends of the assembled sampler. The pressure drop across the sampling device at a given flow rate should be within 5% of the value predicted by Figure 2.

6.1.2 **Personal Sampling Pump.** A calibrated personal sampling pump capable of maintaining the flow rate between 0.2 and 1.0 ℓ/min for 8 hr with a sampler in the sampling line. A wet or dry test meter or glass rotameter capable of measuring a flow rate of 0.2 to 1 ℓ/min to within 5% may be used in the calibration.

6.2 **HPLC and Accessories**

6.2.1 High performance liquid chromatograph (HPLC) equipped with an ultraviolet detector (254 nm) and an injection valve.

6.2.2 A prepacked μBondapak C_{18} column, 0.63 cm i.d. by 30 cm long, Waters Associates, or equivalent. μBondapak C_{18} consists of a monomolecular layer of C_{18}-dichloro- or monochlorosilyl groups bonded to porous silica particles with an average diameter of 10 μm.

6.2.3 Potentiometric strip chart recorder (with an electronic integrator if desired).

6.3 **Glassware**

6.3.1 Glass syringes, 10 μℓ.

6.3.2 Test tubes, 1 mℓ, fitted with polyethylene stoppers.

6.3.3 Separatory funnels, 250 mℓ.

6.3.4 Beakers, 250 mℓ.

6.3.5 Pipettes and volumetric flasks of appropriate sizes for the preparation of standard solutions.

6.4 **Miscellaneous Apparatus**

6.4.1 Clinical centrifuge.

6.4.2 Analytical balance.

6.4.3 Magnetic stirrers.

6.4.4 Test-tube vortex mixer.

7. **Reagents**

7.1 Sulfuric acid, 0.05 *N*.

7.2 Sodium hydroxide, 5 *N*.

7.3 Cyclohexane, distilled in glass (Burdick and Jackson Laboratories, Muskegon, Mich., or equivalent).

7.4 Ethyl acetate, distilled in glass (Burdick and Jackson Laboratories, Muskegon, Mich., or equivalent).

7.5 Acetonitrile, distilled in glass, uv grade (Burdick and Jackson Laboratories, Muskegon, Mich., or equivalent).

7.6 4,4'-Methylene-bis-(2-chloroaniline), MOCA.

CAUTION: MOCA has been classified as a carcinogen and appropriate precautions must be taken in handling this compound. The Occupational Safety and Health Administration has promulgated regulations for its use and handling. These regulations currently serve as an emergency temporary standard and may be found in 29CFR1910.93e.

The commercially available material may require further purification. The following purification procedure is recommended:

7.6.1 Place 0.08 to 0.12 g of MOCA into a 250-mℓ separatory funnel containing 90 mℓ of 0.05 *N* H_2SO_4 and 90 mℓ of cyclohexane.

7.6.2 Shake the mixture 3 min with frequent venting, and allow the phases to separate.

7.6.3 Transfer the acidic phase into another 250-mℓ separatory funnel.

7.6.4 Extract the cyclohexane phase again with 80 mℓ of 0.05 *N* H_2SO_4.

724

7.6.5 Combine the acidic fractions in one separatory funnel.

7.6.6 Extract the combined acidic fractions twice with 30 mℓ of cyclohexane.

7.6.7 Collect all cyclohexane fractions in a beaker and allow them to evaporate in a hood. Destroy the residue with a 4% $KMnO_4$ solution and discard.

7.6.8 Add 5 *N* NaOH to the combined acidic fraction until the solution is alkaline. Extract this solution twice with 60 mℓ of a 9:1 (v/v) cyclohexane:ethyl acetate solution.

7.6.9 Collect the extracts in a beaker and allow them to evaporate in a hood with the aid of a nitrogen purge.

7.6.10 Transfer the purified and dry MOCA residue to a vial, flush the headspace with nitrogen, stopper the vial, and store it in a refrigerator. Determine the purity of the MOCA by a melting point determination and HPLC analysis. The material should be homogeneous by HPLC and give a sharp melting point.

8. **Procedure**

8.1 **Cleaning of Equipment.** Wash all glassware used for the laboratory analysis with detergent; rinse with tap water, distilled water, and methanol, and dry in an oven.

8.2 **Collection and Shipping of Samples**

8.2.1 Immediately before sampling, remove the plastic caps from the ends of the sampler and save them for resealing after sampling.

8.2.2 Select the sampling location. Connect the sorbent end of the sampler to the pump with plastic or rubber tubing. Position the sampler vertically so that air enters the filter section first. Do not sample air through any tubing before it enters the sampler.

8.2.3 Sample the atmosphere at a flow rate between 0.2 and 1.0 ℓ/min for the desired period of time (15 min to 8 hr). Measure the flow rate and sampling time, or the volume of sampled air, as accurately as possible.

8.2.4 Reseal the sampler with the plastic caps immediately after sampling.

8.2.5 Obtain blank samples by handling random collection devices in the same manner as in sampling, but sample no air through them.

8.2.6 Pack the capped tubes tightly before shipping to minimize breakage.

8.2.7 If samples of bulk material, associated with the process under investigation, are to be shipped to the laboratory, do not place them in the same container as the air samples.

8.2.8 Avoid subjecting the exposed samplers to extremes of high temperature or low pressure. As soon as possible place the samplers in a refrigerator or freezer for storage prior to analysis.

8.3 Analysis of Samples

8.3.1 **Preparation of Samples.** Place the glass fiber filter and the silica gel from a sampler in a 1-mℓ test tube. If there is a need for separate analysis, place the two collection stages in separate test tubes. The Teflon rings and stainless steel screens associated with the samplers may be extracted with the appropriate stages or rinsed with the desorption solution. Sampler caps can be discarded.

8.3.2 **Desorption of Samples.** Add 0.5 mℓ of methanol to the test tube containing the sample. Cap the tube, shake it with the vortex mixer, and allow it to stand for 1 hr with intermittent shaking. Then centrifuge the mixture for 10 min. Blanks are treated in the same way.

8.3.3 **Liquid Chromatography Conditions.** Typical operating conditions for the liquid chromatograph are as follows:

- Column pressure, 1400 lb/in.^{2}.
- Sample injection temperature, approximately 22°C.
- Column temperature, approximately 22°C.
- Mobile phase, 8:2 (v/v) acetonitrile:water.
- Flow rate, 1.3 mℓ/min.
- Sample size, 10 μℓ.
- Capacity ratio for MOCA, 0.6.
- Efficiency for MOCA, 1350 theoretical plates.

8.3.4 Injection of Sample. First flush the 10-$\mu\ell$ syringe with methanol to ensure its cleanliness and to wet the barrel and plunger, and then flush it with the sample solution. Carefully draw 10 $\mu\ell$ of sample solution into the syringe to minimize the intake of particulate matter that may plug the injection valve and cause a pressure buildup. No more than a 3% difference in areas from replicate injections is to be expected.

8.3.5 Measurement of Peak Area. Measure the area of the sample peak manually or with an electronic integrator. Estimate the amount of MOCA from a standard curve prepared as described in Section 9.

8.4 Determination of Desorption Efficiency

8.4.1 Importance of Determination. The desorption efficiency for MOCA can vary from one laboratory to another and also from one batch of silica gel or filters to another. Thus, it is necessary to determine the percentage of MOCA that can be recovered from these matrices. The mean desorption efficiency found for MOCA from the specified glass fiber filters and silica gel was 0.91. Desorption efficiency may also vary with amount of MOCA present; therefore, measurements should be made for at least two different amounts of MOCA.

8.4.2 Procedure for Determination of Desorption Efficiency. To prepare a sample, place the filter and silica gel from an unused sampler into a 1-mℓ test tube, and add a 10-$\mu\ell$ aliquot of a solution of MOCA in methanol of the appropriate concentration. Cap the tube with a polyethylene stopper. Prepare at least five samples in this manner and allow them to stand overnight to assure complete adsorption of the amine. Another unused sampler is treated in the same manner except that no MOCA is added to the filter and silica gel. This sample is the blank. Two or three standards are prepared by injecting 10 $\mu\ell$ (same syringe as above) of the MOCA solution into 0.5 mℓ of methanol. These samples, blanks, and standards are analyzed together. The desorption efficiency (D.E.) equals the difference between the average peak area of the samples and the peak area of the blank divided by the peak area of the standards, or

$$\text{D.E.} = \frac{\text{area sample - area blank}}{\text{area standard}}$$

9. Calibration and Standards

9.1 For accuracy in the preparation of standards it is recommended that one standard be prepared in a relatively large volume and at high concentration. The initial standard is prepared by weighing a selected amount of MOCA (*e.g.*, 125 mg) into a 250-mℓ volumetric flask and adding methanol to the calibration mark. Dilutions encompassing the concentration range of interest down to 0.15 μg/0.5 mℓ (0.3 μg/mℓ) are then made from the stock solution. Plot the area under the peak *versus* the weight (in μg) of MOCA in 0.5 mℓ of standard solution.

9.2 The standard solutions should be analyzed under the same liquid chromatographic conditions and during the same period as the unknown samples. This will minimize the effect of day-to-day variations of the detector response or the composition of the mobile phase.

10. Calculations

10.1 From the standard curve, read the weight (in μg) corresponding to the peak area for the particular sample. No volume corrections are needed, because the standard curve is based on μg/0.5 mℓ of extract, and the volume of sample injected into the HPLC is identical to the volume of the standards injected.

10.2 In the event that the blank produces a peak at the same retention time as the MOCA peak in the analysis, the amount of MOCA calculated for the blank must be subtracted from the amount calculated for each sample. This is the net amount of MOCA. No blank value is expected. If one is obtained, it is best to locate its source and eliminate it. See Section 3.3.

10.3 Divide the net amount of MOCA by the desorption efficiency to obtain the total amount (in μg) collected in the samples.

10.4 If the filter and silica gel of a sampler are analyzed separately, add the amounts collected in each to obtain the total amount, W, collected in the sampler.

10.5 The concentration, C, of MOCA in air may be expressed in $\mu g/m^3$:

$$C = \frac{W(\mu g)}{V_S\ (\ell)} \times 1000\ (\ell/m^3)$$

where: V_S = volume (liter) of air sampled.

11. Reference

Morales, R., S. M. Rappaport, R. W. Weeks, Jr., E. E. Campbell, and H. J. Ettinger, "Development of Sampling and Analytical Methods for Carcinogens," July 1-December 31, 1975, LA-6387-PR, Los Alamos Scientific Laboratory, Los Alamos, N. M., 1976.

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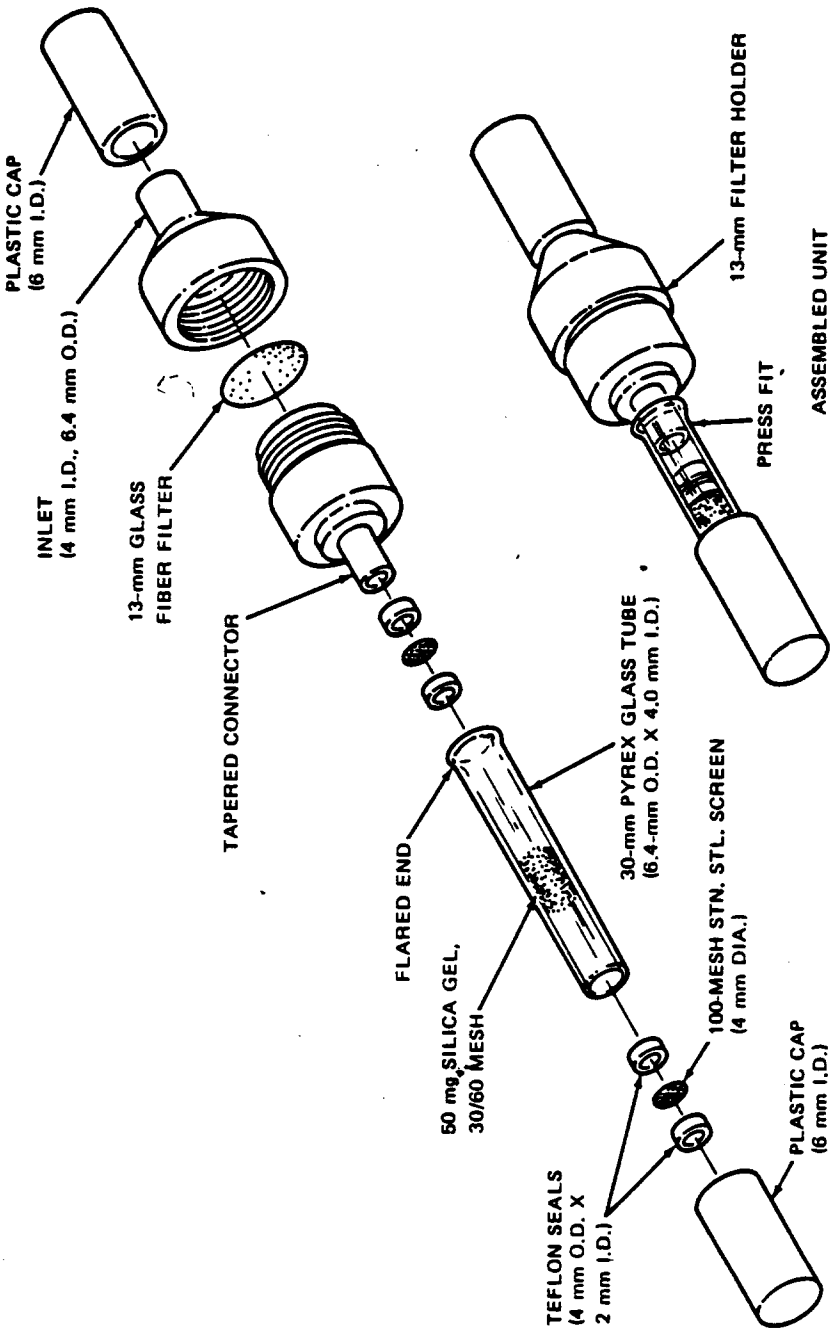


Figure 1. Two-Stage Sampler for MOCA

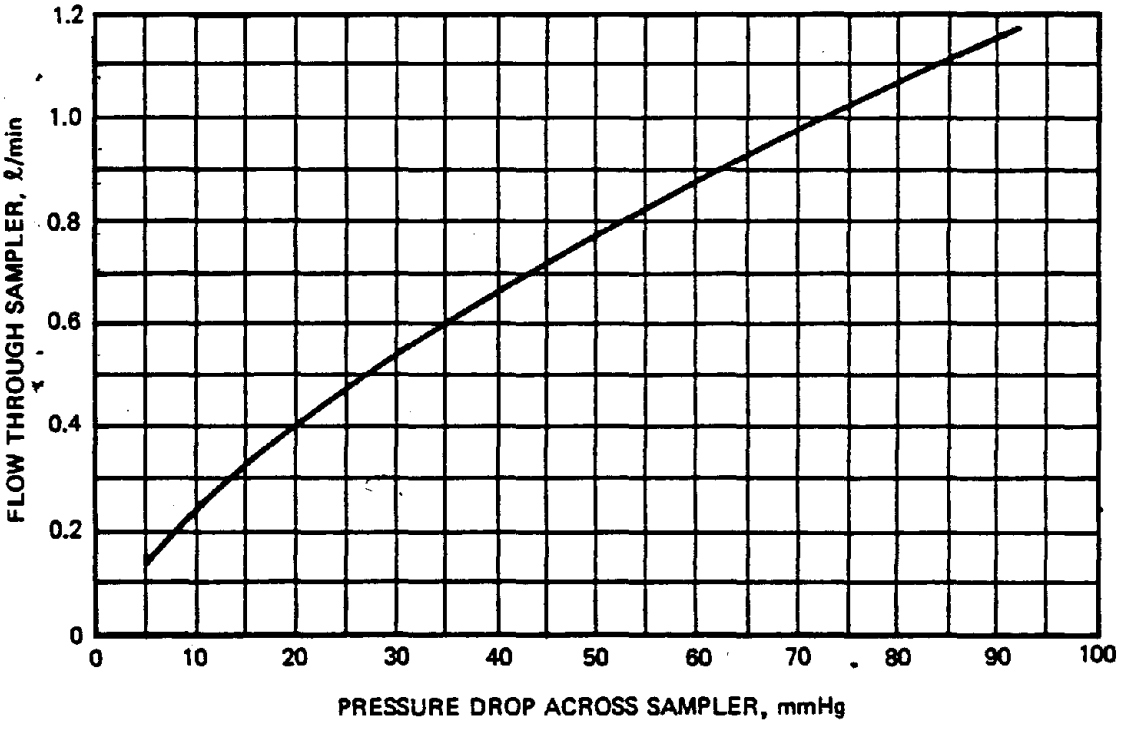


Figure 2. Relationship between Flow Rate and Pressure Drop for MOCA Sampling Device

PROCEDURE FOR THE PREPARATION OF TISSUE FOR ANALYSIS

Measurements Research Branch

Preparation:	Dry homogeneous tissue	**Method No.:**	P&CAM 237
Matrix:	Biological tissue — human lung		
Procedure:	Homogenization, lyophilization		
Date Issued:	2/28/77	**Classification:**	D-Operational
Date Revised:			

1. **Principle of the Procedure**

   (Note: This procedure was developed for processing of lungs. With minor modifications, it can be adapted to other soft tissues.)

   1.1 Whole organ is detached from adjacent structures, trimmed, and weighed. In the case of lung, hilar lymph nodes are dissected out, trimmed, and weighed.

   1.2 The organ is cut into small pieces, water added, and the mixture homogenized.

   1.3 The homogenate is frozen to −50°C and then freeze-dried (lyophilized).

   1.4 The end product is homogeneous, dry tissue. The most commonly used weight basis for tissue analysis is the dry weight; therefore, this material can be analyzed without further data manipulation.

   However, at times ash weight or wet weight bases may be sought, or total burden data. Section 7 details calculations whereby weight ratios (wet/dry/ash) may be determined and subsequent conversions made.

2. **Interferences**

   2.1 The composition of organs is not uniform throughout. For aliquots to be representative of the whole organ, thorough homogenization of the whole organ is required.

   2.2 Metals brought into contact with the tissue may cause gross contamination. It is necessary, therefore, to avoid the use of stainless steel implements or containers. Tantalum (a metal not presently of analytical concern) serves well for knife blades, homogenizer shafts, etc. Epoxy or PVC coating should be used on forceps.

   2.3 Air particulate matter is a source of contamination. A laminar flow clean work station is recommended for use whenever the tissue is exposed.

   2.4 If tissues are fixed (embalmed), trace metals levels are questionable due to the infusion of the blood vessels and the tissues with fluids of unknown composition. We do not do trace metals analysis on embalmed organs.

3. **Advantages and Disadvantages of the Procedure**

   3.1 This procedure results in a homogeneous mixture which can be stored indefinitely. By maintaining a careful record of weights, results may be reported on wet, dry, ash, or total burden bases. Each bulk sample can be used for many analytical procedures. Contamination is rigidly controlled and minimized.

   3.2 The two disadvantages are (a) the time involved in the processing, and (b) the high cost of the necessary equipment.

4. **Apparatus**

4.1 Laminar flow clean work station, Agnew-Higgins, Model 242.

4.2 PVC gloves, unpowdered.

4.3 Hard plastic cutting boards.

4.4 Whirl-Pak plastic bags.

4.5 Virtis homogenizer, Model 60K

4.6 Shaft, blades, screws, lock washers for homogenizer, of high purity tantalum.

4.7 Tissue forceps, coated with PVC or Epoxy.

4.8 Homogenizing flasks, Virtis No. 26-415, 1-liter capacity.

4.9 2-oz. glass vials with plastic snap caps.

4.10 2-liter capacity filter seal cylinders, Virtis F-129-B, F-3, F-C, F-100-C, F-123-6.

4.11 Freeze-drying unit, Virtis Unitrap, Model 10-100.

4.12 Large port vacuum drum, Virtis Model 10-104 LD.

4.13 Cenco Hyvac 7, vacuum pump.

4.14 Top loading balance, 2-kg capacity.

4.15 Storage freezer.

4.16 Quart size screw cap storage jars.

4.17 Parafilm.

4.18 Oxygen plasma asher, International Plasma Co.

4.19 Ashing boats.

4.20 2-oz. screw cap plastic bottles.

5. **Reagents**

5.1 High purity water supply, as Millipore Super-Q.

5.2 Nitric Acid, reagent grade.

6. **Procedure**

6.1 **Cleaning of Equipment.** All glassware must be acid cleaned by soaking, after detergent washing and tap water rinsing, in concentrated nitric acid for no less than 30 minutes. This is followed by tap water rinsing and final rinsings with Super-Q water.

6.2 **Collection and Storage of Samples**

6.2.1 Organs are taken at autopsy without removal of any portions of the organ. They are delivered to the laboratory frozen and sealed in plastic bags. Total organ weight should be taken and recorded (Section 7.2-A).

6.2.2 If shipping from a remote collection site is necessary, they should be packed in an insulated container with sufficient dry ice to maintain the frozen state.

6.3 **Processing of Samples**

6.3.1 Remove lung from freezer and allow it to thaw partially.

6.3.2 Assemble all cutting equipment, homogenizer flask, and hard plastic cutting board in the laminar work station.

6.3.3 Rinse the outer surface of the lung with Super-Q water. Hands should be gloved.

6.3.4 Dissect out the hilar lymph nodes. Trim, weigh, and store in Whirl-Pak bags in freezer.

6.3.5 Trim away bronchii, large airways, and blood vessel structures and discard.

6.3.6 Cut entire organ into small pieces (approximately ¼-inch cubes) and place in a tared homogenizing flask. Weigh and record weights (Section 7.2-C,D).

6.3.7 Add water in the approximate ratio of 2 parts water to 3 parts tissue. Record total weight (Section 7.2-F).

6.3.8 Homogenize in a high speed homogenizer, using tantalum shaft and accessories. A multi-blade shaft assembly is advantageous with fibrous tissue.

6.3.9 Set aside 2 portions of homogenate in small vials to store in freezer.

6.3.10 Transfer the remainder of the homogenate into a tared, 2-liter, filter seal flask and record weight (Section 7.2-J, K). Cap and freeze in a shell freezer set at −50°C.

6.3.11 Refrigerate the freeze drying unit to −50°C and start the vacuum pump. A pressure of 10 microns or less is required.

6.3.12 Attach the cylinder containing the frozen sample homogenate to a port of the freeze dryer. When the cylinder and contents reach room temperature (24 to 36 hours), remove from the drying unit and weigh. Reattach and leave the samples for 2 or 3 hours and reweigh. When constant weight is reached, record the final weight (Section 7.2-N) and transfer sample to an acid-cleaned storage jar.

6.3.13 For ashed tissue, (Section 7.2-S,T), weigh an aliquot of dry tissue in an acid clean boat, and introduce the reaction chamber of the low temperature asher. Ash at 100 watts RF output, with an oxygen flow of 100 liters per minute until the organic matrix is destroyed. Weigh the ash (Section 7.2-W,X).

7. Calculations

7.1 If analytical data based on dry weight of tissue is sought, aliquots of this freeze-dried material may be weighed and used in calculating % by weight, ppm, etc.

7.2 In order to convert analytical data to base other than dry weight, ratios between wet, dry and ashed tissue can be determined as follows.

Data Recorded	Arithmetic Computation
A. Weight Whole Lung	
B. Weight Lymph Nodes	
C. Weight Minced Lung + Flask	
D. Tare Weight of Homogenizer Flask	
E. Weight Minced Lung	$E = C - D$
F. Weight Flask + Minced Lung + Water	
G. Weight Minced Lung + Water	$G = F - D$
H. Ratio Lung/Lung + Water	$H = E/G$
J. Weight Homogenate + Freezer Cylinder	
K. Tare Weight Cylinder	
L. Weight Lung Homogenate	$L = J - K$
M. Weight Lung Tissue in Wet Homogenate	$M = L \cdot H$
N. Weight Freeze-Dried Homogenate + Cylinder	
P. Weight Freeze-Dried Homogenate	$P = N - K$
R. Ratio Dry Lung/Wet Lung	$R = P/M$
For Ash Weight Ratios	
S. Weight Ashing Boat + Freeze-Dried Tissue	
T. Tare Weight Boat	
V. Weight Freeze-Dried Tissue	
W. Weight Ashed Residue + Boat	$V = S - T$

X. Weight Ashed Residue	$X = W - T$
Y. Ratio Ashed Tissue to Freeze-Dried Tissue	$Y = X/V$
Z. Ratio Ashed Tissue to Wet	$Z = R \cdot Y$

8. References

8.1 Carlberg, J. R., J. V. Crable, L. P. Limtiaco, H. B. Norris, J. L. Holtz, P. A. Mauer, and F. R. Wolowicz, "Total Dust, Coal, Free Silica, and Trace Metals Concentrations in Bituminous Coal Miners' Lungs," Am Ind Hyg Assoc J 32:432-40, 1971.

8.2 Versieck, J., A. Speecke, and J. Hoste, "Sampling of Biological Materials," Proceedings of the 7th Materials Research Symposium, National Bureau of Standards. Accuracy in Trace Analysis: Sampling, Sample Handling, Analysis, Volume 1, NBS Special Publication 422.

ASBESTOS FIBERS IN AIR

National Institute for Occupational Safety and Health

Analytical Method

Analyte:	Asbestos fibers	**Method No.:**	P&CAM 239
Matrix:	Air	**Range:**	0.1-60 fibers/cm^2
Procedure:	Filter collection, microscopic count	**Precision (CV_T):**	0.24 to 0.38
Date Issued:	3/30/77	**Classification:**	D (Operational)
Date Revised:			

1. **Principle of the Method**

   1.1 This method describes the equipment and procedures for collecting, mounting, and counting asbestos fibers on cellulose ester membrane filters in the evaluation of personal samples of airborne asbestos fibers. The purpose of the method is to determine an employee's index of exposure to airborne asbestos fibers. The method is primarily a personal monitoring technique, but can be used for area monitoring.

   1.2 The sample is collected by drawing air through a membrane filter by means of a battery powered personal sampling pump. The filter is transformed from an opaque solid membrane to a transparent optically homogeneous gel. The fibers are sized and counted using a phase-contrast microscope at 400-450X magnification.

   1.3 Definitions. Asbestos fiber, for counting purposes, means a particulate which has a physical dimension longer than 5 micrometers and with a length to diameter ratio of 3 to 1 or greater. Asbestos includes chrysotile, cummingtonite-grunerite (amosite), crocidolite, fibrous tremolite, fibrous anthophyllite, and fibrous actinolite.

   1.4 Any laboratory attempting to use this procedure should have at least one counter attend a training course conducted by an experienced, proficient laboratory. Novice, untutored counters, using only published instructions, can easily obtain counts of half those performed by experienced, proficient counters. Large differences between laboratories can be caused by: 1) differences in technique and observing ability among counters and 2) small, but significant, differences between microscopes meeting the basic specifications of Section 6.2. The following procedures are recommended:

      1.4.1 All microscopists who perform asbestos counting should meet together for an "asbestos counting workshop" at least quarterly. This is best accomplished with counters from several laboratories using their own microscopes.

      1.4.2 Each microscopist should count the same series of slides and with the results being compared.

      1.4.3 Differences between counters should be resolved with side-by-side counting of the fields by the different counters.

      1.4.4 Individuals who are found to be persistent outliers over several sessions should be encouraged to seek other tasks in their respective laboratories.

2. Range and Sensitivity

2.1 The usable range is primarily a function of sample volume, microscope count field area, and background airborne particulates. The influence of these variables is discussed in 8.1.3. For a microscope count field area of 0.003 mm^2 (see Figure 1) and a pump flow rate of 1.7 lpm, the optimal fiber densities would be produced over the range of 0.4 fiber/cm^3 (8-hour sample) to about 60 fibers/cm^3 (15-minute sample). For a field area of 0.006 mm^2 (see Figure 2) and a pump flow rate of 1.7 lpm, the optimal range is 0.2 fiber/cm^3 (8-hour sample) to about 30 fibers/cm^3 (15-minute sample). In each case, the optimal detection limits are inversely proportional to pump flow rate.

The upper detection limit can be extended by using sample times less than 15 minutes or using lower flow rates. The lower detection limit can be extended by increasing the flow rate up to about 2.5 lpm. Filter surface fiber densities less than optimal (less than about 0.5 to 1.0 fiber per count field) are still adequate, but will lead to decreased precision for the method (increased coefficient of variation, see Section 4).

The minimum total fiber count in 100 fields considered adequate for reliable quantitation is 10 fibers. Thus, the lower limit of reliable quantitation is 0.1 fiber/cm^3 (100,000 fibers/m^3). For this level, a flow rate of about 2.5 lpm is recommended. For a field area of 0.003 mm^2, the minimum sample time would be about 2 hours. For a field area of 0.006 mm^2, the minimum sample time would be about 1 hour.

2.2 This method considers only fibers with a length to diameter ratio of 3 to 1 or greater and a length greater than 5 micrometers.

3. Interferences

In an atmosphere known to contain asbestos, all particulates with a length to diameter ratio of 3 to 1 or greater, and a length greater than 5 micrometers should, in the absence of other information, be considered to be asbestos fibers and counted as such.

4. Precision and Accuracy

4.1 In the past decade, there have appeared a number of articles examining sources of variation in the asbestos sampling and counting procedure. These include: Lynch et al. (11.1), Weidner and Ayer (11.2), Conway and Holland (11.3), Leidel and Busch (11.4), Beckett and Attfield (11.5), and Rajhans and Bragg (11.6). The sources of variation will be discussed by stages in the membrane filter evaluation procedure.

4.2 **Sources of Variation in the Sampling Process.** These include variations in pump flow rate, proximity of the filter to the employee's body, and filter location (left to right) in the employee's breathing zone.

4.2.1 Section 9.1 requires that the personal sampling pump be calibrated with sufficient accuracy such that the 95% confidence limits on the flow rate are ± 10%. This is equivalent to a coefficient of variation (CV) of about 5%. However, this CV makes a negligible contribution to the total CV for the method due to the relatively large CV of the counting procedure.

4.2.2 Conway and Holland (11.3) concluded that positioning of the filter cassette on the wearer (regarding the angular portions of the filter and their proximity to the wearer) is not a significant factor in determining the fiber distribution on filters.

4.2.3 Weidner and Ayer (11.2) concluded that there is no appreciable difference between samples collected on either the right or left sides of a breathing zone or between samples collected side-by-side, especially for samples with concentrations less than 2.5 fibers/cm^3.

.748

4.3 **Sources of Variation in the Counting Procedure**

4.3.1 Random variations exist in the fiber distribution on a filter wedge (intra-wedge variability). The industrial hygiene literature has seen considerable debate in the last 20 years concerning whether or not the distribution of mineral dust or asbestos fibers on a filter surface is adequately described by a Poisson distribution probability density function. Leidel and Busch (11.4) found excellent agreement between empirical error variance and theoretical variance calculated from the assumption of Poisson distributed true counts. They concluded that there was not excessive variation among count fields for a filter wedge and that clumping of fibers (non-random coalescence) did not occur.

4.3.2 Variations exist in the fiber distribution on the total filter surface (inter-wedge variability) due to the random or non-random distribution of fibers across the total surface of the filter. This type of variation is easily confused with intra-wedge variations. The count procedure does not require counting of multiple sectors of the filter. There may be significant differences between average counts for different wedges, or the fiber distribution variations for the total filter surface may be greater than the variations of the Poisson distribution. If either of these occur experimentally, one must use the experimental variations to estimate the minimum precision of the count procedure. The minimum precision is governed by the variations of the fiber distribution on the total surface of the filter.

Conway and Holland (11.3) concluded the distribution of fibers on filters is not uniform and the distribution of fiber counts is more disperse than Poisson. For their filters which had significant variations in fiber concentrations between sectors (as much as 50-60% of the total filter mean), they described the following relation for the standard deviation of the total number of fibers counted on a wedge (N)

$$\text{empirical } s(N) = 1.6\ (N)^{1/2}$$

where N is about 100. The Poisson standard deviation would be:

$$\text{Poisson } \sigma(N) = (N)^{1/2}$$

Rajhans and Bragg (11.6) in Series I of their study found significant variation between filter segments and rejected the Poisson distribution for the total filter surface. However, in Series II of their study, utilizing various experimental modifications, they found no significant variation between filter segments and no reason to reject the assumption of Poisson distributed fiber counts.

4.3.3 Systematic variations due to differences between microscopes were studied by Leidel and Busch (11.4). In their study using five different brands of microscopes, they found no significant differences among four, but the fifth gave counts approximately 45% higher on the average than the other four.

4.3.4 Variations due to differences between counters should be examined at three levels: experienced counters occasionally counting, experienced counters routinely counting, and inexperienced (new or untutored) counters. Leidel and Busch (11.4) studied five experienced counters, with one counting only occasionally. There were no significant differences among three of the counters, but a fourth was 16% lower than the first three. The fifth, who occasionally counted, averaged 27% higher than the first three. Conway and Holland (11.3) studied three experienced counters and three inexperienced counters. They found statistically significant differences between the means of both the experienced and inexperienced counters that typically were in the range plus or minus 5 to 15%. They concluded that experience as a fiber counter is not a significant parameter affecting intercounter variations.

Rajhans and Bragg (11.6) found no significant differences among means of five experienced counters in Series I of their study. But in their carefully controlled Series II, an analysis of variance showed significant variations between counters that were plus or minus 1 to 15%.

4.3.5 Variations between laboratories are most likely due to systematic biases and are not a significant additional source of random variations. Any additional variations are most likely due to differences in counting technique. Beckett and Attfield (11.5) observed that standard counters improved greatly after personal instruction; also new counters, after instruction, tended to overcompensate and get exceedingly high counts. Additionally, they found that counts from an experienced laboratory that had not had contact with other laboratories performing the same analysis were as far from the standard values as were the counts by new counters.

4.4 Sources of variations between samples taken at different times on one employee during one work shift can affect the exposure estimate for that employee. These are primarily due to a) differences in exposure concentrations during the day, b) differences in location of the employee within the plant, and c) differences in work operation performed by the employee during the day. These sources of variation can be controlled by proper choice of sampling strategy. Refer to Leidel and Busch (11.7) and Leidel, Busch, and Lynch (11.8) for an extended discussion of sampling strategies. Interday temporal variations can affect the exposure estimates obtained on different days. Refer to Leidel, Busch, and Crouse (11.9) for a discussion of this type of variation.

4.5 Until recently, the total coefficient of variation (CV_T) for the sampling and counting procedure was best estimated from the work of Conway and Holland (11.3). The conclusions of their study included:

4.5.1 The precision of their procedure for filters not containing an abundance of fine fibers can be estimated by a coefficient of variation of 16.2%. This value includes variation among counters and observed interaction effects.

4.5.2 The accuracy of the procedure for similar filters may be estimated for a 100-fiber count by a coefficient of variation of 21.4%. This assumes that the contribution of the overall variance from the nonuniform fiber distribution is additive.

4.5.3 A high percentage of very fine fibers on the filter can significantly affect the standard deviation and confidence limits for counts by different counters. After combining variations in fiber concentrations over the entire filter with those for different counters, it was concluded:

a. For filters with a low concentration of fine fibers, the coefficient of variation is estimated at 21% and the 95% confidence interval is ± 43%.

b. For filters with a high concentration of fine fibers, the coefficient of variation is estimated at 25% and the 95% confidence interval is ± 50%.

Lynch, Kronoveter, and Leidel (11.1) have also reported on variations of the method. Their intralaboratory study utilized the data from a large number of dust counts made by different methods by experienced counters over a period of years in an epidemiologic study of the asbestos products industry. They concluded that the standard deviation of counts of fibers longer than 5 micrometers on membrane filters could be estimated from the relation $\sigma = (N)^{0.581}$. Thus for counts of about 100 fibers, the coefficient of variation could be estimated at about 15.2% and the 95% confidence limits at ± 30.4%. These values are lower than the values reported by Conway and Holland (11.3).

Recently, the Johns-Manville Corporation conducted an in-house investigation of the asbestos count method (11.10). The study data contained total fiber counts for over

100 filters with each filter counted by two to five counters. From the Johns-Manville data, NIOSH calculated over 100 estimates of the count CV for the method (11.11). The NIOSH CV estimates included random intrafilter variations and intercounter variations, but did not include random pump flow rate variations. It was found that the count coefficient of variation (all random variations except for pump variations) was a function of the total fiber count. NIOSH then included a CV of 0.05 for random pump variations (see Section 9.1) in the CV-estimator equation to obtain a CV_T-estimator. The CV_T-estimator line is plotted on Figure 3 for total fiber counts in the range 10 to 100 fibers. Or the following equation can be used:

$$CV_T = [\text{antilog}_{10}(-0.215 - 0.203\,(\log_{10}FB)) + 0.0025]^2$$

where FB is total fiber count as discussed in Section 10.

Figure 3 demonstrates that for a total fiber count of 100, the best CV_T is attainable with the appropriate sampling times given in 8.1.3 and the count rules in 8.3.9. When making decisions regarding compliance with the OSHA asbestos exposure standards in 29 CFR 1910.1001, the statistical procedures given in Leidel et al. (11.11) should be followed. The procedures are based on statistical theory and assumptions given in References 11.12, 11.13.

Because of the possibility of systematic biases due to differences between microscopes, counters, and laboratories as discussed above, it is strongly recommended that any laboratory counting asbestos should participate in an interlaboratory quality control program that includes the counting of standard reference filters. These standard filters are available from NIOSH through the Proficiency Analytical Testing (PAT) Program. The PAT Program is used by the American Industrial Hygiene Association (AIHA) as part of its Laboratory Accreditation Program. Each laboratory's quality control program must include protocols for routinely adjusting and calibrating sampling and counting equipment plus training and evaluation programs for counters.

5. Advantages and Disadvantages of the Method

5.1 The method is intended to give an index of employee exposure to airborne asbestos fibers of specified dimensional characteristics.

5.2 It is not meant to count all asbestos fibers in all size ranges or to differentiate asbestos from other fibrous particulates.

6. Apparatus

6.1 Sampling Equipment

The personal sampling equipment train consists of 1) personal sampling pump, 2) tubing, 3) clothing spring clip, 4) tubing-to-field monitor metal adaptor, and 5) field monitor (filter and holder).

6.1.1 Personal Sampling Pump. The pump must be capable of sampling at 1.0 to 2.5 liters per minute (lpm) against a flow resistance of 7.5 inches of water (1.4 cm Hg) for 8 continuous hours on a fully charged battery.

6.1.2 Tubing. Laboratory tubing such as rubber or plastic with 6-mm bore and about 100 cm length.

6.1.3 Clothing Spring Clip. The clip attaches the rubber tubing to the lapel or shirt of the individual being monitored.

6.1.4 Tubing-to-field Monitor Adaptor. A short metal adaptor with ridges on one end to grip the inside of the tubing. The other end is designed for a pressure fit into the field monitor.

6.1.5 Field Monitor (Filter and Holder). The only field monitor currently considered acceptable by NIOSH is manufactured by the Millipore Corporation. The unit con-

743

sists of 1) a three section styrene plastic case designated Millipore Aerosol Monitor Case, 2) a 37-mm diameter plain white cellulose ester membrane filter designated Millipore AA (pore size of 0.8 micrometer), 3) a support pad, and 4) two plastic sealing caps. If a large number of samples are to be taken, it may be less expensive to reuse the plastic cases. Great care must be taken in the cleaning and reassembly process. The outside mating surfaces of the field monitors may be covered with a "shrink-fit" band to provide proper sealing and a writing surface for filter identification.

6.2 **Optical Equipment and Microscope Features**

6.2.1 Microscope body with binocular head.

6.2.2 10X Huygenian eyepieces are recommended. Other eyepieces can be substituted if necessary. Wide field eyepieces can be used; however, wide field eyepieces may yield a count field area less than 0.003 mm^2 with the Porton reticle. This is not always desirable from the standpoint of obtaining optimum sampling times (see Section 8.1.3). If wide field eyepieces are used, it is preferable to use the Patterson Globe and Circle reticle to obtain a larger count field area.

6.2.3 Koehler illumination (preferably built-in with provisions for adjusting light intensity).

6.2.4 A Porton reticle is recommended. Others such as the Patterson Globe and Circle can be substituted.

6.2.5 Mechanical stage.

6.2.6 Phase-Contrast condenser with a numerical aperture (N.A.) equal to or greater than the N.A. of the objective.

6.2.7 40-45X phase contrast achromatic objective (N.A. 0.65 to 0.75).

6.2.8 Phase-ring centering telescope or Bertrand lens.

6.2.9 Green or blue filter, if recommended by microscope manufacturer.

6.2.10 Stage micrometer with 0.01 mm subdivisions.

6.2.11 For general guidance on phase contrast microscopy, consult Needham (11.12), Clark (11.15) and McCrone (11.14).

6.3 **Filter Mounting Equipment.** Experience has shown that certain equipment is useful for efficient sample mounting. The following items are recommended for extracting and mounting a portion of the filter for counting.

6.3.1 Microscope slides. 2.5 by 7.5 cm glass slides are most commonly used. Sample number, data, initials, etc., can be conveniently written on a frosted end slide.

6.3.2 Cover Slips. Cover slips are a necessary part of the slide mount and optical system. The shape should be appropriate for the size of the filter wedge. The appropriate cover slip depends upon the objective to be used. Ordinarily, objectives are optically corrected for a #1½ (0.17 millimeter) thickness cover slip. Improper cover glass thickness will detract from the final image quality.

6.3.3 Scalpel. A scalpel is needed to cut out a portion of the filter to be examined. A number-ten curved blade scalpel is recommended.

6.3.4 Tweezers. A pair of fine-tipped tweezers is used to remove the membrane filter slice from the field monitor and place it upon the slide.

6.3.5 Lens Tissue. To insure cleanliness, a lint-free tissue is recommended. This tissue should also be used for wiping mounting tools and for cleaning slides and cover slips.

6.3.6 Glass Rod. A fire-polished glass rod may be used to spread the mounting solution on the slide.

6.3.7 Wheaton Balsam Bottle. This special glass container has a glass top which prevents contamination of the mounting solution. A glass rod is included for dispensing the solution.

7. **Reagents**

Chemicals should be reagent grade, free from particles and color, conforming to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.

7.1 Dimethyl phthalate

7.2 Diethyl oxalate

Avoid getting the mounting solution on the skin. Wash skin promptly with soap and water if skin contact occurs.

8. **Procedure**

8.1 **Sampling**

8.1.1 General Information

Guidelines for the monitoring of employee exposures to industrial atmospheres are given in Reference 11.8. The Federal requirements for monitoring employee exposure to airborne asbestos are found in 29 CFR 1910.1001.

8.1.2 Mounting the Sampling Pump on the Worker

Fasten the sampling pump to the worker's belt and fasten the field monitor to the lapel or shirt front (as close to the breathing zone as is practical). Remove the top cover of the plastic monitor, then invert the monitor making certain the exposed filter is facing downward. Turn the pump on and adjust to the calibrated flow rate (1.0 to 2.5 lpm). Record the following information in a logbook.

1. Filter number
2. Pump start time and date
3. Flow rate
4. Subject's name and job title
5. Type of operation or process
6. Ventilation controls and is the worker wearing a respirator approved for asbestos?

The pump should be checked periodically during the sampling period for proper operation and flow rate.

8.1.3 Optimum Sampling Times

The requirement for the minimum count of 100 fibers or 20 fields in 8.3.9 was determined to be the best compromise to achieve adequate precision for the airborne fiber estimate and reasonable counting times. An optimum fiber density of about 1 to 5 fibers per microscope count field is recommended. To estimate appropriate sampling times for feasible counting and optimal counting, one must consider the following constraints:

1. microscope count field area (generally 0.003 to 0.006 mm^2)
2. pump flow rate (typically 2.5 lpm maximum)
3. average airborne fiber concentrations
4. counting rule range of 20 to 100 fields
5. adequate fiber density to obtain a minimum count of 10 fibers in 100 fields, which is the least total fiber count that yields an acceptable count precision
6. background airborne particulate levels that can reduce the count precision due to an obscuring of fibers on the filter surface

145

The preceding constraints were considered in drawing Figures 1 and 2. These figures were developed from the following relationship:

$$\text{sampling time} = \frac{(FB/FL)\ (ECA/MFA)}{(FR)\ (AC)\ (1000)} \quad \text{minutes}$$

where:

FB/FL = 1 to 5 fibers/field

ECA = effective collecting area of filters (855 mm^2 for 37-mm filter with effective diameter of 33 mm)

MFA = microscope field area (generally 0.003 to 0.006 mm^2)

FR = Pump flow rate (generally 1.0 to 2.5 lpm)

AC = Air concentration of fibers in fibers/cm^3.

Figure 1 (microscope field area = 0.003 mm^2) and Figure 2 (microscope field area = 0.006 mm^2) show optimum and feasible sampling times for a pump flow rate of 1.7 lpm. Each individual responsible for sampling asbestos should prepare a similar chart for his particular pump flow rate and microscope field area before sampling is performed to aid in estimating proper sampling times. On Figures 1 and 2, the areas with solid shading lines are generally the optimum conditions for counting. The broken shading lines are for conditions very close to optimal.

However, feasible counting conditions may extend down to about 0.1 fiber/field and and above 5 fibers/field. Recommended sampling times are most strongly influenced by background airborne particulate levels, once all the other constraints have been estimated. For heavy particulate levels, it may be necessary to limit each filter to about 60 to 180 minutes sampling duration. Each individual responsible for sampling should work closely with the microscopist to attain as high as possible filter surface fiber densities (up to about 5 fibers/field), while avoiding filter surface background particulate levels that create very difficult or impossible counting conditions. If one has very little idea of airborne fiber and particulate levels, the best procedure is to take several long samples (as one 8-hour or two consecutive 4-hour samples) in conjunction with several short samples (as four consecutive 2-hour or eight consecutive 1-hour samples). If the longer samples prove very difficult to count, the microscopist will have the shorter samples to fall back on.

From Figures 1 and 2, it can be seen that there are certain sampling times which will yield optimum fiber densities on the filter for almost all airborne fiber concentrations from 1 to 10 fibers/cm^3. These optimum times have been calculated and are presented in Figure 4. Note that the optimum times given by Figure 4 are approximate and can be varied by as much as $\pm$ 25%. The nomogram is intended as a guide to be used where no prior knowledge of the air concentration is available.

8.1.4 End of Sampling Period

Remove the field monitor, replace the plastic top cover and the small end caps, and store the monitor. Always shut off the pump when changing monitors to avoid contaminating or damaging the pump. Record the pump shutoff time and flow rate in the logbook.

8.1.5 Blanks

With each batch (25 to 50 filters) of samples sent for analysis, submit two unopened field monitors which have been subjected to the same treatment as the samples except that they were not exposed to the sampling environment. Label these as blanks. If the blanks yield fiber counts greater than 5 fibers/100 fields, then the entire sampling procedure should be examined carefully for the cause of contamination. The

mounting solution of Section 8.2.1 should also be examined for contamination and/or crystal growth.

8.1.6 Shipping

The field monitors in which the samples are collected should be shipped in a rigid container with sufficient packing material to prevent crushing.

8.1.7 Numbers of Samples

When sampling for the Federal ceiling standard of 10 fibers (>5μm)/cm³, [29 CFR 1910.1001(b) (3), effective July 7, 1972], only one sample (15 minutes maximum duration) is necessary, theoretically. However, several samples should be taken during expected periods of peak air concentrations to allow for detection of gross sampling or counting errors.

When sampling for determination of noncompliance with the Federal 8-hour TWA standard of 2 fibers (>5μm)/cm³, [29 CFR 1910.1001(b) (2)], one should continuously sample as large a portion of the work day as is feasible for airborne concentrations of about 2 to 10 fibers/cm³. However, for a lower airborne concentration such as 0.5 fiber/cm³, one sample might require 4 to 8 hours sampling time in order to get the proper filter fiber density (Section 8.1.3). For this situation, the 8-hour TWA exposure would be determined from one 8-hour or two 4-hour samples as appropriate.

8.2 Sample Preparation

8.2.1 Preparation of Mounting Solution

A very important part of the sample evaluation is the mounting process. This process involves a special mounting medium of prescribed viscosity. The proper viscosity is important in order to expedite filter dissolving and still minimize particle migration. After the sample has been mounted, an elapsed time of approximately sixty minutes is needed before the sample is ready for evaluation.

Combine the dimethyl phthalate and diethyl oxalate in a one to one ratio by volume and pour into a Wheaton balsam bottle. Add approximately 0.05 (± 0.005) grams of new membrane filter per milliliter of solution to reach the necessary viscosity. The mixture must be stirred periodically until the filters have dissolved and a homogeneous mixture is formed. The normal shelf life of the mounting solution is about three months. Twenty milliliters of mounting solution will prepare approximately 300 samples.

8.2.2 Sample Mounting

Cleanliness is important! A dirty working area may result in sample contamination and erroneous counts. The following steps should be followed when mounting a sample.

1. Clean the slides and cover slips with lens tissue. Lay each slide down on a clean surface with the frosted end up. It is a good practice to rest one edge of the cover slip on the slide and the other edge on the working surface. By doing this, you keep the bottom surface (the one which contacts the filter) from becoming contaminated.
2. Wipe all the mounting tools clean with lens tissue and place them on a clean surface (such as lens tissue). All tools should be wiped clean prior to mounting each sample.
3. Using the glass rod supplied with the Wheaton balsam bottle, apply a drop of mounting solution onto the center of the slide. It may be necessary to adjust the quantity of solution so that after the cover slip has been placed on top, the solution extends only slightly beyond the filter boundary. If the quantity is greater than this, particle migration may occur.

143

4. Using another glass rod, spread the mounting media into a triangular shape. The size of this triangle should coincide with the dimension of the filter wedge.
5. Separate the middle and bottom sections of the field monitor case to expose the filter. Cut a triangular wedge from the center to the edge of the filter using the scalpel. The size of the wedge should approximate one-eighth of the filter surface. The filter can be very carefully removed from the cassette for cutting, but this should only be done with great care.
6. Grasp the filter wedge with the tweezers on the perimeter of the filter which was clamped between the monitor case sections. Do not touch the filter with your fingers. Place the wedge, **sample side up,** upon the mounting medium.
7. Pick up a clean cover slip with tweezers and carefully place it on the filter wedge. Once this contact has been made, **do not reposition the cover slip.**
8. Label the slide with the sample number and current date before proceeding to the next filter. On the bottom (backside) of the slide, trace the perimeter of the filter wedge with a felt tip marking pen. This will enable the counter, after the filter has become transparent, to stay within the filter perimeter when counting.
9. The sample should become transparent within fifteen minutes. If the filter appears cloudy, it may be necessary to press very lightly on the cover slip. This is rarely necessary; however, counting should not be started until an hour after the mounting. This allows the microscopic texture of the filter to become invisible to microscope viewing.
10. Discard the sample mount after two days if it has not been counted. Crystals appearing similar to asbestos fibers may begin to grow at the mounting media/air interfaces. They seldom present any problems if the slide is examined before two days. In any case, stay away from the filter's edges when counting and sizing.

8.3 **Counting of Fibers**

8.3.1 Place the slide on the mechanical stage of the microscope and position the center of the wedge under the objective lens and focus upon the sample. Start counting from one end of the wedge and progress along a radial line to the other end (count in either direction from perimeter to wedge tip). Random fields are selected, without looking into the eyepieces, by slightly advancing the slide in one direction with the mechanical stage control.

8.3.2 It is essential to continually scan over a range of focal planes (generally the upper 10 to 15 micrometers of the filter surface) with the fine focus control during each field count. This is especially necessary for asbestos fibers due to their impaction into the filter matrix.

8.3.3 On most airborne samples, asbestos fibers will generally have fiber diameters less than one micrometer. Therefore, it is necessary to look carefully for faint fiber images.

8.3.4 Regularly check phase ring alignment.

8.3.5 When an agglomerate (mass of material) covers a significant portion of the field of view (approx 1/6 or greater) reject the field and select another. (Do not include it in the number of fields counted.) However, report the fact as it may have meaning on other data collection.

8.3.6 Bundles of fibers are counted as one fiber unless both ends of the fiber can be clearly resolved.

8.3.7 Count only fibers with a length to width ratio greater than or equal to 3:1.

8.3.8 Count only fibers greater than 5 micrometers in length. (Be as accurate as possible in accepting fibers near this length.) Measure curved fibers along the curve to estimate the total length.

8.3.9 Count as many fields as necessary to yield a total count of at least 100 fibers. Exceptions: a) count at least 20 fields even if you count more than 100 fibers, and b) stop at 100 fields even if you haven't reached 100 fibers.

8.3.10 For fibers that cross either one or two sides of the counting field, the following procedure is used to obtain a representative count.

COUNT any fiber greater than 5 micrometers in length, that lies entirely within the counting area. COUNT as "½ fiber" any fiber with only one end lying within the counting area. DO NOT COUNT any fiber crossing any two sides.

Reject and do not count all other fibers. Refer to Figures 5 through 10. Note that the fibers in Figures 5 through 10 are not representative of the appearance of most asbestos fibers. Most fibers have a very faint image.

9. Calibration and Standards

9.1 Sampling Train Calibration

The accurate calibration of the sampling pump is essential to the correct calculation of the air volume sampled. The frequency of calibration is dependent on the use, care, and handling to which the pump is subjected. Pumps must be recalibrated if they have just been repaired, misused, or received from the manufacturer. If the pump receives hard usage, more frequent calibration may be necessary. Ordinarily, pumps should be calibrated in the laboratory both before they are used in the field and after they have been used to collect a large number of field samples.

The accuracy of calibration is dependent upon the type of instrument used as a reference. The choice of a calibration instrument will depend largely on where the calibration is performed. For laboratory testing, a 1-liter buret used as a soap bubble flow meter or wet-test meter is recommended. Other standard calibrating instruments, such as a spirometer, Marriott's bottle, or dry gas meter can be used. The calibration should be of sufficient precision that the 95% confidence limits on the flow rate are ± 10% (95% of the flow rates will fall within ± 10% of the calibrated value).

Instructions for calibration with the soap bubble flow meter follow. The sampling train used (pump, hose, filter cassette) in the pump calibration should be the same as the one used in the field.

9.1.1 Check the voltage of the pump battery with a voltmeter both with the pump off and while it is operating to assure adequate voltage for calibration. If necessary, charge the battery to manufacturer's specifications.

9.1.2 Fill a beaker with 10 ml of soap solution.

9.1.3 Connect the filter cassette inlet to the top of the buret with a length of hose.

9.1.4 Turn the pump on and moisten the inside of the soap bubble meter by immersing the open end of the buret into the soap solution and drawing bubbles up the inside of the buret. Perform this task until the bubbles are able to travel the entire length of the buret without breaking.

9.1.5 Adjust the pump rotameter to provide a flow between 1.5 to 2.5 lpm.

9.1.6 With a water manometer, check that the pressure drop across the filter is less than 13 inches of water (about 1 inch of mercury).

9.1.7 Start a soap bubble up the buret and measure the time it takes for the bubble to travel a minimum volume of 1 liter.

9.1.8 Repeat the procedure in 9.1.7 at least three times, average the results, and calculate the calibrated flow rate by dividing the volume traveled by the soap bubble by the elapsed time. If the range between the highest and lowest of the three flow rates is greater than about 0.33 lpm, then the calibration should be repeated since it is likely that the precision is not adequate.

9.1.9 Data required for the calibration include the volume measured, elapsed time, pressure drop, air temperature, atmospheric pressure (or elevation), pump serial number, date, and name of person performing the calibration.

9.1.10 Corrections to the flow rate for pumps with rotameters may be necessary if the pressure (elevation) or temperature where the samples are collected (actual flow rate) differs significantly from that where the calibration was performed (indicated flow rate). Actual flow rates at time of sampling may be calculated for a linear scale rotameter by using the following correction formula:

$$Q_{actual} = Q_{indicated} \sqrt{\frac{P_{cal}}{P_{actual}} \cdot \frac{T_{actual}}{T_{cal}}}$$

where both pressure (P) and temperature (T) are in absolute units such as:

psia = psig + 14.7
deg Rankin = deg Fahrenheit + 460
deg Kelvin = deg Celsius + 273

9.2 Microscope Setup

9.2.1 Porton Reticle and the Counting Field

The asbestos fiber count procedure consists of comparing fiber length to the diameters of calibrated circles of a Porton reticle, and counting all fibers greater than 5 micrometers in length lying within a given counting field area. The Porton reticle is a glass plate inscribed with a series of circles and rectangles. The left half of the reticle is divided into six rectangles constituting the counting field. The counting field is illustrated in Figures 5 through 10.

9.2.2 Placement in Eyepiece

The Porton reticle is placed inside the Huygenian eyepiece where it rests on the field-limiting diaphragm. If other types of eyepieces are used, it may be necessary to insert a counting collar for retaining the reticle. The reticle should always be kept clean, since dirt on the reticle is in focus and could complicate the counting and sizing process.

9.2.3 Stage Micrometer

The Porton reticle cannot be used for counting until it has been properly calibrated with a stage micrometer. Most stage micrometer scales are approximately two millimeters long and are divided into units of one-hundredth of a millimeter (ten micrometers).

9.2.4 Microscope Adjustment

When adjusting the microscope, follow the manufacturer's instructions while observing the following guidelines.

1. The light source image must be in focus and centered on the condenser iris or annular diaphragm.
2. The particulate material to be examined must be in focus.
3. The illuminator field iris must be in focus, centered on the sample, and opened only to the point where the field of view is illuminated.
4. The phase rings (annular diaphragm and phase-shifting elements) must be concentric.

9.2.5 Porton Reticle Calibration Procedure

Each eyepiece-objective-reticle combination on the microscope must be calibrated. Should any of the three be changed (disassembly, replacement, zoom adjustment, etc.), the combination must be recalibrated. Calibration may change if interpupillary dis-

tance is changed. For proper calibration, the following procedure should be followed closely.

With a 10X objective in place, place the stage micrometer on the mechanical stage, focus the millimeter scale, and center the image. Change to the 40–45X objective and adjust the first millimeter scale division to coincide with the left boundary of the Porton rectangle. Measure the distance between the left and extreme right boundaries of the Porton rectangle, estimating any portion of the final division. This measurement represents 200 L units. The rectangle is 100 L units on the short vertical dimension. The calculated "L" is inserted into the formula $D = L(2^N)^{1/2}$ where "N" is the circle number (indicated on the reticle) and "D" is the circle diameter. Since the circle diameters vary logarithmically, every other circle doubles in diameter. For example, circle number three is twice the diameter of number one; number four is twice the diameter of number two. When the circle sizes have been determined, the counting field area which consists of the left six smaller rectangles can be calculated from the relation 10,000 L^2. This completes the reticle calibration for this specific objective-eyepiece-reticle combination.

Example for Porton Reticle

The following calibration was obtained for a pair of 10X Huygenian eyepieces and a 43X objective:

200 L = 0.148 mm = 148 micrometers

100 L = 0.074 mm = 74 micrometers

One L-unit = 0.74 micrometers

Thus Circle #1 has a diameter $D = L(2^N)^{1/2} = 0.74(2^1)^{1/2} = 0.74 \quad (1.414) = 1.05$ micrometers.

Then our circle diameter calibration table looks like:

Diameter of Circle #1 = 1.05 micrometers
#2 = 1.48
#3 = 2.09
#4 = 2.96
#5 = 4.19
#6 = 5.92

Field area = (10,000) (L^2) = (100 L) (100 L) = (0.074) (0.074) = 0.0055 mm^2

Thus fibers with a length greater than a distance halfway between the diameters of the #5 and #6 circles would be counted.

If a Patterson Globe and Circle reticle is used, a different calculation procedure is required. The circle diameters are related as follows. The #25 circle diameter is (0.1) (reticle length).

The circle diameters are proportional to the ratio of their numbers. Thus the #20 circle diameter is (20/25) or 0.8 times the #25 circle diameter.

10. Calculations

10.1 The average airborne asbestos fiber concentration estimated by the filter sample may be calculated from the following formula:

$$AC = \frac{[(FB/FL) - (BFB/BFL)]\ (ECA)}{(1000)\ (FR)\ (T)\ (MFA)}$$

161

where:

AC = Airborne fiber concentration in (fibers > 5 μm)/cm^3.
BFB = Total number of fibers counted in the BFL fields of the blank or control filters in fibers > 5 μm.
BFL = Total number of fields counted on the blank or control filters.
ECA = Effective collecting area of filter (855 mm^2 for a 37-mm filter with effective diameter of 33 mm).
FR = Pump flow rate in liters/min (lpm).
FB = Total number of fibers counted in the FL fields in fibers > 5 μm.
FL = Total number of fields counted on the filter.
MFA = Microscope count field area in mm^2 (generally 0.003 to 0.006).
T = Sample collection time in minutes.

10.2 Recount criteria. It is very desirable for a counter to conduct a "blind recount" for about 1 in every 10 filter wedges (slides) counted. Alternatively, a second counter could perform the blind recount. In training sessions for novice counters, the trainee should conduct a blind recount for filter wedges counted by an experienced, proficient counter. In all cases, we will observe differences between the first and second counts of the same filter wedge. Most of these differences will be due to chance alone, that is, due to the random variability (precision) of the count method. Statistical recount criteria enable us to decide whether observed differences can reasonably be explained due to chance alone or are probably due to systematic differences between counters or microscopes or due to some other biasing factor.
The following recount criterion is for a pair of counts that estimate some airborne fiber concentration (AC) in fibers/cm^3. The criterion is given at the type-I error level. That is, there is a 5% maximum risk that we will reject a pair of counts for the reason that one might be biased, when the large observed difference is really due to chance.
Reject a pair of counts because one might be biased if:

$$(AC_2 - AC_1) \text{ exceeds } 2.77(\overline{AC})(CV_{FB})$$

where:

AC_1 = lower estimated airborne fiber concentration
AC_2 = higher estimated airborne fiber concentration
$\overline{AC}$ = average of the two airborne concentration estimates
CV_{FB} = average CV for the two concentration estimates which are a function of the total fiber count (FB) in each case. Use the relation in Section 4 or Figure 3.

For a pair of counts on the same filter, reject the pair because one might be biased if:

$$(FB_2 - FB_1) \text{ exceeds } 2.77(\overline{FB})(CV_{\overline{FB}})$$

where:

FB_1 = lower fiber count on the filter (total fibers)
FB_2 = higher fiber count on the filter (total fibers)
$\overline{FB}$ = average of the two total fiber counts
$CV_{\overline{FB}} = CV_T$ for the value FB. Use the relation in Section 4 or Figure 3.

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753

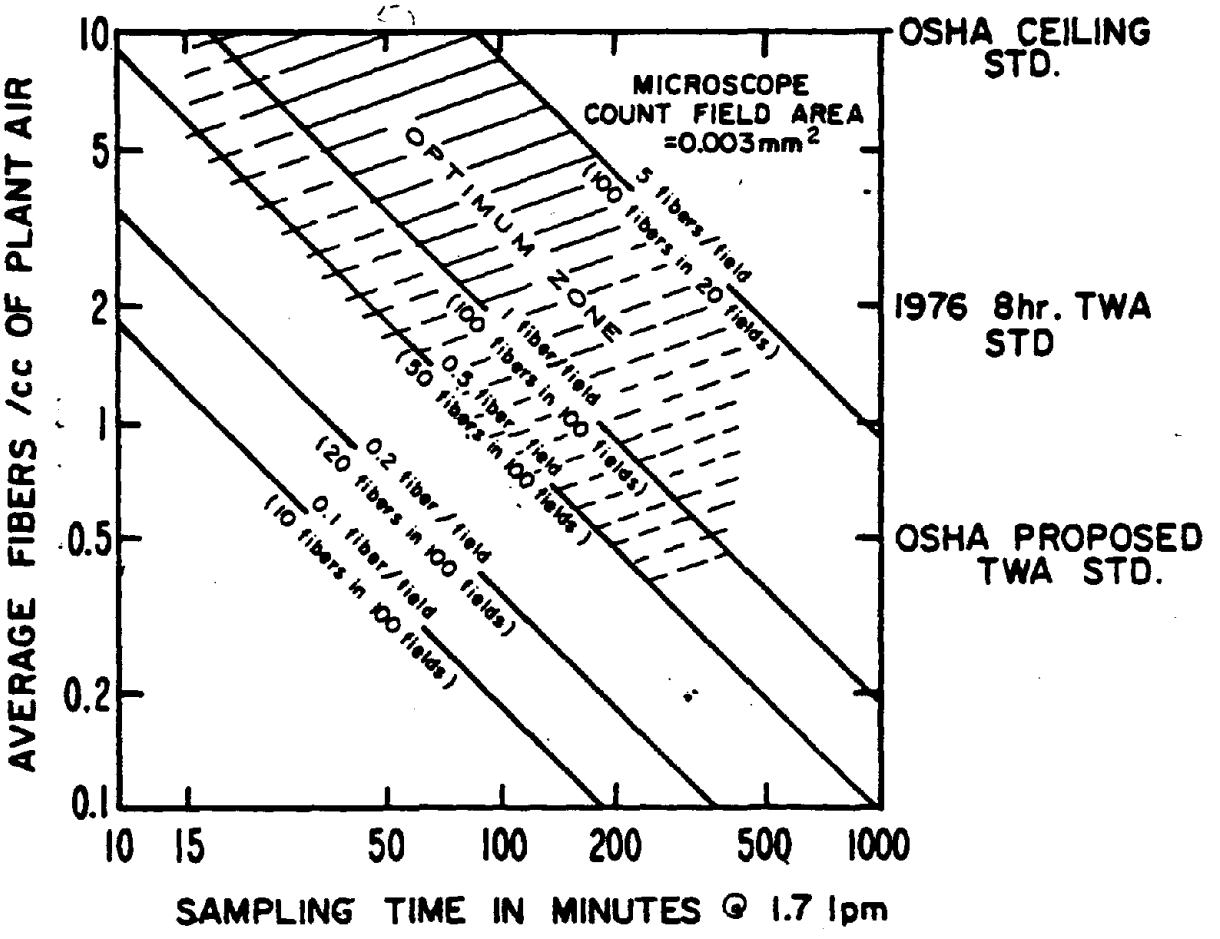


FIGURE 1. Optimum Sampling Times for airborne asbestos where microscopic field area = 0.003 mm²

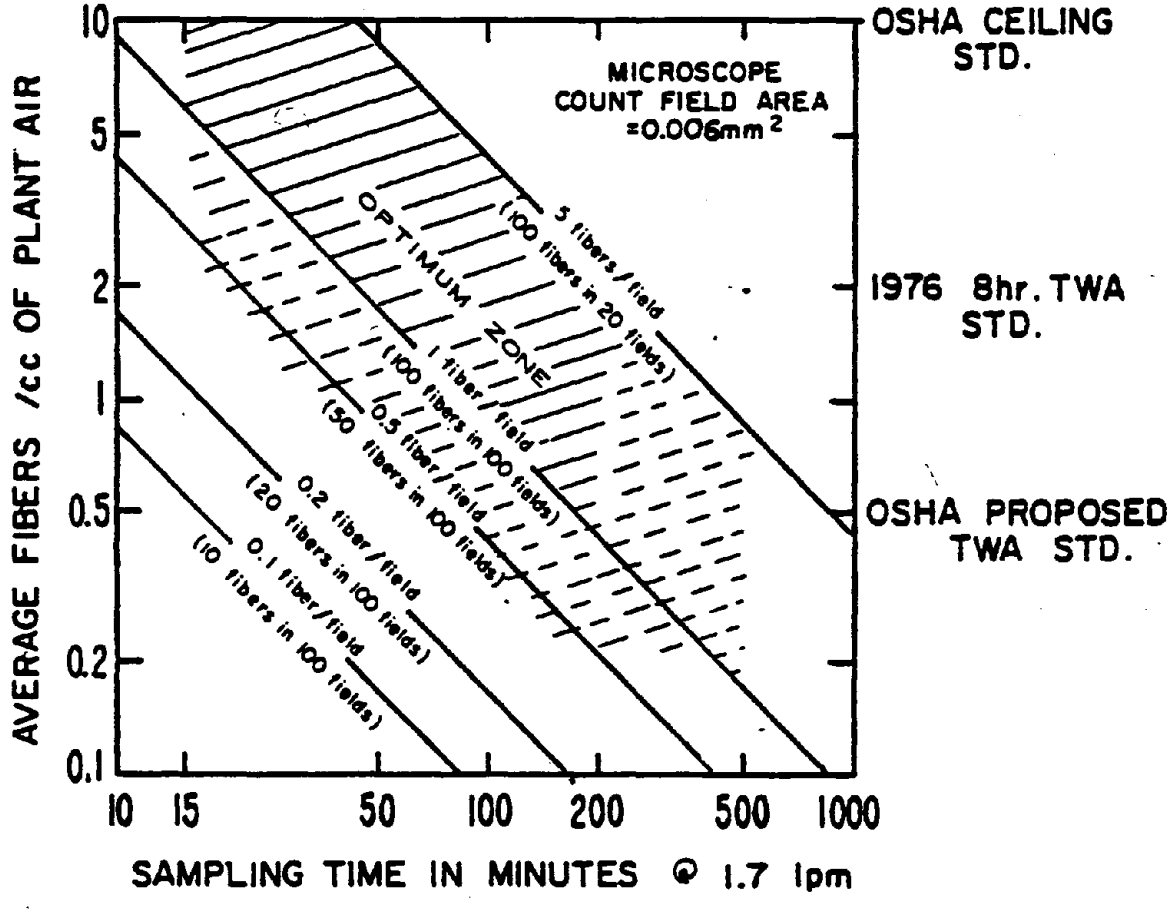


FIGURE 2. Optimum sampling times for airborne asbestos where microscopic field area = 0.006 mm²

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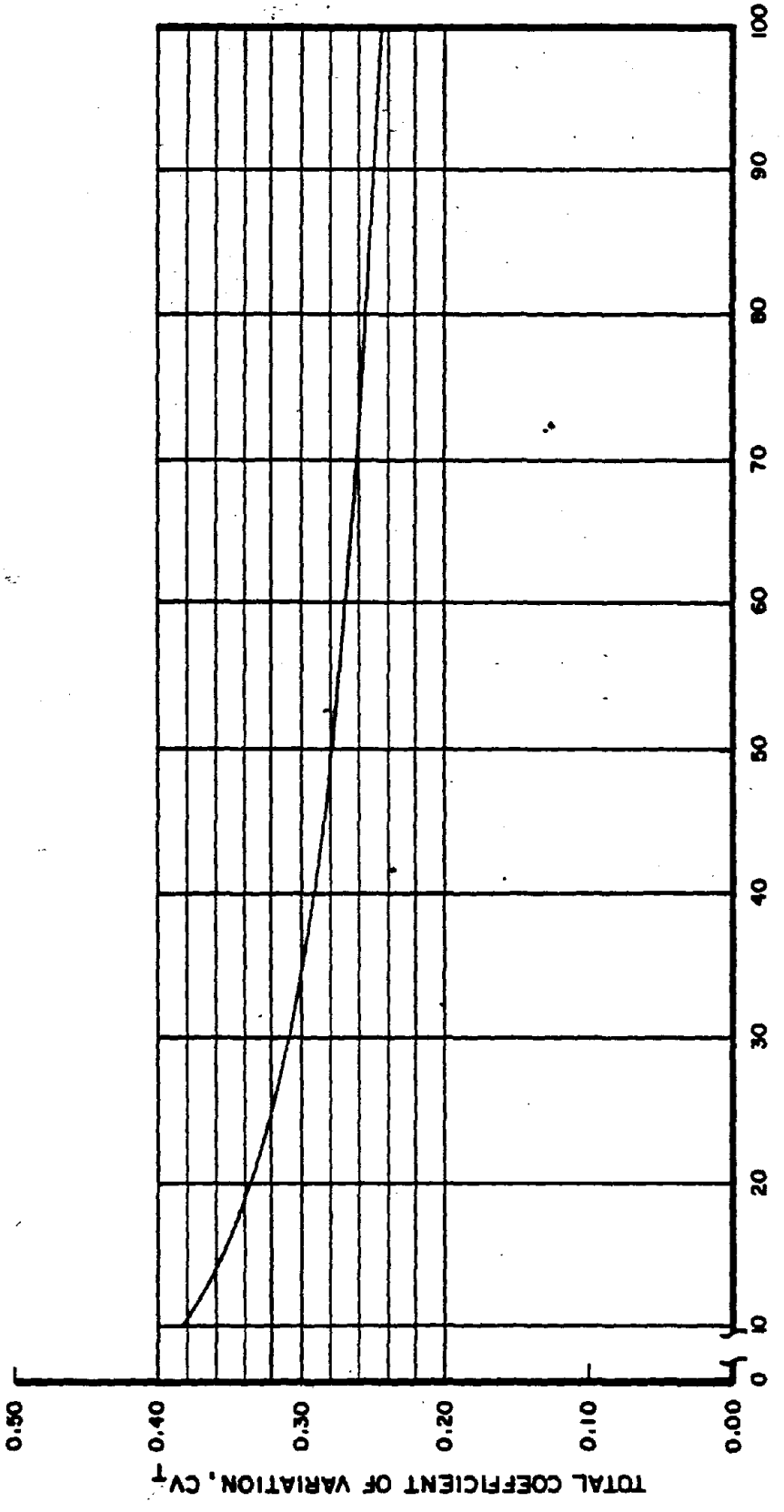


FIGURE 3. Total coefficient of variation as a function of total fiber count

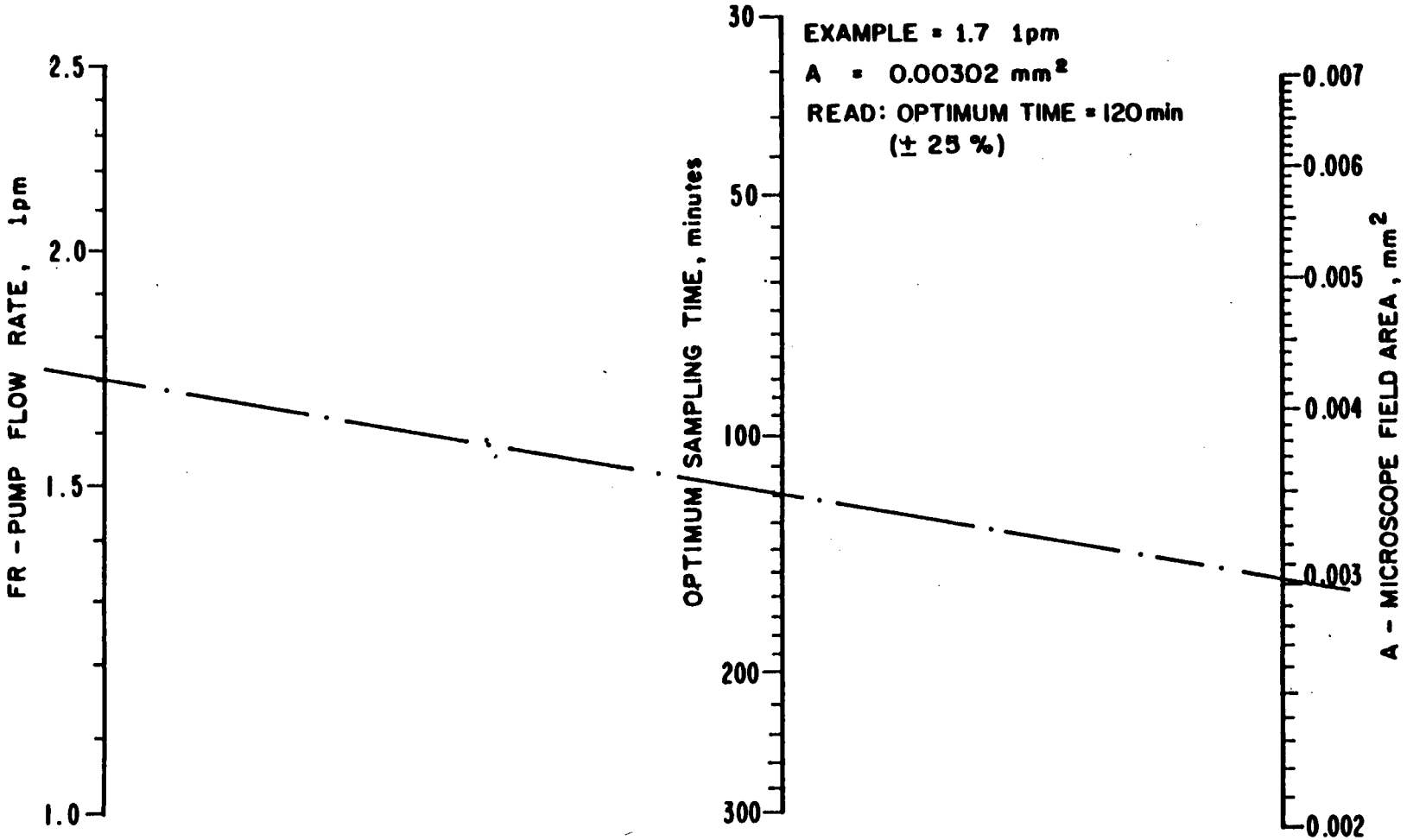


FIGURE 4. Nomogram of optimum sampling times for airborne asbestos fibers in concentrations of 1 to 10 fibers/cm³

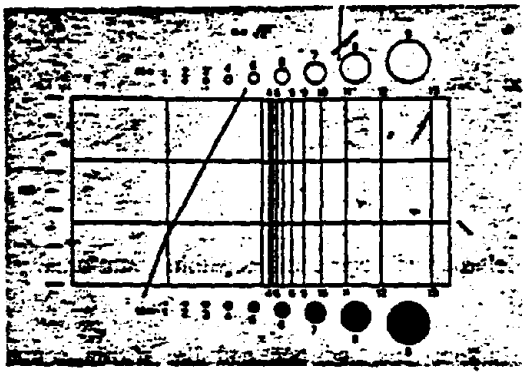


FIGURE 5

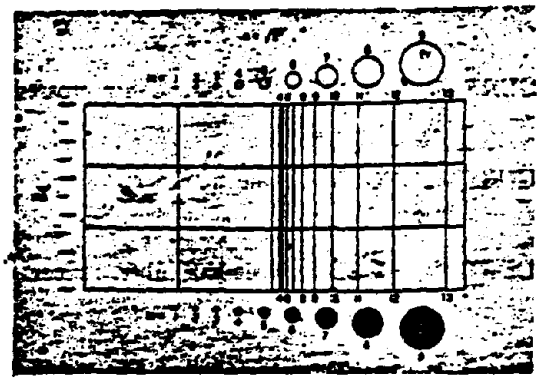


FIGURE 6

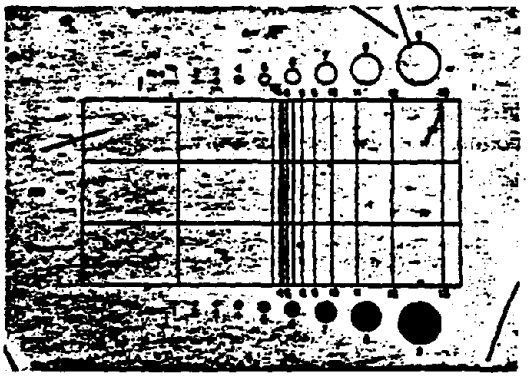


FIGURE 7

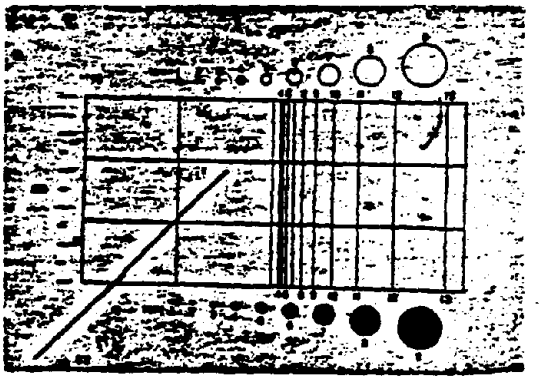


FIGURE 8

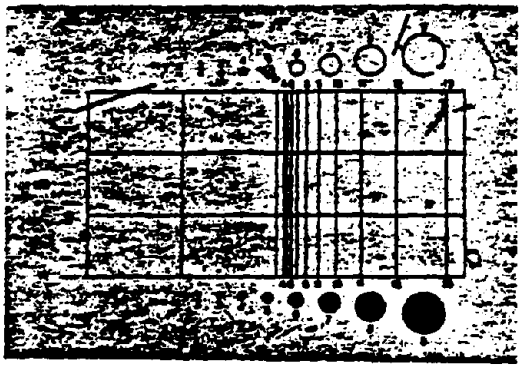


FIGURE 9

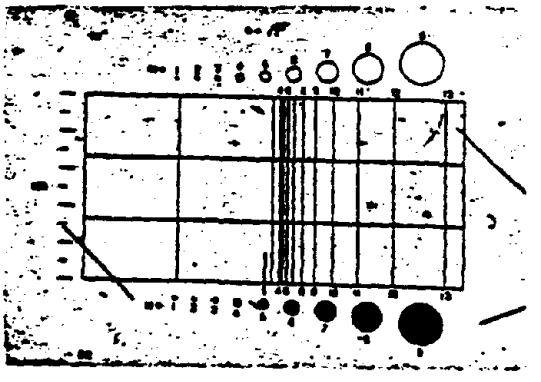


FIGURE 10

LIST OF FIGURES

(5 through 10)

FIGURE 5. DO NOT COUNT. Fiber crosses top and bottom sides.

FIGURE 6. COUNT. One fiber.

FIGURE 7. COUNT. One-half fiber. Fiber crosses left side and one end lies within count area.

FIGURE 8. COUNT. One-half fiber. Fiber crosses bottom side and one end lies within count area.

FIGURE 9. DO NOT COUNT. Fiber crosses two sides.

FIGURE 10. DO NOT COUNT. Fiber crosses two sides (bottom left corner).
COUNT. One-half fiber. Fiber crosses bottom side and one end lies within count area.
COUNT. One fiber (top right corner).

SODIUM HYDROXIDE IN AIR

Measurements Research Branch

Analytical Method

Analyte:	Sodium Hydroxide	**Method No.:**	P&CAM 241
Matrix:	Air	**Range:**	1 mg/m³-4 mg/m³
Procedure:	Bubbler collection, electrometric titration	**Precision (CV_T):**	0.06 at 2 mg/m³
Date Issued:	12/1/76	**Classification:**	E (Proposed)
Date Revised:			

1. **Principle of the Method**
   - 1.1 NaOH is collected by passing a measured volume of air through 7.5×10^{-3} N HCl in a fritted glass bubbler.
   - 1.2 NaOH is determined (as total alkali) by an electrometric back titration with 0.1 N NaOH.
2. **Range and Sensitivity**
   - 2.1 The effective range for this method is 1 to 4 mg/m³ of air, based on a sample size of 420 liters.
   - 2.2 Maximum concentration is a function of the absorptive capacity of the HCl. Four milligrams/sample of NaOH is the recommended maximum.
3. **Interferences**
   - 3.1 The most common interference is HCO_3^- caused by the absorption of CO_2 from the air. This is minimized by the use of dilute HCl as the sample absorbing solution.
   - 3.2 Other acids and bases present, affecting pH balance, will interfere. If the electrometric titration curve is plotted and no extra inflection points are observed, the sample may be considered free of significant interferences. Acid interferences will cause erroneously high results. Base interferences will cause low results.
4. **Precision and Accuracy**
   - 4.1 The analytical precision varies with the level of NaOH concentration. Validation tests show that at 4 mg/m³, CV = 0.036; at 2 mg/m³, CV = 0.055; and at 1 mg/m³, CV = 0.145.
   - 4.2 The bias, derived from the analysis of spiked samples, varies with concentration level. The average recovery of spiked samples equivalent to a 420-l air sample at a concentration of 1.2 mg/m³ was 77%; at 1.9 mg/m³ was 92%; at 2.9 mg/m³ was 95%.
5. **Advantages and Disadvantages of the Method**
   - 5.1 The method is for the determination of total alkali, and hence is non-specific for NaOH.
   - 5.2 It is difficult to predict the contaminants that might appear under field-sampling conditions.
   - 5.3 In order to collect sufficient sample, several hours sampling time are required.
   - 5.4 The analytical procedure is relatively simple to perform and requires little specialized equipment.
   - 5.5 The method measures total alkali, which is the harmful agent in NaOH exposure (as opposed to measuring Na^+).

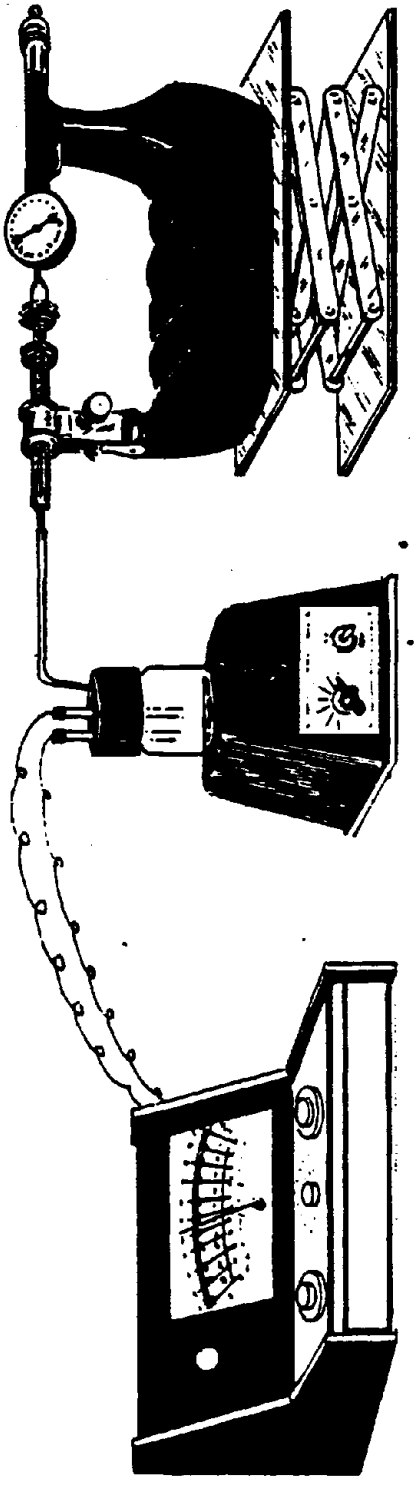


FIGURE 1. Microburet assembly for electrometric titration of sodium hydroxide

762

6. **Apparatus**

6.1 Sampling Equipment

6.1.1 Pump: A personal sampler pump whose flow rate can be determined accurately, ±5%, at the recommended flow rate. The pump must be calibrated with a representative sampling train in line.

6.1.2 Bubbler: A midget bubbler filled with exactly 15 ml of 7.5×10^{-3} Normal hydrochloric acid. A Teflon baffle should be installed on the bubbler stem at the 25-ml level.

6.2 pH Meter with expanded scale readout, equipped with a single junction reference electrode and a glass pH electrode

6.3 Fleakers™, 150 ml, with caps

6.4 Magnetic Stirrer

6.5 Laboratory Jack

6.6 Syringe Microburet

7. **Reagents**

All reagents must be analytical regent grade or better.

7.1 Ascarite, fine mesh, for CO_2 traps.

7.2 Double deionized water, boiled and capped under an Ascarite trap.

7.3 Sodium Hydroxide, 0.1 Normal. The solution is made by diluting a 50% solution of NaOH, using water as in 7.2. Four milliliters of a concentrated solution diluted to one liter will yield a solution of 0.1 normality. Store under an Ascarite trap.

7.4 Hydrochloric Acid, 7.5×10^{-3} Normal. Dilute 1.25 ml of concentrated HCl (Reagent Grade) to two liters.

7.5 Sodium Carbonate, Na_2CO_3, Primary standard grade.

7.6 Potassium Acid Phthalate, $KHC_8H_4O_4$, Primary standard grade.

8. **Procedure**

8.1 **Cleaning of Equipment.** Since interferences occur with acids and bases, all glassware, after washing, should be rinsed several times with double deionized water and oven dried.

8.2 **Collection and Shipping of Samples**

Sample Size: To evaluate exposures at the standard, a minimum sample size of 350 liters is recommended. Sample at a rate of 1.0 liter per minute. At least 700 μg of sodium hydroxide should be collected. If more than 4 milligrams of sodium hydroxide are collected, the excess sodium hydroxide will not be detected because all the hydrochloric acid in the collecting liquid will be consumed.

8.2.1 Connect the bubbler to the pump with a piece of flexible tubing. If personal sampling is being done, the midget bubbler is placed in a midget bubbler holder and fastened to the worker in his breathing zone.

8.2.2 Turn on the pump and set the flow rate to 1.0 liter per minute according to the manufacturer's directions. Periodically check the flow rate and readjust as needed. Terminate sampling if unable to readjust. In addition, check the level of solution in the bubbler. If less than 10 ml of solution remains in the bubbler, fill the bubbler back up to the 15 ml mark with distilled water.

8.2.3 After sampling, disconnect and seal off the bubbler outlets with parafilm, and tape the ground glass joint. Care should be taken to prevent spillage and minimize loss by evaporation at all times.

766

8.2.4 Record ambient temperature and pressure. If ambient pressure is unavailable, record the elevation.

8.2.5 Use one bubbler as a blank. Handle it the same as any other bubbler but do not draw air through it. Label this solution as a blank.

8.2.6 Shipping Instructions: Ship midget bubblers in a container that is designed to prevent damage during shipment.

Special Instruction: Under no circumstances is additional acid solution to be added to the bubbler. The addition of more acid solution will be a negative interference.

8.3 **Analysis of Samples**

8.3.1 Transfer the solution in the bubbler quantitatively to a 150-ml Fleaker™, washing the bubbler stem and impinger cell thoroughly with distilled deionized water to obtain a final volume of approximately 100 ml.

8.3.2 Titrate with the standardized 0.1 N NaOH past the pH 7 endpoint, to a pH of about 10, using the system shown in Figure 1. Record pH readings (expanded scale) for each incremental addition of NaOH and plot the titration curve.

8.3.3 Titrate a blank water solution in the same manner.

9. **Calibration and Standards**

9.1 The hydrochloric acid (7.5×10^{-6} N) is standardized against primary standard grade sodium carbonate. The electrometric titration system is used with a 50-ml buret and the second inflection of the curve used for the endpoint. The theoretical pH for this second endpoint is 3.7. However, it should be determined experimentally in each case. Accurately weigh approximately 0.018 gram of sodium carbonate for the standardization.

9.2 The sodium hydroxide (approximately 0.1 N) is standardized against primary standard grade potassium acid phthalate using an electrometric titration and a 50-ml buret. Accurately weigh approximately 0.9 gram of potassium acid phthalate.

10. **Calculations**

10.1 To calculate normality of the HCl, the following formula is used:

$$N_{HCl} = \frac{g\ Na_2CO_3}{(0.053)V_{HCl}}$$

where:

N_{HCl} = Normality of the HCl (from Section 9.1)

V_{HCl} = ml of HCl consumed to second inflection point

0.053 = Volumetric factor for Na_2CO_3, milliequivalent weight of Na_2CO_3.

10.2 To calculate normality of the NaOH, the following formula is used:

$$N_{NaOH} = \frac{gKHC_8H_4O_4}{(0.20423)\ V_{NaOH}}$$

where:

N_{NaOH} = Normality of the NaOH solution (from Section 9.2)

V_{NaOH} = ml of NaOH consumed to the inflection point

0.20423 = Volumetric factor for $KHC_8H_4O_4$, milliequivalent weight for $KHC_8H_4O_4$.

10.3 To calculate the μg NaOH in the sample, the following formula is used:

$$W_{NaOH} = \frac{(V_B - V_s)\,(N_{HCl})\,(6 \times 10^5)}{V_B}$$

where:

W_{NaOH} = μg NaOH in the sample

*V_B = μl of standard NaOH required to neutralize 15 ml of standard HCl

V_s = μl standard NaOH required to neutralize sample

N_{HCl} = Normality calculated (from Section 10.1)

*V_B may be calculated from the normality of HCl and NaOH by the formula $V_1N_1 = V_2N_2$. Calculated value should agree closely with blank titration.

10.4 For personal sampling pumps with rotameter only, the following correction should be made.

$$V_{corr} = f \times t \sqrt{\frac{P_1}{P_2} \times \frac{T_2}{T_1}}$$

where:

f = flow rate sampled

t = time (minutes)

P_1 = pressure during calibration, ambient (mm Hg)

P_2 = pressure at sampling site

T_1 = temperature during calibration (°K)

T_2 = temperature at sampling site (°K)

10.5 The corrected concentration of NaOH is expressed:

$$\text{mg NaOH/m}^3 = \frac{\text{mg NaOH in sample}}{V_{corr}} \qquad \begin{matrix}\text{(Sec 10.3)} \\ \text{(Sec. 10.4)}\end{matrix}$$

11. **References**

11.1 1972 Annual Book of ASTM Standards: Water, Atmospheric Analysis: Part 23, D.1067-70, Standard Methods of Test for Acidity and Alkalinity of Water, Method A: Electrometric Titration.

11.2 1972 Annual Book of ASTM Standards: Water, Atmospheric Analysis: Part 23, E.200-67, Standard Methods for Preparation, Standardization, and Storage of Standard Solutions for Chemical Analysis.

11.3 Sweet, D. V., J. C. Haartz, and M. S. Hawkins, "Determination of Sodium Hydroxide Aerosol in Industrial Hygiene Samples", paper in preparation.

165

PHOSPHORUS IN AIR

Measurements Research Branch

Analytical Method

Analyte:	Phosphorus ("yellow," "white")	**Method No.:**	P&CAM 242
Matrix:	Air	**Range:**	0.005 – 5.0 mg/m³
Procedure:	Filter/impinger (xylene).• collection, FPD gas chromatography	**Precision:**	0.033 RSD (analytical)
Date Issued:	12/6/76	**Classification:**	E (Proposed)
Date Revised:			

1. **Principle of the Method**

   Phosphorus (yellow, white) from the air is trapped in xylene contained in a midget impinger. The xylene solution is maintained at 10 ml by addition of solvent, if necessary. This xylene solution is subjected to flame photometric gas chromatographic analysis that is phosphorus specific. The red and black forms of phosphorus do not interfere. All references in the method are to white phosphorus, which is also called yellow phosphorus.

2. **Range and Sensitivity**

   2.1 The linear range of the flame photometric detector is 0.05 ng to 50.0 ng, corresponding to 0.005 to 5 mg/m³ for a 50-liter sample.

   2.2 When a 50-liter air sample is collected in 10 ml xylene, a 2 μl (1-6 μl) injection is conveniently used for determinations in the range 0.005 to 5.0 mg/m³. A detection limit of 0.05 ng is readily attainable. The sensitivity will depend upon changing: (1) the volume of the air sampled, (2) the volume of the final xylene solution, or (3) the size of the aliquot injected into the gas chromatograph.

3. **Interferences**

   Phosphorus compounds having retention times close to that of phosphorus will interfere with the analysis.

4. **Precision and Accuracy**

   4.1 The precision for the analytical method is ± 0.033 mg/m³ for samples ranging from 0.64 mg/m³ to 1.12 mg/m³. This range is based upon 6 sets of samples, 3 per set, taken at 0.4 to 0.55 lpm with a volume range of 16-34 liters.

   4.2 The accuracy of this method has not been estimated.

5. **Advantages and Disadvantages of the Method**

   5.1 The method is very sensitive and the detector exhibits high specificity for phosphorus compounds. The analysis is performed directly on the compound of interest. Separation and quantitation are accomplished in a reasonable amount of time.

   5.2 The cost of the equipment and supplies may tax the budget of some laboratories. The sensitivity of the equipment depends on careful adjustment of the operating parameters. Contamination can occur through equipment and/or reagents.

6. Apparatus

6.1 Sampling Equipment. The sampling unit for the impinger collection method consists of the following components:

6.1.1 A prefilter unit that consists of the filter medium and cassette filter holder.

6.1.2 A set of two midget impingers containing the absorbing solvent.

6.1.3 A pump suitable for delivering desired flow rates. The sampling pump is protected from solvent condensation by a tube loosely packed with a plug of fine glass wool and inserted between the exit arm of the impinger and the pump.

6.1.4 An integrating volume meter such as a wet test meter or dry gas meter.

6.1.5 Thermometer

6.1.6 Manometer

6.1.7 Stopwatch

6.2 Filter cassette with glass-fiber filter, 0.8 μm, 37 mm

6.3 Forceps

6.4 Beakers, 100 ml

6.5 Volumetric flasks for standards

6.6 Volumetric pipet, 10 ml

6.7 Pipet bulb, rubber

6.8 Perkin-Elmer 3920-B gas chromatograph, or equivalent (11.1, 11.2, 11.3), with attendant equipment, including a phosphorus flame photometric detector.

6.9 Gas chromatography column constructed from 6 ft × 4 mm I.D. silanized borosilicate glass or 6 ft × 2 mm I.D. borosilicate glass packed with one of the following:

6.9.1 3% SE-30 on 80/100 mesh Gas Chrom Q

6.9.2 3% OV-1 on 100/120 mesh Gas Chrom Q (11.1)

6.9.3 3.8% SE-30 on 80/100 mesh Chromosorb W-HP (11.2)

6.9.4 3.0% SE-30 on 80/100 mesh Chromosorb W-HP (11.3)

6.9.5 3% OV-1 on 100/120 mesh Chromosorb W-HP (11.1)

6.9.6 4% DC-200 on 100/120 mesh Gas Chrom Q

Columns 6.9.4 and 6.9.5 are preferred. Columns 6.9.1 through 6.9.5 were conditioned by heating for one day at the recommended temperature under the appropriate carrier gas flow rate (30 ml/minute), then cooled to 170°C and treated with 10-50 μl of a silylation agent. The column may then be primed with 5-μl injections of 2 mg/ml phosphorus in xylene stock solution. Priming should only be performed when adequate column conditioning by silylation fails to provide the minimum detection limit.

6.10 Syringes, 5 μl, 10μl and 100μl

6.11 Spatula, blade

7. Reagents

7.1 Distilled water

7.2 Acetone, wash

7.3 Xylene, chromatoquality; this is absorbing solvent

7.4 Phosphorus (yellow, white), stick, ⅝-inch diameter, of known purity

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware used should be scrupulously cleaned and given a final xylene rinse immediately before use. If colorimetric methods are to be used as secondary analysis, special cleaning should be performed with non-phosphate detergents.

8.2 **Collection and Shipping of Samples**

8.2.1 Pipette 10 ml of the absorbing solvent (section 7) into the midget impinger.

8.2.2 Connect the impinger (via the adsorption tube) to the personal sampling pump and prefilter assembly with a short piece of flexible tubing. The minimum amount of tubing necessary to make the joint between the prefilter and the impinger should be used. The air being sampled should not be passed through any other tubing or equipment before entering the impinger.

8.2.3 Turn on the sampling pump to begin sample collection. Care should be taken to measure the flow rate, time and/or volume as accurately as possible. The sample should be taken at a flow rate of 0.5 to 1.0 lpm. A sample size of not more than 96 liters and no less than 24 liters should be collected. The minimum volume of air sampled will allow the measurement of at least 1/10 times the TLV, 0.01 mg/m³ (760 mm Hg, 250°C). A flow rate of 0.3 lpm may be employed where solvent evaporation appears significant; however, this is well below impinger flow/rate efficiency maximum.

8.2.4 After sampling, the impinger stem can be removed and cleaned. Tap the stem gently against the inside wall of the impinger bottle to recover as much of the sampling solvent as possible. Wash the stem with approximately 1 ml of unused absorbing solvent and add the washing to the impinger. Then the impinger is sealed with a hard, non-reactive stopper (preferably Teflon). Do not seal with rubber. The stoppers on the impingers should be tightly sealed to prevent leakage during shipping. If it is preferred to ship the impingers with the stems in, the outlets of the stem should be sealed with Parafilm or other non-rubber covers, and the ground glass joints should be sealed (i.e., taped) to secure the top.

8.2.5 Care should be taken to avoid or minimize loss by spillage or evaporation at all times. Refrigerate samples if analysis cannot be done within a day. Exclusion of light to the sample is advised. Should more than 1 ml of the sample be lost to spillage, the sample should be discarded.

8.2.6 Whenever possible, hand delivery of the samples is recommended. Otherwise, special impinger shipping cases designed by NIOSH should be used to ship the samples.

8.2.7 A blank impinger should be handled as the other samples (fill, seal, and transport) except that no air is sampled through this impinger.

8.2.8 The filter cassettes are capped and placed in an appropriate cassette shipping container. One filter disc should be handled like the other samples (seal and transport) except that no air is sampled through, and this is labeled as a blank.

8.3 **Analysis of Samples**

8.3.1 The sample in 9 to 10 ml of xylene is transferred quantitatively to a 10 ml volumetric flask. Washings from the sample container are used to obtain exactly 10 ml solution.

8.3.2 Transfer the glass filter to a 100 ml beaker with a clean pair of forceps. Pipette 10 ml of xylene and agitate with a glass rod. Quantitatively transfer the xylene to a volumetric flask (10 ml) and dilute to the mark (if necessary).

8.3.3 Inject a 1 to 6 μl aliquot of the xylene solution, filter and impinger respectively, into the gas chromatograph and obtain a chromatogram. The chromatographic conditions are:

Column Temperature (6.9.1 to 6.9.5)	80°C
Injection Port Temperature	200°C
Detector Temperature	200-225°C
Transfer line and switching valve (11.3)	235°C
Gas Carrier (He) Flow	80 ml/min 4-mm I.D. column
	40 ml/min 2-mm I.D. column

Retention time of phosphine is approximately 0.3 minutes, whereas phosphorus retention time is greater than 3.0 minutes at these conditions.

8.3.4 The solvent-vent sample injection technique is not necessary if a "hyperventilated oxygen" flame (11.4) is used. Triplicate injections should be made if the solvent shoulder blends into the phosphorus peak.

9. **Calibration and Standards**

9.1 Prepare at least four standard solution in the concentration range 100 ng/ml to 10,000 ng/ml from a stock solution of 2 mg/ml phosphorus in xylene. Should the samples be more concentrated than the most concentrated standard, then additional standards should be made until the samples are bracketed by standards.

Note: Extreme care should be taken to avoid skin contact with phosphorus and phosphorus solutions; the handling of xylene should be performed in a well-ventilated area using gloves to avoid skin contact.

9.2 Make triplicate injections of aliquots of each standard solution onto the gas chromatograph and determine the peak areas. Peak height provides a reasonable measure of peak area when extremely sharp peaks are obtained.

9.3 Plot the amount (μg) of phosphorus seen by the detector versus the peak area (or peak height). A straight line passing through the origin should result. If these conditions are not observed, either the linear range of the detector has been exceeded or a system malfunction has occurred. An extrapolated plot may not be used to determine the phosphorus content of samples.

Note: At 80°C, the plot may not intersect at the origin; however, if the intersection of the x or y axis by the line is (in mm) less than the baseline noise (in mm) then acceptable results are obtainable. Lowering the column temperature to 60°C generally results in a line that intersects at the origin without a significant change in slope.

9.4 Injections of standards should be interspersed with the sample injections, so a watch can be maintained on detector sensitivity. A calibration curve must be prepared daily as flame characteristics change from day to day.

10. **Calculations**

10.1 Determine the total amount, in μg, of phosphorus present in the filter and impinger separately, and combine μg for total.

$$\mu g_f = \mu g_o \times \frac{\text{solution volume}}{\text{injection volume}}$$

$$\text{total } \mu g = \mu g_f + \mu g_i$$

where

μg_o = micrograms of phosphorus determined from calibration curve based on peak area (peak height) response

μg_f = micrograms of phosphorus on filter

μg_i = micrograms of phosphorus from impinger

solution volume = volume in microliter (μl) of the final xylene solution (generally 10,000 μl)

injection volume = volume in μl of the aliquot of the final xylene solution injection into the gas chromatograph.

10.2 Convert the volume of air sampled to standard conditions at 25°C and 760 mm Hg.

$$V_s = V \times \frac{P}{760 \text{ mm Hg}} \times \frac{298°C}{(273°C + T°C)}$$

V_s = volume of air in liters at 25°C and 760 mm Hg

V = volume of air in liters as measured

P = barometric pressure in mm Hg

T = temperature of air in degrees centigrade

10.3 The concentration of phosphorus can be expressed in μg per liter or mg per m^3.

$$mg/m^3 = \mu g/\text{liter}$$

$$mg/m^3 = \frac{\text{total } \mu g \text{ phosphorus}}{V_s} \qquad \left(\frac{\text{Section 10.1}}{\text{Section 10.2}}\right)$$

11. References

11.1 Barrett, W. J., H. K. Dillon, and R. H. James, "Development of Methods for the Determination of Elemental Phosphorus and Phosphine in Air", Third Bimonthly Report, HEW Contract No. 210-75-0047, January 30, 1976.

11.2 Bohl, C. D. and E. F. Kaelble, "Determination of Elemental Phosphorus in Air by Gas Chromatography", Am Ind Hyg Assoc J 34:306, 1973.

11.3 Addison, R. F. and R. G. Ackman, "Direct Determination of Elemental Phosphorus by Gas-Liquid Chromatography", J Chromatogr 47:421, 1970.

11.4 Burgett, C. A. and L. E. Green, "Improved Flame Photometric Detection without Solvent Flameout", J Chromatogr Sci 12:356, 1974.

BENZIDINE IN AIR

Measurements Research Branch

Analytical Method

Analyte:	Benzidine and benzidinium sulfate	**Method No.:**	P&CAM 243
Matrix:	Air	**Range:**	3-130 $\mu g/m^3$ for a 50-liter air sample
Procedure:	Collection on glass fiber filter and silica gel, elution with triethylamine in methyl alcohol, analysis by HPLC	**Precision:**	7% relative standard deviation
Date Issued:	3/25/77	**Classification:**	E (Proposed)
Date Revised:			

1. **Principle of the Method**

   1.1 A known volume of air is drawn through a two-stage sampler consisting of a high-efficiency glass fiber filter followed by a bed of silica gel to collect benzidine or benzidinium sulfate aerosol and/or vapor.

   1.2 The filter and sorbent sections of the sampler are transferred to stoppered tubes and the benzidine or benzidinium sulfate is desorbed with a solution of triethylamine in methyl alcohol. This desorbing solution converts benzidinium sulfate to free benzidine.

   1.3 An aliquot of this solution is injected into a high performance liquid chromatograph (HPLC).

   1.4 Peak areas are determined and compared with a calibration curve obtained from injections of standard solutions of benzidine.

2. **Range and Sensitivity**

   2.1 The range of the method is 0.15-6.5 μg/sample (3-130 $\mu g/m^3$ for a 50-liter air sample) using 0.5 ml of desorbing solution and a 10-μl injection into the liquid chromatograph. The upper limit can be extended by increasing the volume of desorbing solvent, decreasing the sensitivity of the detector, or decreasing the aliquot injected into the liquid chromatograph.

   2.2 The limit of detection is 0.05 μg/sample.

3. **Interferences**

   3.1 Aniline has the same retention time as benzidine and will interfere in the analysis.

   3.2 In solutions containing benzidine or its sulfate at concentrations of 8 ng/μl, the following compounds were found not to interfere when their concentrations were $\leq$ 16 ng/μl: o-, p-, and m-chloroaniline, 4,4′-methylenedianiline, β-naphthylamine, 3-chloro-4-aminotoluene, 3,3′-dichlorobenzidine, 4,4′-methylenebis(2-chloroaniline), hydrazobenzene, and 1,2- and 1,4-naphthoquinone.

   3.3 Any compound sampled with benzidine and having the same retention time as benzidine will interfere with the analysis. This type of interference often can be eliminated by changing the operating conditions of the chromatograph, e.g., by changing the composition of the mobile phase or by solvent programming.

4. Precision and Accuracy

4.1 Filters spiked with 0.2 to 2.0 μg of benzidine and stored at $-15°C$ yielded mean recoveries of 97% over an 11-day period. Recoveries dropped to 89% and 75% after 15 and 21 days, respectively. Recoveries of 98% were obtained through 21 days for filters spiked with comparable amounts of benzidinium sulfate and stored at $-15°C$.

4.2 The pooled estimate of the relative standard deviation was 7% based upon 29 samples collected from test atmospheres containing benzidine at concentrations of 21 $\mu g/m^3$ (nine 16-liter samples), 28 $\mu g/m^3$ (ten 49-liter samples) and 63 $\mu g/m^3$ (ten 48-liter samples). The sampling rate was 0.8 liter/min and the environment was maintained at 30°C and 80% relative humidity. (Benzidine was generated as a poly-disperse aerosol with a MMAD of $\approx$2.4 μm and a σ_g of $\approx$1.6.)

4.3 The accuracy of the method has not been determined.

5. Advantages and Disadvantages of the Method

5.1 The sampler is a small portable device containing no liquids which can be used to sample the air in the breathing zone of a worker. Environmental conditions of temperature and humidity do not significantly affect the method. Desorption of the collected sample is simple and the analysis is accomplished by a rapid instrumental technique. Possible interferences can be obviated by changing the composition of the mobile phase or by solvent programming.

5.2 At a flow rate of 0.2 liter/min, the linear velocity through the 4-mm inlet of the filter holder is 26.5 cm/s. It is not known if this throat velocity is sufficient for the capture of all particles of analytical importance.

5.3 This method has not been field-tested.

5.4 Samples containing benzidine showed a tendency to degrade even when stored at $-15°C$. (Section 4.1)

6. Apparatus

6.1 Calibrated personal air sampling pump that can be maintained for 8 hr at a flow rate of 0.2 liter/min with the sampler in line.

6.2 Sampler, equivalent to that shown in Fig. 1. The sampler consists of two sections, a high-efficiency glass fiber filter followed by a 50-mg bed of silica gel. The first section, a 13-mm type A-E glass fiber filter (Gelman Instrument Co., Ann Arbor, MI) is contained in a 13-mm filter holder (SX00-013-00, Millipore Corp., Bedford, MA). The sorbent tube is a 30-mm section of Pyrex glass, 6.4-mm O.D. $\times$ 4-mm I.D. flared on one end, and containing 50 mg of GC grade silica gel (D-08, 30/60 mesh, 720-760 m^2/g, 4.3 g/cc; Applied Science Laboratories, Inc., State College, PA). The sorbent is held by 3.5-mm diameter, 100-mesh stainless steel screens and 4-mm O.D. Teflon rings. To connect the two stages, the filter holder is pressed into the flared end of the sorbent tube. The pressure drop across samplers at various flow rates should be within 5% (relative standard deviation) of values extrapolated from Fig. 2. Plastic caps of 6-mm I.D. seal the ends of the sampler.

6.3 Insulated container suitable for transporting samples packed in dry ice.

6.4 Dry ice.

6.5 High performance liquid chromatograph equipped with an ultraviolet detector (254 nm) and injection valve.

6.6 μBondapak C_{18} column (10-μm), 4.0-mm I.D. $\times$ 30-cm (Waters Associates, Milford, MA), or equivalent.

6.7 Potentiometer strip chart recorder.

6.8 Test tubes (1-ml) fitted with polyethylene stoppers.

6.9 Glass syringes, 10-μl.

6.10 Pipettes of convenient sizes for the preparation of standard solutions.

6.11 Volumetric flasks of convenient sizes for the preparation of standard solutions.

6.12 Clinical centrifuge.

6.13 Analytical balance.

6.14 Test-tube shaker (vortex type).

7. Reagents

All reagents should be of analytical reagent quality or better.

7.1 Methyl alcohol (UV grade), distilled in glass.

7.2 Water, distilled.

7.3 Mobile Phase: 3/2 (v/v) methyl alcohol in water.

7.4 Triethylamine.

7.5 Desorbing Solution: 0.17% (v/v) triethylamine in methyl alcohol.

7.6 Benzidine, analytical standard quality.

8. Procedure

8.1 **Cleaning of Equipment** All glassware used for the laboratory analysis is washed with detergent, rinsed with tap water, distilled water, and methyl alcohol, and dried in an oven.

8.2 **Calibration of Personal Sampling Pumps** Each pump should be calibrated with a representative sampler in line to minimize errors in volume measurement. A bubble flow meter or other suitable flow measuring device may be used.

8.3 **Collection and Shipping of Samples**

8.3.1 Immediately before sampling, the plastic caps are removed from the end of the sampler and saved for resealing after sampling.

8.3.2 The sorbent end of the sampler is connected to the pump with plastic or rubber tubing. The sampler is positioned vertically during sampling. Sampled air must not pass through any tubing before entering the sampler.

8.3.3 The atmosphere is sampled at a flow rate of 0.2 liter/min for the desired period of time. The flow rate and sampling time, or the volume of sampled air, must be measured as accurately as possible.

8.3.4 The temperature, pressure, and humidity of the atmosphere being sampled is measured and recorded.

8.3.5 The sampler is resealed with the plastic caps immediately after sampling.

8.3.6 Blank samples are obtained by handling random collection devices in the same manner as in sampling, except that no air is drawn through them.

8.3.7 Samples shipped to the laboratory should be packed tightly to minimize breakage and cooled with dry ice.

8.3.8 If samples of bulk material, associated with the process under investigation, are to be shipped to the laboratory, they should not be placed in the same container as the air samplers or blanks.

8.3.9 The samples should be stored at temperatures at or below −15°C prior to analysis. Because of the instability of the analyte, the samples should be analyzed as soon as possible after collection.

8.4 **Analysis of Samples**

8.4.1 Preparation of Samples. Place the glass fiber filters and silicia gel in separate 1-ml test tubes or other suitable vials. Teflon rings and stainless steel screens associated

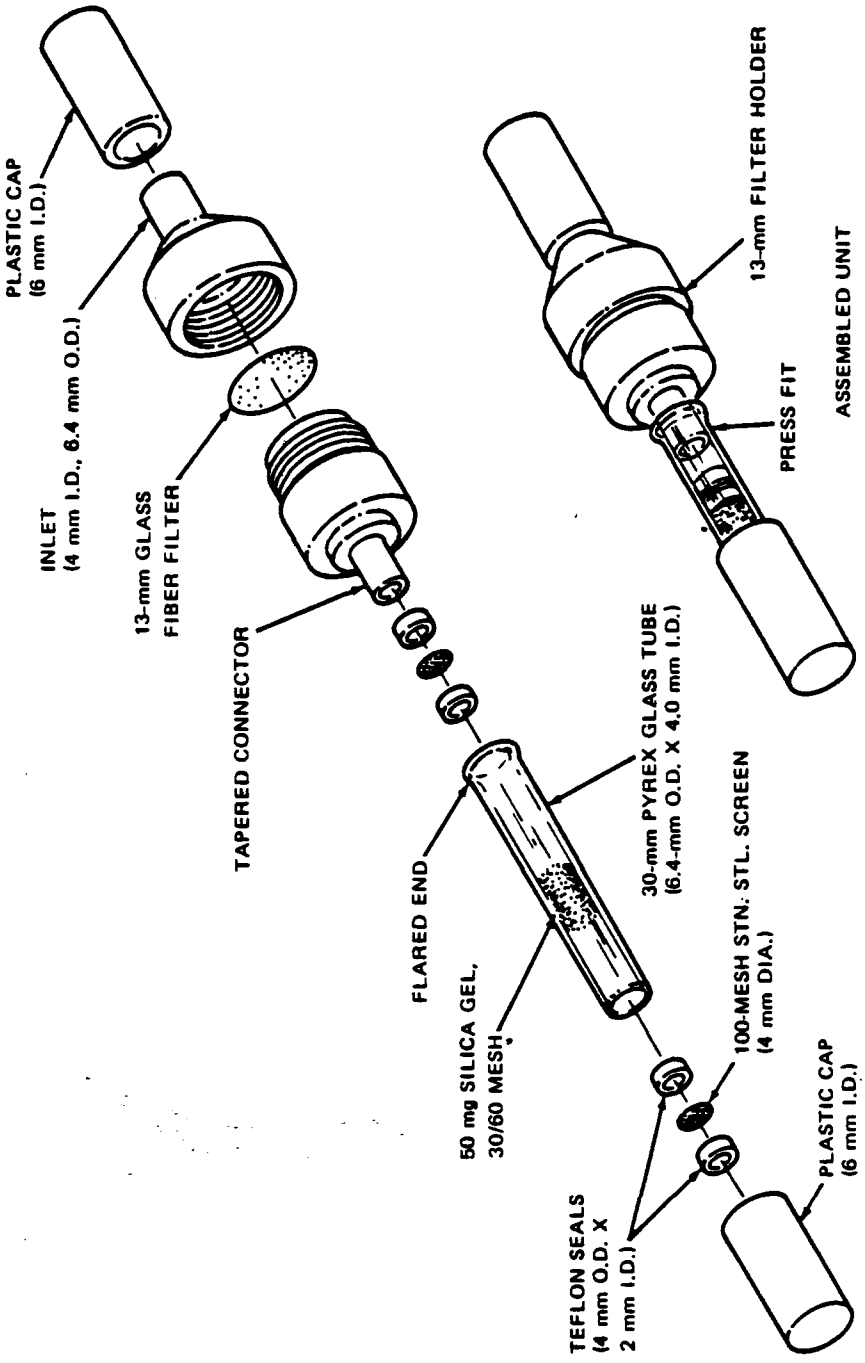


FIGURE 1. Two-stage sampler

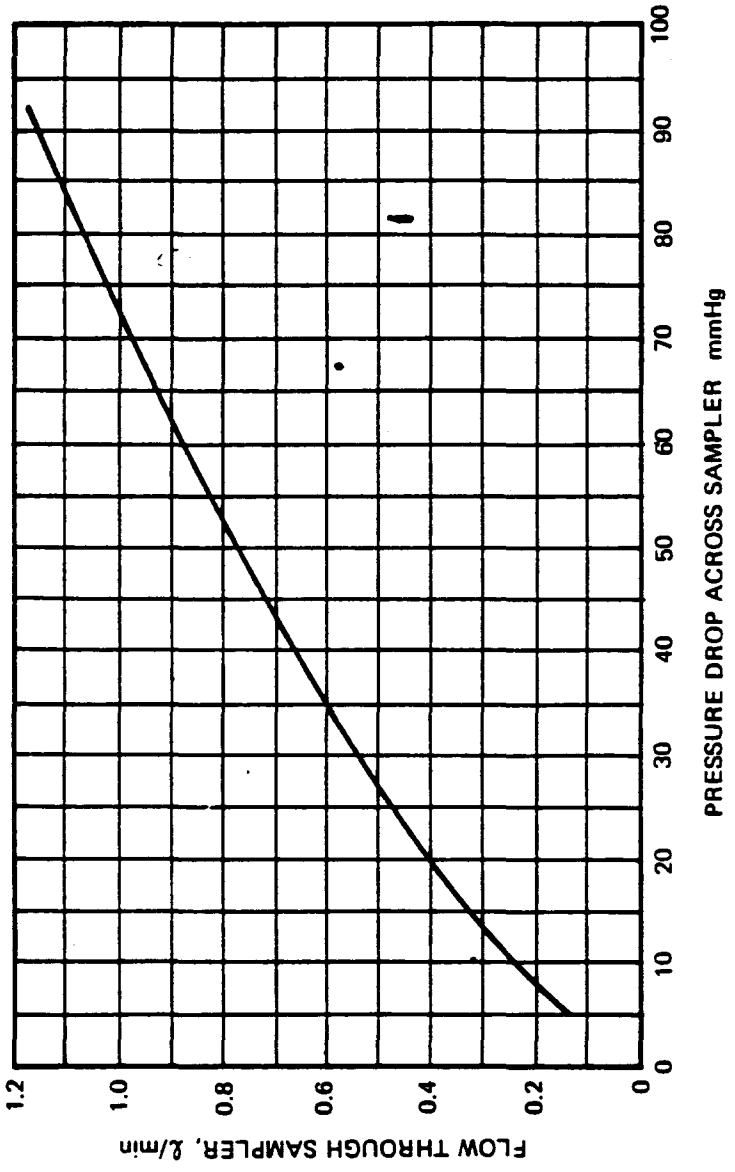


FIGURE 2. Relationship between flow rate and pressure drop for sampling device

with the samplers may be analyzed with the appropriate stages or rinsed with the desorbing solution. Sampler caps can be discarded.

8.4.2 Desorption of Samples. Add 0.5 ml of desorbing solution to each vial containing a sampler stage. Cap the vials and shake them with a test-tube shaker. Allow them to stand for one hour with intermittent shaking. Centrifuge for 10 min. Treat blanks in the same manner.

8.4.3 Liquid Chromatographic Conditions

Column:	μBondapak C_{18} (30 cm × 4.0 mm I.D.)
Mobile Phase:	Methyl alcohol/water, 3/2 (v/v)
Flow Rate:	1.5 ml/min, 1200 psi
Temperature:	~23°C
Detector:	UV (254 nm), 0.04 Absorbance units full scale
Injection Volume:	10-μl
Efficiency:	784 theoretical plates
Capacity Ratio:	~2.4

8.4.4 Injection. Flush the 10-μl syringe first with methyl alcohol (to insure its cleanliness and to wet the barrel and plunger) and then with the sample solution. Carefully draw 10 μl of sample solution into the syringe (to minimize the intake of particulate matter) and inject into the HPLC. No more than 3% difference in peak areas for replicate injections is to be expected.

8.4.5 Measurement of Peak Area. The area of the sample peak is either measured manually or with an electronic integrator. Preliminary results are read from a standard curve prepared as discussed in Section 9.

8.5 **Efficiency of Desorption**

8.5.1 Importance of Determination. The efficiencies of desorption for benzidine and its sulfate can vary from one laboratory to another and also from one batch of silica gel or filters to another. Thus, it is necessary to determine the percentage of benzidine and the sulfate that can be recovered from these matrices. The mean efficiency of desorption for benzidine from the specified glass filters and silica gel has been found to be ≧ 90% when desorbed within 7 days after sampling. The efficiency of desorption may also vary with the amount of benzidine present; therefore, measurements should be made for at least two amounts in the range of the sample size.

8.5.2 Procedure for Determining the Efficiency of Desorption. Place the filters and silica gel from unused samplers in 1-ml test tubes. Add to each a known amount of benzidine in 10-μl of methyl alcohol using the same microliter syringe for each. Five filter and silica gel samples are thus prepared at each of two different levels in the range of interest. Cap the tubes and allow them to stand overnight to assure complete adsorption of the amine on the matrix. Treat a parallel blank in the same manner (no analyte is added). Desorb and analyze "samples" and blanks in exactly the same manner as described in Section 8.4. Prepare two or three standards at these same levels by injecting 10 μl (same syringe as above) of the benzidine solutions into 0.5 ml of desorbing solution. Analyze these solutions with the samples.

$$\text{Efficiency of desorption (D)} = \frac{\text{Area}_{(\text{sample})} - \text{Area}_{(\text{blank})}}{\text{Area}_{(\text{standard})}}$$

9. **Calibration and Standards**

9.1 For accuracy in the preparation of standards, it is recommended that one standard be prepared in a relatively large volume and at high concentration. The initial standard is prepared

by weighing a selected amount of benzidine (e.g., 125 mg) into a 250-ml volumetric flask and adding the desorbing solution to the calibration mark. Dilutions encompassing the concentration range of interest down to 0.3 ng/μl are then made from the stock solution.

CAUTION: Benzidine has been identified as a carcinogen. Appropriate precautions must be taken in handling this compound to avoid personnel exposure and area contamination.

9.2 The standard solutions should be analyzed under the same liquid chromatographic conditions and during the same time period as the samples. This will minimize the effect of variations of the detector response or the mobile phase.

9.3 A standard curve for benzidine is prepared by plotting the average peak area for a standard against the concentration of the standard in μg/0.5 ml.

10. **Calculations**

10.1 In the event that the blank produces a peak with the same retention time as benzidine, the analyst should determine the source of the interference and eliminate or compensate for it.

10.2 Read the mass (M) in μg of benzidine present in the sample from the calibration curve.

10.3 The corrected mass (W) is determined by

$$W = \frac{M}{D}$$

where D is the efficiency of desorption for that sampler stage.

10.4 Add the masses of benzidine found in the two sampler stages to determine the total mass (W_t) collected.

10.5 The concentration of benzidine in air (C, $\mu g/m^3$) is given by

$$C(\mu g/m^3) = \frac{W_t\ (\mu g)}{V\ (\text{liter})} \times \frac{10^3\ \text{liter}}{m^3}$$

where V is the sampling volume in liters.

10.6 This procedure calculates air concentrations as the mass of benzidine per volume of air. If benzidinium sulfate is the compound sampled, a weight correction must be made. The conversion factor (1.52) is the ratio of the molecular weights. Thus, the weight of benzidinium sulfate is (W'_t) is

$$W'_t = 1.52\ W_t$$

11. **Reference**

11.1 Weeks, R. W., Jr., R. Morales, S. M. Rappaport, E. E. Campbell, and H. J. Ettinger, "Development of Sampling and Analytical Methods for Carcinogens: January-September 1976", Los Alamos Scientific Laboratory, Los Alamos, New Mexico, 1977.

POLYCHLORINATED BIPHENYLS (PCB) IN AIR

Measurements Research Branch

Analytical Method

Analyte:	Polychlorinated biphenyls (PCB)	**Method No.:**	P&CAM 244
Matrix:	Air	**Range:**	0.01 - 10 mg/m³
Procedure:	Adsorption on Florisil®, hexane desorption, gas chromatography with electron capture detection	**Precision:**	4.4% RSD (Analytical)
Date Issued:	10/1/76	**Classification:**	E (Proposed)
Date Revised:			

1. **Principle of the Method**

   1.1 Sampling. A measured volume of air is drawn through a small sorbent tube containing Florisil which adsorbs the PCB present in the air sample.

   1.2 Standard Analytical Procedure. The collected PCB are desorbed in hexane and the resulting solutions are analyzed using gas chromatography with electron capture detection. The concentration of PCB relative to a PCB standard (Aroclor) is read from a standard curve.

   1.3 The Webb-McCall Analytical Procedure. If the air sample is found to differ significantly in composition from the PCB standard, the method of Webb and McCall is used (11.2 and 11.4). Using gas chromatography with electron capture detection, the concentration of PCB is determined by comparison with a standard supplied by Webb. The weight percent of each peak in this standard is known and thus the weight for each corresponding peak in the sample can be calculated, regardless of overall composition.

2. **Range and Sensitivity**

   2.1 Standard Analytical Procedure. The estimated useful range of this method is 0.01 to 10 mg/m³. The minimum detectable quantity of PCB (Aroclor 1016) from a standard curve was found to be 32 picograms per injection. In laboratory experiments, no breakthrough was detected after sampling 48 liters of 10 mg/m³ of Aroclor 1016 at 75% relative humidity at a sampling rate of 200 cc/min through a 100-mg bed of Florisil. Field samples analyzed by this method ranged from 0.1 to 1.5 mg/m³.

   2.2 The Webb-McCall Analytical Procedure. This procedure has not been evaluated by NIOSH, but probably has the same detection limit and range as the standard analytical procedure.

3. **Interferences**

   3.1 Strict measures to avoid contamination are required when using the electron capture detector. Foremost the syringe must be thoroughly cleaned after each injection. Hexane used for desorption and back flush should be injected periodically for a purity check. It should show no peaks having retention times greater than 45 seconds, providing that not more than 5 μl have been injected.

   3.2 Any compound which has nearly the same retention time on the GC column as one of the PCB is an interference. This type of interference can often be overcome by changing the GC

operating conditions or by selecting another column. Retention time data on a single column, or even on a number of columns, cannot be considered as conclusive proof of chemical identity. It is important, therefore, that a sample of the bulk mixture of PCB be submitted at the same time as the sample tubes so that chemical identification of possible interferences can be made.

3.3 Chlorinated pesticides, such as DDT, DDE, etc., have been reported as interferences which arise due to coextraction with PCB during workup of environment samples such as water, tissue, soil, or biological fluids. In the case of personal air sampling in an industrial environment, these interferences would not be present unless these were being manufactured in the same area. Thus, unless these chlorinated pesticides are known to specifically be present, there need be no extra cleanup or separation step for these materials.

3.4 Sulfur-containing compounds in petroleum products have been reported as interferences (11.5).

4. **Precision and Accuracy**

4.1 The volume of air sample can be measured to within ± 1% if a pump with a calibrated volume indicator is used. Volumes calculated from initially set flow rates may be less accurate (± 5%) because of changes in flow rates during sampling.

4.2 The front section of the Florisil tube has a 100% collection efficiency for 50-l air samples at airborne concentrations up to 10 mg/m^3.

4.3 No loss of PCB was observed in sealed tubes or desorbed samples stored for two months.

4.4 The precision of the analysis is dependent upon the precision and sensitivity of the technique used to quantitate the gas chromatograph peaks of samples and standards. Known quantities of PCB which were spiked on Florisil and allowed to sit overnight were quantitatively (100%) recovered. The precision of the standard analytical procedure is indicated by a relative standard deviation of 4.4% for 27 spiked samples.

4.5 The accuracy of these procedures is not known. Accuracy depends upon the ability to separate and identify each compound in the PCB mixture and compare each with a known standard. Many of the isomers in the commercial PCB mixtures have not been separated or identified, nor are standards of these compounds available. Therefore, the task of assessing accuracy is very difficult. Despite this drawback, the Webb and McCall procedure has produced good results (11.3).

5. **Advantages and Disadvantages of the Method**

5.1 The sampling device is small, portable, and involves no liquids. Interferences are minimal, and most of those which do occur can be eliminated by altering chromatographic conditions. The tubes are analyzed by means of a quick instrumental method.

5.2 The capacity of the solid sorbent sampling device for PCB is large and recovery from the sorbent is quantitative.

5.3 A substantial difference in air sample composition and the commercial PCB mixture being used will result in a large error when the standard analytical procedure is used, since the standardization is based on comparison with the PCB mixture being used. The Webb-McCall Analytical Procedure should provide a more accurate quantitation since standardization is based on a factor. Thus, a weight can be assigned to a percent weight/peak specific peak in the chromatogram of the sample.

6. **Apparatus**

6.1 A personal-sampling pump which operates in the range of 50-200 cc/min is used. These pumps should be calibrated with a representative sorbent tube in the sampling line. A wet or dry test meter or a glass rotameter capable of measuring a flow rate of 200 cc/min within ± 5% may be used in the calibration.

6.2 Sorbent Tubes. Each glass tube is at least 7 cm long with 4 mm inside diameter and contains two sections of 30-48 mesh deactivated Florisil. The front section is preceded by glass wool and contains 100 mg and the backup section contains 50 mg. Urethane foam is placed between the sections and after the backup section. The ends of the tube are flame sealed to prevent contamination before use.

6.3 Gas chromatograph equipped with an electron capture detector (^{63}Ni foil).

6.4 Glass column (6 ft × 4 mm I.D.) packed with 3% SE-30 or 3% OV-1 on 80/100 mesh Supelcoport or Gas Chrom Q.

6.5 Vials, 20-ml, with aluminum-lined caps.

6.6 Microliter syringe, 10-μl.

6.7 Volumetric flasks, 10-ml, with glass stoppers.

6.8 A mechanical or electronic integrator or a recorder and some method for determining peak area.

7. **Reagents**

ACS reagent grade or better.

7.1 Hexane, Pesticide Quality.

7.2 Florisil® (Registered Trademark of the Floridin Co.), 30-48 mesh chromatographic adsorbent, deactivated. Florisil 30-60 mesh is sieved to the proper mesh size. Prior to packing tubes, dry a weighed amount of Florisil at 105°C for 45 minutes. After cooling to room temperature, the Florisil is added to a round bottom flask which can be attached to a rotary evaporator. Three milliliters of water per 100 gm of Florisil (i.e., 3% w/w water) are added and the mixture is turned in the rotary evaporator for one hour or until it is free flowing.

7.3 Nitrogen, prepurified.

7.4 Standard PCB mixtures. A sample of the specific PCB mixture under study or being used in the environment which was sampled is used as the standard. Webb-McCall Aroclor PCB standards in isoctane may be obtained from Dr. Ronald G. Webb, Southeast Environmental Research Laboratory, National Environmental Research Center-Corvallis, Environmental Protection Agency, Athens, Georgia 30601.

7.5 p,p'-DDE [1,1-Dichloro-2,2-bis(p-chlorophenyl)ethene].

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware used for the laboratory analysis should be detergent washed and thoroughly rinsed with tap water, distilled water, pesticide grade acetone, and finally pesticide grade hexane and dried.

8.2 **Collection and Shipping of Samples**

8.2.1 Immediately before sampling, break each end of the sorbent tube so as to provide an opening at least one-half the internal diameter of the tube (2 mm).

8.2.2 The smaller section of Florisil is used as a backup and is positioned nearest the sampling pump.

8.2.3 The sorbent tube is vertical during sampling.

8.2.4 Air being sampled is not passed through any hose or tubing before entering the sorbent tube.

8.2.5 The air sample is taken at a flow rate of 200 cc/min or less to attain the total sample volume required. The recommended sample volume is 50 liters.

8.2.6 The sorbent tube is capped with the supplied plastic caps immediately after sampling. Under no circumstances are rubber caps to be used.

8.2.7 One tube is handled in the same manner as the sample tubes (break, seal, and ship) except that no air is pumped through it. Label this tube as a blank.

8.2.8 The tubes are packed tightly before shipping to minimize breakage in transit. The tubes are not subjected to extremes of high temperatures or low pressure during shipment.

8.2.9 Samples of the PCB whose presence in the environment is suspected are submitted to the laboratory performing the analyses. These bulk materials are not transported in the same container as the samples or blank tubes. If possible, a bulk air sample is shipped for qualitative identification purposes.

8.3 **Analysis of Samples**

8.3.1 Preparation of Samples. Each tube is scored with a file and the glass wool and front section are placed in a clean, dry vial. The separating urethane foam plug, the back section of the sorbent, and the retaining urethane foam plug are placed in a second clean, dry vial.

8.3.2 Desorption of Samples. Prior to analysis, 5.0 ml of hexane is pipetted into each vial. Florisil particles should not be allowed to cling to the glass above the solvent. A minimum of 10 minutes desorption time is required before analysis.

8.3.3 Preliminary Analysis. An injection of 1 μl hexane backflush and 1 μl of sample is made at the conditions specified under 8.3.4. If the sample is too concentrated, further dilutions will be necessary to bring the concentration of the sample solution into the linear range of the electron capture detector. Once the concentration of the sample is appropriately adjusted, the chromatogram of this sample should be compared to that of a standard to determine if the air sample is qualitatively different in composition from the standard. If there is little difference in the two chromatograms, then proceed via the Standard Analytical Procedure (8.3.5). If there is a significant difference in the two chromatograms, the Webb and McCall Analytical Procedure is followed (8.3.6).

8.3.4 Gas Chromatographic conditions for PCB determination by the Standard Analytical Procedure are:

1. Nitrogen carrier gas flow, 90 ml/min
2. Injector temperature, 300°C
3. Interface and detector temperatures both 325°C
4. Column temperature, 200°C

8.3.5 Standard Analytical Procedure. An aliquot of the sample solution is injected under the gas-chromatographic conditions in 8.3.4. The solvent flush technique is used for injection. The volume of back flush is *1 μl and the volume of standard or sample solution is equal to 4 μl. Larger volumes may give a solvent peak which interferes with sample components. The syringe must be cleaned with hexane after each injection. The peak heights or areas of at least five prominent peaks in the sample chromatogram are selected and compared with those of the standard PCB mixture and the weight of PCB present is calculated.

8.3.6 The Webb-McCall Analytical Procedure. An aliquot of the sample solution is injected under the gas-chromatographic conditions in 8.3.4. The solvent flush technique is used for injection. The volume of backflush is 1 μl and the volume of the standard or sample solution is equal to 4 μl. Larger volumes may give a solvent peak which interferes with sample components. The syringe must be cleaned with hexane after each injection. An aliquot of p,p'-DDE (0.02 ng/μl) is also injected under the same conditions and the relative retention time (RRT) of each peak is determined using p,p'-

DDE as the reference standard equal to 100. Retention time (RT) is the distance between the first appearance of the solvent peak and the maximum of the peak of interest. Thus,

$$RRT = \frac{RT \times 100}{RT\,(DDE)}$$

where RT = retention time of peak of interest

RT (DDE) = retention time of DDE.

The area of each peak in the sample is compared with the appropriate standard curve of the peak having the same RRT and the concentration is read from the curve. If the sample contains more than one Aroclor, use Figure 1 to identify the Aroclor standard to be used.

8.4 **Determination of Desorption Efficiency.** In various experiments, 5.46 μg and 3.42 μg (3 sets) of PCB (Aroclor 1016) in hexane were spiked on 100 mg sections of Florisil and allowed to sit overnight. Desorption with 5 ml of hexane the next day gave recoveries of 95.73%, 100.78%, 98.39%, and 102.12% (overall average, 99.3%). Thus, results of sample analysis need not be corrected by desorption efficiency.

9. **Calibration and Standards**

9.1 **Standard Analytical Procedure.** The particular PCB mixture being used in the work area in which air samples were taken is used as the standard. Standards are prepared in hexane at concentrations ranging from 8 to 500 ng/ml (within the linear range of the electron capture detector — upper limit is approximately 3 ng per injection). Calibration curves should be established daily since the electron capture detector response may vary from day to day. The standard curve is plotted in terms of concentration (ng/ml) versus area. Since the injection volumes of the standard and the sample are identical, the concentration of the sample can be read directly from the standard curve.

9.2 **The Webb-McCall Analytical Procedure.** The standard Aroclor mixtures supplied by Webb (see 7.4) are prepared at concentrations ranging from 0.008 to 0.5 ng/μl. Calibration curves are established daily since the electron capture detector responses may vary from day to day. Standard curves for each peak to be used in the determination are constructed in terms of concentration (ng/ml) versus area. The concentration of each peak may be determined with the use of Tables 1-6 (taken from Ref. 11.2) by multiplying the total concentration of the Aroclor standard by the appropriate mean-weight percent factor and dividing by 100.

10. **Calculations**

10.1 **Standard Analytical Procedure.** The following are the steps in the calculation of air concentrations of PCB determined with PCB mixture as the standard.

10.1.1 The height or area of several selected prominent peaks (at least 5) in the chromatogram are added, compared to the total height or area of those same peaks in the standard, and the concentration of the sample solution is read from the standard curve.

10.1.2 The concentration, in ng/ml, of the sample solution is multiplied by the total volume, in ml, of the sample solution and the weight of the PCB in the sample is calculated. Corrections for the blanks are made if necessary.

10.1.3 The weights found on the front and the back sections of the tube are summed to find the total weight of the PCB in the air sample.

10.1.4 The total weight of the PCB in the air sample is divided by the volume, in liters, of air sampled and the air concentration is reported in ng/l or the equivalent units, $\mu g/m^3$.

10.2 **The Webb-McCall Analytical Procedure.** The following are the steps in the calculation of air concentration of PCB determined using the method of Webb and McCall.

10.2.1 The height or area of each peak in the chromatogram is compared to the corresponding standard curve for that peak from the Webb-McCall standard and the concentration of that particular PCB (represented by that peak) is read from the standard curve.

10.2.2 The concentrations of each PCB are summed. This total concentration of the PCB is then multiplied by the total volume, in ml, of the sample solution and the weight of the PCB in the sample is calculated. Corrections for blanks should be made if necessary.

10.2.3 The weights found on the front and the back section of tube are summed to find the total weight of the PCB in the air sample.

10.2.4 The total weight of the PCB in the air sample is divided by the volume, in liters, of air sampled and the air concentration is reported in ng/l or the equivalent units, $\mu g/m^3$.

11. **References**

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TABLE 1

Composition of Aroclor 1221*

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]	
11	31.8	15.8	1	
14	19.3	9.1	1	
16	10.1	9.7	2	
19	2.8	9.7	2	
21	20.8	9.3	2	
28	5.4	13.9	2 3	85% 15%
32	1.4	30.1	2 3	10% 90%
37 40	1.7	48.8	3 3	
Total	93.3			

[a]Retention time relative to p,p'-DDE = 100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b]Standard deviation of 17 results as a percentage of the mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

*Taken from Reference 11.2

781

TABLE 2

Composition of Aroclor 1232*

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]	
11	16.2	3.4	1	
14	9.9	2.5	1	
16	7.1	6.8	2	
20	17.8	2.4	2	
21			2	
28	9.6	3.4	2	40%
			3	60%
32	3.9	4.7	3	
37	6.8	2.5	3	
40	6.4	2.7	3	
47	4.2	4.1	4	
54	3.4	3.4	3	33%
			4	67%
58	2.6	3.7	4	
70	4.6	3.1	4	90%
			5	10%
78	1.7	7.5	4	
Total	94.2			

[a]Retention time relative to p,p'-DDE = 100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b]Standard deviation of four results as a mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

*Taken from Reference 11.2

749

TABLE 3

Composition of Aroclor 1242*

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
11	1.1	35.7	1
16	2.9	4.2	2
21	11.3	3.0	2
28	11.0	5.0	2 } 25% 3 } 75%
32	6.1	4.7	3
37	11.5	5.7	3
40	11.1	6.2	3
47	8.8	4.3	4
54	6.8	2.9	3 } 33% 4 } 67%
58	5.6	3.3	4
70	10.3	2.8	4 } 90% 5 } 10%
78	3.6	4.2	4
84	2.7	9.7	5
98	1.5	9.4	5
104	2.3	16.4	5
125	1.6	20.4	5 } 85% 6 } 15%
146	1.0	19.9	5 } 75% 6 } 25%
Total	98.5		

[a]Retention time relative to p,p'-DDE = 100. Measured from first appearance of solvent.

[b]Standard deviation of six results as a percentage of the mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

*Taken from Reference 11.2

TABLE 4

Composition of Aroclor 1248*

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
21	1.2	23.9	2
28	5.2	3.3	3
32	3.2	3.8	3
47	8.3	3.6	3
40	8.3	3.9	3 } 85% 4 } 15%
47	15.6	1.1	4
54	9.7	6.0	3 } 10% 4 } 90%
58	9.3	5.8	4
70	19.0	1.4	4 } 80% 5 } 20%
78	6.6	2.7	4
84	4.9	2.6	5
98	3.2	3.2	5
104	3.3	3.6	4 } 10% 5 } 90%
112	1.2	6.6	5
125	2.6	5.9	5 } 90% 6 } 10%
146	1.5	10.0	5 } 85% 6 } 15%
Total	103.1		

[a]Retention time relative to p,p'-DDE = 100. Measured from first appearance of solvent.

[b]Standard deviation of six results as a percentage of the mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

*Taken from Reference 11.2

789

TABLE 5

Composition of Aroclor 1254*

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]	
47	6.2	3.7	4	
54	2.9	2.6	4	
58	1.4	2.8	4	
70	13.2	2.7	4	25%
			5	75%
84	17.3	1.9	5	
98	7.5	5.3	5	
104	13.6	3.8	5	
125	15.0	2.4	5	70%
			6	30%
146	10.4	2.7	5	30%
			6	70%
160	1.3	8.4	6	
174	8.4	5.5	6	
203	1.8	18.6	6	
232	1.0	26.1	7	
Total	100.0			

[a]Retention time relative to p,p'-DDE = 100. Measured from first appearance of solvent.

[b]Standard deviation of six results as a percentage of the mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

*Taken from Reference 11.2

TABLE 6
Composition of Aroclor 1260*

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
70	2.7	6.3	5
84	4.7	1.6	5
98, 104	3.8	3.5	[d] 5: 60%, 6: 40%
117	3.3	6.7	6
125	12.3	3.3	5: 15%, 6: 85%
146	14.1	3.6	6
160	4.9	2.2	6: 50%, 7: 50%
174	12.4	2.7	6
203	9.3	4.0	6: 10%, 7: 90%
232, 244	9.8	3.4	[e] 6: 10%, 7: 90%
280	11.0	2.4	7
332	4.2	5.0	7
372	4.0	8.6	8
448	0.6	25.3	8
528	1.5	10.2	8
Total	98.6		

[a]Retention time relative to p,p'-DDE = 100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b]Standard deviation of six results as a percentage of the mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

[d]Composition determined at the center of peak 104.

[e]Composition determined at the center of peak 232.

*Taken from Reference 11.2

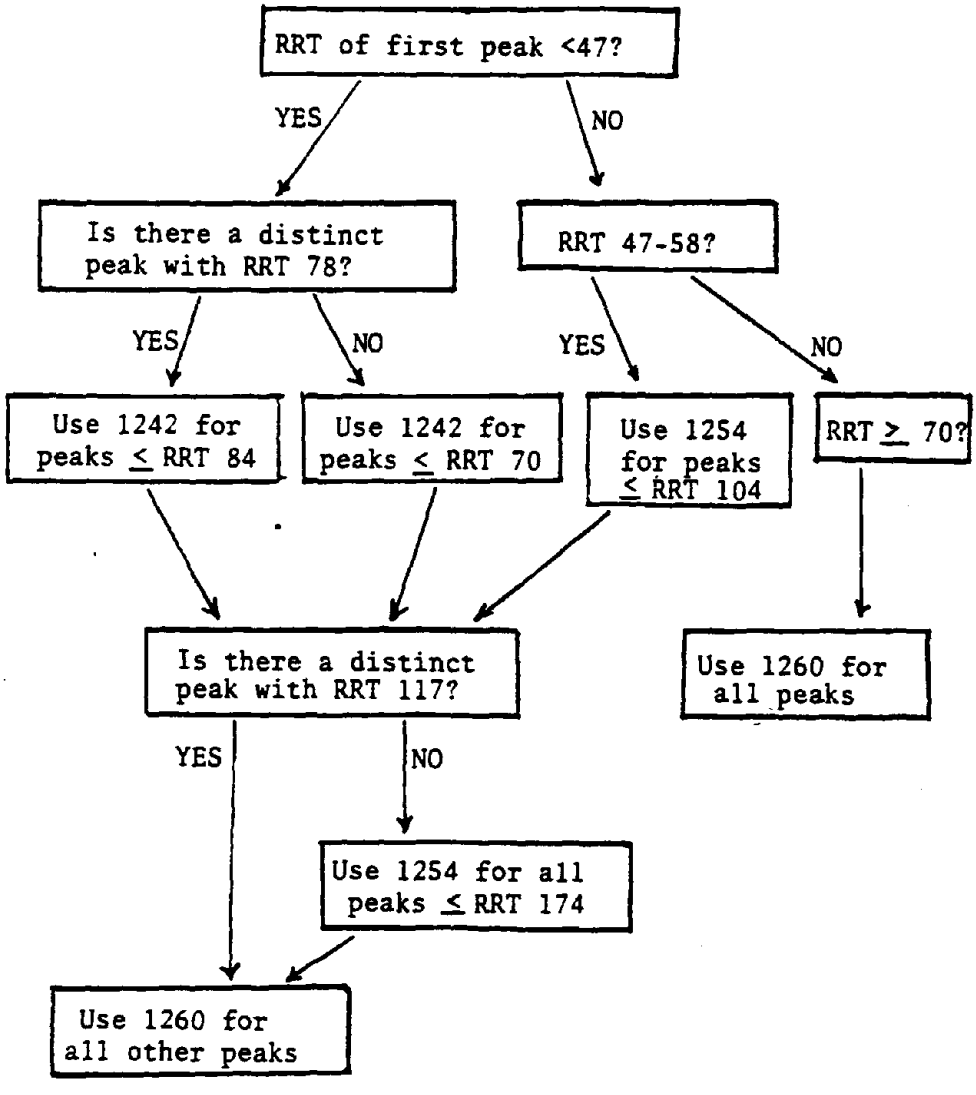


FIGURE 1. Chromatogram Division Flowchart (from Reference 11.2)

CHRYSOTILE ASBESTOS IN BULK SAMPLES

Measurements Research Branch

Analytical Method

Analyte:	Chrysotile Asbestos	**Method No.:**	P&CAM 245
Matrix:	Bulk sample	**Range:**	0.1-10 mg/ sample
Procedure:	Thermal Analysis (DTA)	**Precision(CV):**	0.12 at 3.8 mg (analytical)
Date Issued:	1/1/77	**Classification:**	E (Proposed)
Date Revised:			

1. **Principle of the Method**

   The bulk sample of asbestos is heated to 850°C in a DTA apparatus. The amount of chrysotile present is proportional to the height of the exotherm occurring at approximately 830°C, which represents recrystallization of chrysotile to forsterite. An alternative measurement can be made on the dehydroxylation endotherm at about 630°C.

2. **Range and Sensitivity**

   2.1 The range of this method is from 0.1 to 10 mg chrysotile/sample. Larger quantities may be determined but care should be taken that standard curves be obtained using the same crucible geometry, filling technique, chemical matrix, and instrumental parameters as with the samples.

   2.2 The approximate detection limit is 0.1 mg chrysotile asbestos. This corresponds to approximately 0.2μV potential difference between sample and reference when using Pt vs. 90% Pt-10% Rh thermocouples. Increased sensitivity may be obtained by using more sensitive thermocouples such as W vs. 74% W-26% Re or by increasing the heating rate. A maximum heating rate of 15°C/min is recommended, however.

3. **Interferences**

   3.1 Lizardite, a non-asbestiform serpentine, gives a similar DTA curve (11.1).

   3.2 If large amounts of dolomite ($CaMg(CO_3)_2$) or calcite ($CaCO_3$) are present, sharp endotherms at 790-810°C will occur and interfere with the chrysotile exotherm at 830°C.

4. **Precision and Accuracy**

   4.1 Precision. A calibration curve of 10 standards covering the range 1-10 mg chrysotile had a linear correlation coefficient of 0.982. The intercept and slope (and associated standard deviations) were, respectively, -0.22 μV (± 0.22 μV) and 0.44 μV/mg (± 0.03 μV/mg). For 3.8-mg samples, the CV was 0.12.

   4.2 Accuracy has not been determined.

5. **Advantages and Disadvantages of the Method**

   5.1 The method is relatively free of common interferences.

   5.2 Expensive thermal analysis equipment is required and a moderate degree of skill is needed.

   5.3 The method is not sensitive enough for personal samples and is therefore restricted to bulk samples.

6. **Apparatus**

6.1 Thermal analyzer, with 90 Pt/10Rh-Pt thermocouples, platinum crucibles and furnace capable of heating at 5-15°C per minute to a temperature of 850°C. The instrument must have a sensitive DTA readout, with detection limit of 1 microvolt or better, and an accurate temperature measurement readout. Simultaneous measurement of mass is desirable, but not essential. A means of atmosphere control should be available.

6.2 Analytical balance.

7. **Reagents**

7.1 Standard chrysotile asbestos.

7.2 Aluminum oxide powder, reagent grade.

8. **Procedure**

8.1 **Cleaning of Equipment**

The platinum crucibles should be cleaned, if necessary, by heating in a beaker with potassium bisulfate ($KHSO_4$) until the $KHSO_4$ melts. The beaker is removed from the heat and the $KHSO_4$ allowed to solidify around the crucibles. The $KHSO_4$ is then dissolved in distilled water and the cleaned crucibles removed and dried.

8.2 **Collection of Samples**

8.2.1 For settled dust or bulk samples, select a representative sample and screen out gross particles. Grinding or sieving is not necessary, but an approximate match in particle size between standards and samples is required.

8.2.2 If gross amounts of water-soluble matter are present, the sample should be stirred with distilled water in a beaker, and then filtered through a 0.8 μm membrane filter. The solid material recovered on the filter should be oven-dried for 1 hour at 120°C and then weighed out for analysis.

8.3 **Analysis of Samples**

8.3.1 Weigh a 1-50 mg sample into a clean crucible and press the sample down several times with a glass stirring rod so that it makes good thermal contact with the bottom of the crucible. Cover the sample with a layer (approximately 3 mm thick) of dry, powdered aluminum oxide. Do not use a vibrator or other means to cause the aluminum oxide to settle around the sample, because vibration will cause the sample to rise to the top of the aluminum oxide powder thereby losing thermal contact with the base of the crucible. Transfer the sample carefully to the thermal analyzer, placing it on the sample thermocouple.

8.3.2 Using a duplicate crucible, weigh out powdered aluminum oxide in an amount approximately equal to that of the contents of the sample crucible. The actual amount used should be adjusted by preliminary runs to produce as flat a baseline as possible. Place the reference crucible on the reference thermocouple.

8.3.3 Check the alignment of the crucibles in the furnace (when the balance pan is released, if applicable). If necessary, adjust the thermocouple stick so that it is centered on the axis of the furnace.

8.3.4 If applicable, adjust the tare weights and position the weight trace to a convenient position on the chart recorder.

8.3.5 If applicable, select the atmosphere desired and adjust gas flows. The recommended atmosphere is dry air flowing at 5.72 l/hr.

8.3.6 Select heating rate and upper temperature limit. Sensitivity is enhanced by higher heating rates, but a maximum rate of 15°C/min is recommended. In order to decrease the time for analysis, a rate of 25°C/min can be used until the furnace reaches ap-

proximately 700°C. At that time, the rate should be reduced. The upper temperature limit should be set to approximately 875°C.

8.3.7 Measure the peak height of the exotherm produced by recrystallization (to forsterite) of chrysotile at approximately 800-830°C. The peak temperature is dependent upon heating rate and thermocouple configuration; peak temperature increases with increasing heating rate.

9. **Calibration and Standards**

9.1 Prepare a calibration curve of at least four points from standard chrysotile, using the same type crucibles and instrumental settings as for the samples. Plot a calibration curve of DTA peak height vs. μg of chrysotile.

9.2 If the sample contains a major non-chrysotile component, it may be necessary to use the method of standard additions (11.2 and 11.3). In this case, at least two additional samples, spiked with approximately 1X and 2X the amount of chrysotile contained in them, are analyzed.

10. **Calculations**

10.1 From the calibration curve of DTA peak height in μV vs. chrysotile in μg, read the quantity of chrysotile present in the sample.

10.2 If the method of standard additions is used, it is necessary to plot μV vs. amount of chrysotile added to the sample. The X-axis intercept is then the amount of chrysotile present in the unspiked sample.

11. **References**

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796

3,3'-DICHLOROBENZIDINE IN AIR

Measurements Research Branch

Analytical Method

Analyte:	3,3'-dichlorobenzidine and 3,3'-dichlorobenzidine dihydrochloride	**Method No.:**	P&CAM 246
Matrix:	Air	**Range:**	3-140 $\mu g/m^3$ for a 50-liter air sample
Procedure:	Collection on glass fiber filter and silica gel, elution with triethylamine in methyl alcohol, analysis by HPLC.	**Precision:**	7% relative standard deviation
Date Issued:	4/13/77	**Classification:**	E (Proposed)
Date Revised:			

1. **Principle of the Method**
   - 1.1 A known volume of air is drawn through a two-stage sampler consisting of a high-efficiency glass fiber filter followed by a bed of silica gel to collect 3,3'-dichlorobenzidine aerosol and/or vapor.
   - 1.2 The filter and sorbent sections of the sampler are transferred to stoppered tubes and the 3,3'-dichlorobenzidine is desorbed with a solution of triethylamine in methyl alcohol. This desorbing solution converts 3,3'-dichlorobenzidine dihydrochloride to free dichlorobenzidine.
   - 1.3 An aliquot of this solution is injected into a high performance liquid chromatograph (HPLC).
   - 1.4 Peak areas are determined and compared with a calibration curve obtained from injections of standard solutions of 3,3'-dichlorobenzidine.
2. **Range and Sensitivity**
   - 2.1 The range of the method is 0.2-7.0 μg/sample (3-140 $\mu g/m^3$ for a 50-liter air sample) using 0.5 ml of desorbing solution and a 15-μl injection into the liquid chromatograph. The upper limit can be extended by increasing the volume of desorbing solvent, decreasing the sensitivity of the detector, or decreasing the aliquot injected into the liquid chromatograph.
   - 2.2 The limit of detection is 0.05 μg/sample.
3. **Interferences**
   - 3.1 The retention time for 4,4'-methylenebis(2-chloroaniline) is close to that of 3,3'dichlorobenzidine and will interfere with the analysis.
   - 3.2 In solutions containing dichlorobenzidine or its dihydrochloride at concentrations of 24 ng/μl, the following compounds were found not to interfere when their concentrations were $\leq$ 60 ng/μl: o, p, and m-chloroaniline, 4,4'-methylenedianiline, β-naphthylamine, benzidine, aniline, N-methylaniline, 2-aminotoluene (o-toluidine), and 3,3'-dimethylbenzidine (o-tolidine).
   - 3.2 Any compound sampled with 3,3'-dichlorobenzidine and having the same retention time as 3,3'-dichlorobenzidine will interfere with the analysis. This type of interference often can be eliminated by changing the operating conditions of the chromatograph, e.g., by changing the composition of the mobile phase or by solvent programming.

4. **Precision and Accuracy**

4.1 The pooled estimate of the relative standard deviation was 7%, based upon 28 samples collected from test atmospheres containing 3,3'-dichlorobenzidine at concentrations of 20 $\mu g/m^3$ (ten 51-liter samples), 66 $\mu g/m^3$ (eight 51-liter samples) and 130 $\mu g/m^3$ (ten 51-liter samples). The sampling rate was 0.8 liter/min, and the environment was maintained at 30°C and 80% relative humidity. (3,3'-dichlorobenzidine was generated as a polydisperse aerosol with a MMAD of $\simeq$2.4 μm and a σ_g of $\simeq$1.5.)

4.2 The accuracy of the method has not been determined.

5. **Advantages and Disadvantages of the Method**

5.1 The sampler is a small portable device containing no liquids which can be used to sample the air in the breathing zone of a worker. Environmental conditions of temperature and humidity do not significantly affect the method. Desorption of the collected sample is simple and the analysis is accomplished by a rapid instrumental technique. Possible interferences can be obviated by changing the composition of the mobile phase or by solvent programming.

5.2 At a flow rate of 0.2 liter/min the linear velocity through the 4-mm inlet of the filter holder is 26.5 cm/s. It is not known if this throat velocity is sufficient for the capture of all particles of analytical importance.

5.3 This method has not been field-tested.

6. **Apparatus**

6.1 Calibrated personal air sampling pump that can be maintained for 8 hr at a flow rate of 0.2 liter/min with the sampler in line.

6.2 Sampler, equivalent to that shown in Fig. 1. The sampler consists of two sections, a high-efficiency glass fiber filter followed by a 50-mg bed of silica gel. The first section, a 13-mm type A-E glass fiber filter (Gelman Instrument Co., Ann Arbor, MI) is contained in a 13-mm filter holder (SXOO-013-00, Millipore Corp., Bedford, MA). The sorbent tube is a 30-mm section of Pyrex glass, 6.4-mm O.D. × 4-mm I.D., flared on one end and containing 50 mg of GC grade silica gel (D-08, 30/60 mesh, 720-760 m^2/g, 4.3 g/cc; Applied Science Laboratories, Inc., State College, PA). The sorbent is held by 3.5-mm diameter 100-mesh, stainless steel screens and 4-mm O.D. Teflon rings. To connect the two stages, the filter holder is pressed into the flared end of the sorbent tube. The pressure drop across samplers at various flow rates should be within 5% (relative standard deviation) of values extrapolated from Fig. 2. Plastic caps of 6-mm I.D. seal the ends of the sampler.

6.3 High performance liquid chromatograph equipped with an ultraviolet detector (254 nm) and injection valve.

6.4 μBondapak C_{18} column (10-μm), 4.0-mm I.D. × 30 cm. (Waters Associates, Milford, MA), or equivalent.

6.5 Potentiometer strip chart recorder.

6.6 Test tubes (1-ml) fitted with polyethylene stoppers.

6.7 Glass syringes, 25-μl.

6.8 Pipettes of convenient sizes for the preparation of standard solutions.

6.9 Volumetric flasks of convenient sizes for the preparation of standard solutions.

6.10 Clinical centrifuge.

6.11 Analytical balance.

6.12 Test-tube shaker (vortex type).

7. **Reagents**

All reagents must be of analytical reagent quality or better.

7.1 Methyl alcohol (UV grade), distilled in glass.

7.2 Acetonitrile (UV grade), distilled in glass.

7.3 Mobile Phase: 7/3 (v/v) acetonitrile in distilled water.

7.4 Triethylamine.

7.5 Desorbing Solution: 0.17% (v/v) triethylamine in methyl alcohol.

7.6 3,3′-Dichlorobenzidine, analytical standard quality.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware used for the laboratory analysis is washed with detergent, rinsed with tap water, distilled water, and methyl alcohol, and dried in an oven.

8.2 **Calibration of Personal Sampling Pumps.** Each pump should be calibrated with a representative sampler in line to minimize errors in volume measurement. A bubble flow meter or other suitable flow measuring device may be used.

8.3 **Collection and Shipping of Samples**

8.3.1 Immediately before sampling, the plastic caps are removed from the ends of the sampler and saved for resealing after sampling.

8.3.2 The sorbent end of the sampler is connected to the pump with plastic or rubber tubing. The sampler is positioned vertically during sampling. Sampled air must not pass through any tubing before entering the sampler.

8.3.3 The atmosphere is sampled at a flow rate of 0.2 liter/min for the desired period of time. The flow rate and sampling time, or the volume of sampled air, must be measured as accurately as possible.

8.3.4 The temperature, pressure, and humidity of the atmosphere being sampled is measured and recorded.

8.3.5 The sampler is resealed with the plastic caps immediately after sampling.

8.3.6 Blank samples are obtained by handling random collection devices in the same manner as in sampling, except that no air is drawn through them.

8.3.7 Samples shipped to the laboratory should be packed tightly to minimize breakage.

8.3.8 If samples of bulk material, associated with the process under investigation, are to be shipped to the laboratory, they should not be placed in the same container as the air samplers or blanks.

8.4 **Analysis of Samples**

8.4.1 Preparation of Samples. Place the glass fiber filters and silica gel in separate 1-ml test tubes or other suitable vials. Teflon rings and stainless steel screens associated with the samplers may be analyzed with the appropriate stages or rinsed with the desorbing solution. Sampler caps can be discarded.

8.4.2 Desorption of Samples. Add 0.5 ml of desorbing solution to each vial containing a sampler stage. Cap the vials and shake them with a test-tube shaker. Allow them to stand for one hour with intermittent shaking. Centrifuge for 10 min. Treat blanks in the same manner.

8.4.3 Liquid Chromatographic Conditions

Column:	μBondapak C_{18} (30 cm × 4.0-mm I.D.)
Mobile Phase:	Acetonitrile/water, 7/3 (v/v)
Flow Rate:	1.5 ml/min, 1200 psi
Temperature:	~ 23°C
Detector:	UV (254 nm), 0.04 Absorbance units full scale
Injection Volume:	15 μl
Efficiency:	1876 theoretical plates
Capacity Ratio:	1.3

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8.4.4 Injection. Flush the 25-μl syringe first with methyl alcohol (to insure its cleanliness and to wet the barrel and plunger) and then with the sample solution. Carefully draw 15 μl of sample solution into the syringe (to minimize the intake of particulate matter) and inject into the HPLC. No more than 3% difference in peak areas for replicate injections is to be expected.

8.4.5 Measurement of Peak Areas. The area of the sample peak is either measured manually or with an electronic integrator. Preliminary results are read from a standard curve prepared as discussed in Section 9.

8.5 **Efficiency of Desorption**

8.5.1 Importance of Determination. The efficiencies of desorption for 3,3′-dichlorobenzidine and its dihydrochloride can vary from one laboratory to another and also from one batch of silica gel or filters to another. Thus, it is necessary to determine the percentage of 3,3′-dichlorobenzidine that can be recovered from these matrices. The mean efficiency of desorption for 3,3′-dichlorobenzidine from the specified glass filters and silica gel has been found to be ≥90% when desorbed within 30 days after sampling. The efficiency of desorption may also vary with the amount of 3,3′-dichlorobenzidine present; therefore, measurements should be made for at least two amounts in the range of the sample size.

8.5.2 Procedure for Determining the Efficiency of Desorption. Place the filters and silica gel from unused samplers in 1-ml test tubes. Add to each a known amount of 3,3′-dichlorobenzidine in 10 μl of methyl alcohol, using the same microliter syringe for each. Five filter and silica gel samples are thus prepared at each of two different levels in the range of interest. Cap the tubes and allow them to stand overnight to assure complete adsorption of the amine on the matrix. Treat a parallel blank in the same manner (no analyte is added). Desorb and analyze "samples" and blanks in exactly the same manner as described in Section 8.4. Prepare two or three standards at these same levels by injecting 10 μl (same syringe as above) of the 3,3′-dichlorobenzidine solutions into 0.5 ml of desorbing solution. Analyze these solutions with the samples.

$$\text{Efficiency of desorption (D)} = \frac{\text{Area}_{(\text{sample})} - \text{Area}_{(\text{blank})}}{\text{Area}_{(\text{standard})}}$$

9. **Calibration and Standards**

9.1 For accuracy in the preparation of standards, it is recommended that one standard be prepared in a relatively large volume and at high concentration. The initial standard is prepared by weighing a selected amount of 3,3′-dichlorobenzidine (e.g., 125 mg) into a 250-ml volumetric flask and adding the desorbing solution to the calibration mark. Dilutions encompassing the concentration range of interest down to 0.4 ng/μl are then made from the stock solution.

CAUTION: 3,3′-dichlorobenzidine has been identified as a carcinogen. Appropriate precautions must be taken in handling this compound to avoid personnel exposure and area contamination.

9.2 The standard solutions should be analyzed under the same liquid chromatographic conditions and during the same time period as the samples. This will minimize the effect of variations of the detector response or the mobile phase.

9.3 A standard curve for 3,3′-dichlorobenzidine is prepared by plotting the average peak area for a standard against the concentration of the standard in μg/0.5 ml.

10. **Calculations**

10.1 In the event that the blank produces a peak with the same retention time as 3,3′-dichloro-

800

benzidine, the analyst should determine the source of the interference and eliminate or compensate for it.

10.2 Read the mass (M) in μg of 3,3′-dichlorobenzidine present in the sample from the calibration curve.

10.3 The corrected mass (W) is determined by

$$W = \frac{M}{D}$$

where D is the efficiency of desorption for that sample stage.

10.4 Add the masses of 3,3′-dichlorobenzidine found in the two sampler stages to determine the total mass (W_t) collected.

10.5 The concentration of 3,3′-dichlorobenzidine in air (C, $\mu g/m^3$) is given by

$$C(\mu g/m^3) = \frac{W_t\ (\mu g)}{V\ (\text{liter})} \times \frac{10^3\ \text{liter}}{m}$$

where V is the sampling volume in liters.

10.6 This procedure calculates air concentrations as the mass of 3,3′-dichlorobenzidine per volume of air. If 3,3′-dichlorobenzidinium dihydrochloride is the compound sampled, a weight correction must be made. The conversion factor (1.29) is the ratio of the molecular weights. Thus, the weight of 3,3′-dichlorobenzidine dihydrochloride (W_t') is

$$W_t' = 1.29\ W_t$$

11. **Reference**

11.1 Weeks, R. W., Jr., R. Morales, S. M. Rappaport, E. E. Campbell, and H. J. Ettinger, "Development of Sampling and Analytical Methods for Carcinogens: January-September 1976", Los Alamos Scientific Laboratory, Los Alamos, New Mexico, 1977.

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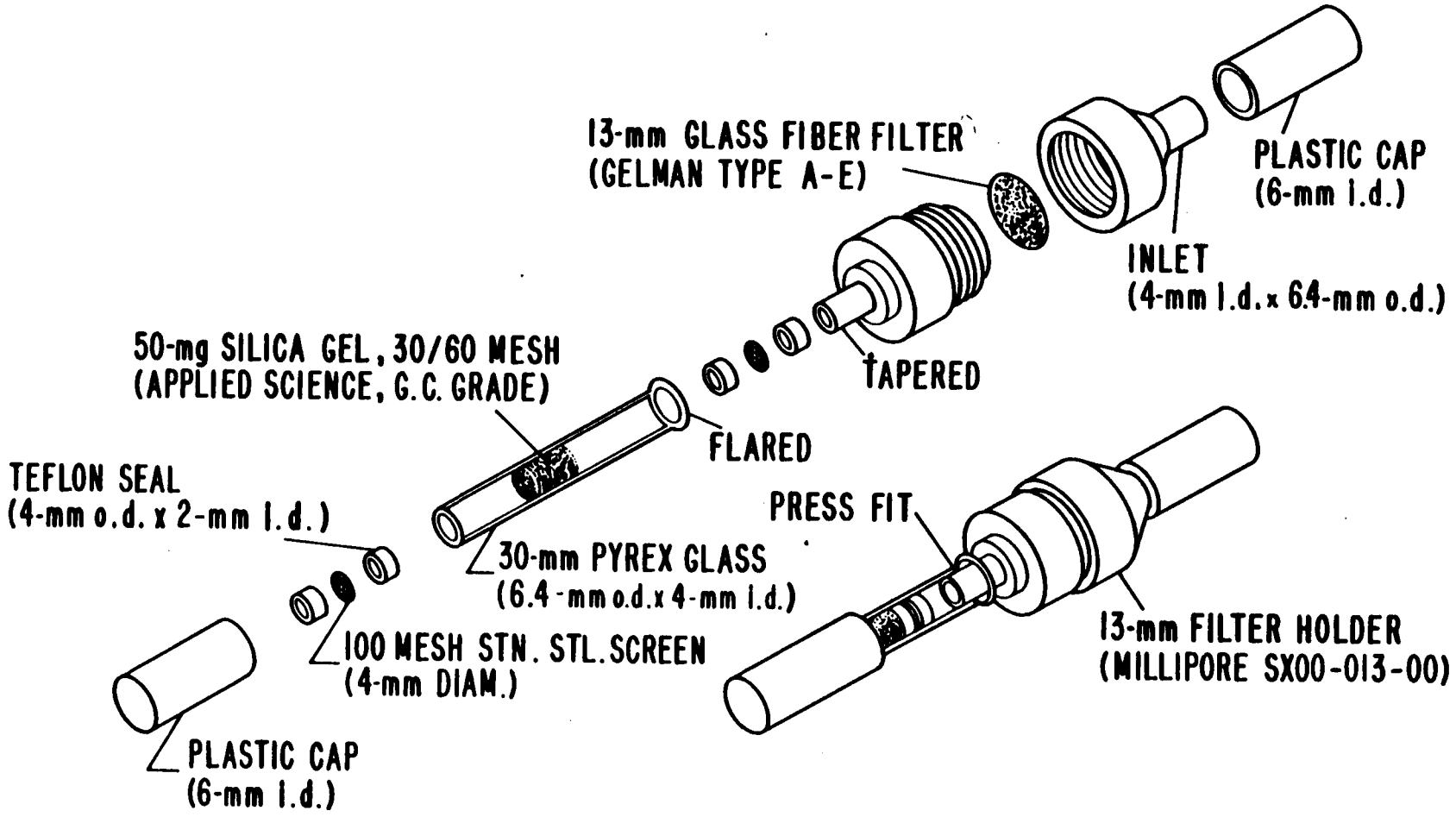


FIGURE 1. Two-stage sampler

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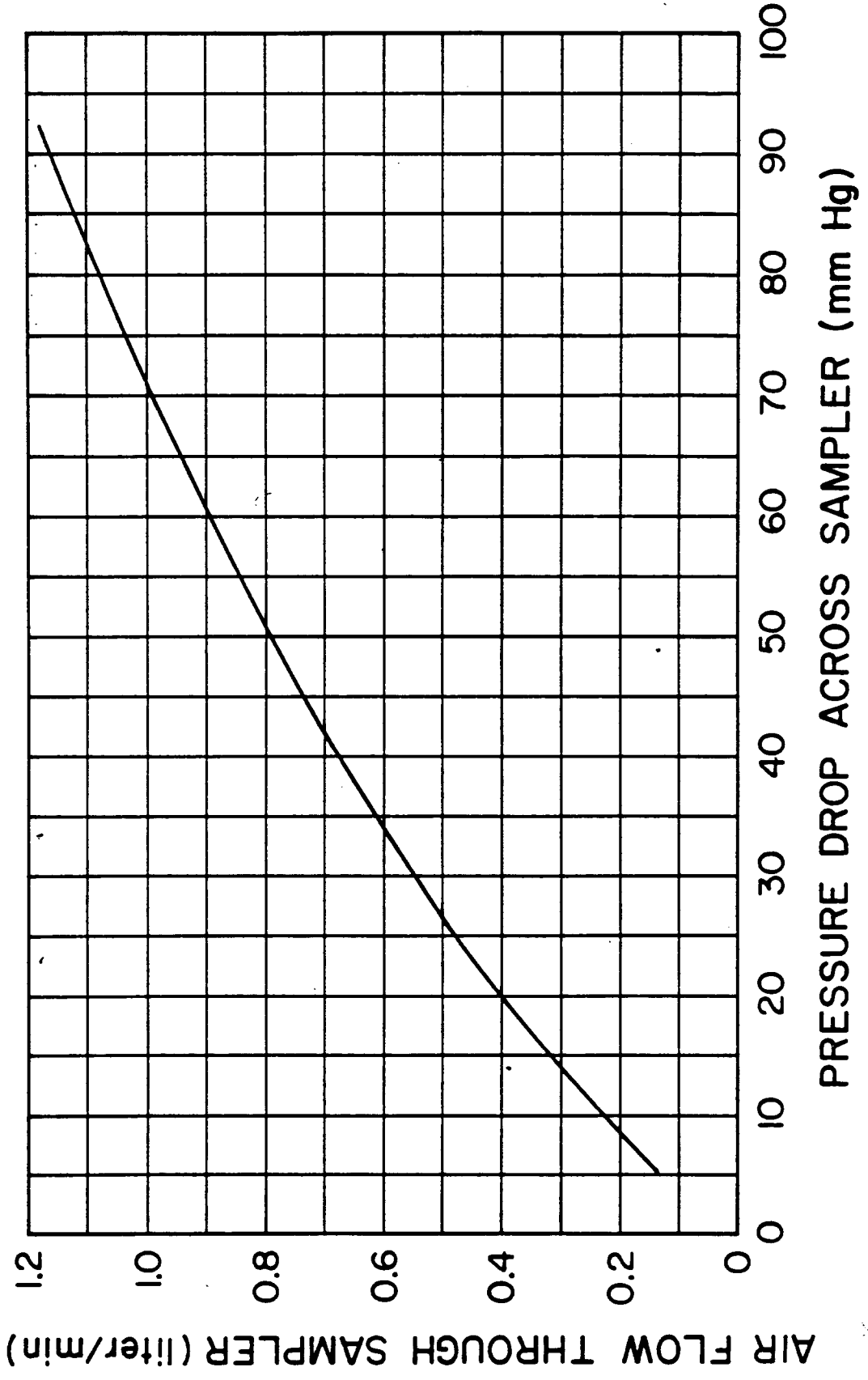


FIGURE 2. Pressure drop across sampler vs. flow rate

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METHYL ALCOHOL IN AIR

Measurements Support Branch

Analytical Method

Analyte:	Methyl Alcohol	**Method No.:**	P&CAM 247
Matrix:	Air	**Range:**	135-534 mg/m³
Procedure:	Adsorption on silica gel, desorption with water, GC	**Precision ($\overline{CV}_T$):**	0.065
Date Issued:	10/1/76	**Classification:**	D (Operational)
Date Revised:			

1. **Principle of the Method**
   - 1.1 A known volume of air is drawn through a large silica gel tube to trap the organic vapors present.
   - 1.2 The silica gel in the tube is transferred to a small, stoppered sample container and the analyte is desorbed with water.
   - 1.3 An aliquot of the desorbed sample is injected into a gas chromatograph.
   - 1.4 The area of the resulting peak is determined and compared with areas obtained from the injection of standards.
   - 1.5 This method is similar to NIOSH Method S59 but is recommended when sampling is done in high relative humidity or when a large concentration of methanol is expected.
2. **Range and Sensitivity**
   - 2.1 This method was validated over the range of 135-534 mg/m³ at an atmospheric temperature of 25°C and a relative humidity of 65 to 90%. Under the conditions of a 5-liter sample size, the probable range of this method is 25-2000 mg/m³. The method is capable of measuring much smaller amounts if the desorption efficiency is adequate. Desorption efficiency must be determined over the range used.
   - 2.2 The upper limit of the range of the method is dependent on the adsorptive capacity of the silica gel tube. This capacity varies with the concentration of the analyte and other substances in the air. The first section of the silica gel tube was found to hold 4.6 mg of methanol in a 85-90% relative humidity atmosphere when sampled at a flowrate of 50 ml/minute for 175 minutes.

     Breakthrough occurred at this time, i.e., the concentration of the analyte in the effluent was 5% of that in the influent. (The silica gel tube consists of three sections of silica gel held in place by teflon plugs, each separated by a 12 mm air gap. See Section 6.2).

     If a particular atmosphere is suspected of containing a large amount of contaminant, a smaller sampling volume should be taken.
3. **Interferences**
   - 3.1 When the amount of water in the air is so great that condensation actually occurs in the tube, organic vapors will not be trapped efficiently.
   - 3.2 When two or more compounds are known or suspected to be present in the air, such information, including their suspected identities, should be transmitted with the sample.

3.3 It must be emphasized that any compound which has the same retention time as the specific compound under study at the operating conditions described in this method is an interference. Retention time data on a single column cannot be considered as proof of chemical identity.

3.4 If the possibility of interference exists, separation conditions (column packing, temperature, etc.) must be changed to circumvent the problem.

4. **Precision and Accuracy**

The Coefficient of Variation ($\overline{CV}_T$) for the total analytical and sampling method in the range of 135-534 mg/m^3 was 0.065.

5. **Advantages and Disadvantages of the Method**

5.1 The sampling device is small, portable, and involves no liquids. Interferences are minimal, and most of those which do occur can be eliminated by altering chromatographic conditions. The tubes are analyzed by means of a quick, instrumental method. The method can also be used for the simultaneous analysis of two or more compounds suspected to be present in the same sample by simply changing gas chromatographic conditions from isothermal to a temperature-programmed mode of operation.

5.2 One disadvantage of the method is that the amount of sample which can be taken is limited by the number of milligrams that the tube will hold before overloading. When the sample value obtained for the backup section of the silica gel tube exceeds 25% of that found on the front section, the possibility of sample loss exists.

5.3 Furthermore, the precision of the method is limited by the reproducibility of the pressure drop across the tubes. This drop will affect the flowrate and cause the volume to be imprecise, because the pump is usually calibrated for one tube only.

5.4 The advantage of this method over method S59 is the increased sample weight before overloading and decreased effect of high humidity.

6. **Apparatus**

6.1 A calibrated personal sampling pump whose flow can be determined accurately (±5%) at the recommended flowrate (11.3).

6.2 Silica gel tubes: glass tube with both ends flame sealed, 15 cm long with a 10-mm O.D. and an 8-mm I.D., containing 3 sections of 20/40 mesh silica gel held by teflon plugs and separated by air. The absorbing section contains 700 mg of silica gel held in teflon plugs and screen. The second section is 12 mm from the absorbing and contains 150 mg of silica gel held by teflon plugs and screen. The third section is 12 mm from the second and is the same composition as the second. The pressure drop across the tube must be less than one inch of mercury at a flowrate of 1 liter per minute.

6.3 Gas chromatograph equipped with a flame ionization detector.

6.4 Column (10 ft × ⅛ in stainless steel) packed with 10% FFAP on 80/100 Chromosorb W-AW.

6.5 An electronic integrator or some other suitable method for determining peak areas.

6.6 Five milliliter glass sample containers with glass stoppers or Teflon-lined caps. If an automatic sample injector is used, the sample injector vials can be used.

6.7 Microliter syringes: 10-μl, and other convenient sizes for making standards.

6.8 Pipets: 3.0-ml delivery type.

6.9 Volumetric flasks: 10 ml or convenient sizes for making standard solutions.

7. **Reagents**

7.1 Eluent: Distilled water.

7.2 Methyl Alcohol (reagent grade).

7.3 Purified nitrogen.

7.4 Prepurified hydrogen.

7.5 Filtered compressed air.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware used for the laboratory analysis should be detergent washed and thoroughly rinsed with tap water and distilled water.

8.2 **Calibration of Personal Pumps.** Each personal pump must be calibrated with a representative silica gel tube in the line. This will minimize errors associated with uncertainties in the sample volume collected.

8.3 **Collection and Shipping of Samples**

8.3.1 Immediately before sampling, break the ends of the tube to provide an opening at least one-half the internal diameter of the tube (4 mm).

8.3.2 The smaller sections of silica gel are used as a back-up and should be positioned nearest the sampling pump.

8.3.3 The silica gel tube should be placed in a vertical direction during sampling to minimize channeling through the silica gel.

8.3.4 Air being sampled should not be passed through any hose or tubing before entering the silica gel tube.

8.3.5 A maximum sample size of 5 liters is recommended. Sample at a flowrate of 0.20 liters per minute or less. The flowrate should be known with an accuracy of at least ±5%.

8.3.6 The temperature and pressure of the atmosphere being sampled should be recorded. If the pressure reading is not available, the elevation should be recorded.

8.3.7 The silica gel tubes should be capped with the supplied plastic caps immediately after sampling. Under no circumstances should rubber caps be used.

8.3.8 One tube should be handled in the same manner as the sample tube (break, seal, and transport), except that no air is sampled through this tube. This tube should be labeled as a blank.

8.3.9 Capped tubes should be packed tightly and padded before they are shipped to minimize tube breakage during shipping.

8.3.10 A sample of the suspected compound should be submitted to the laboratory in glass containers with Teflon-lined caps. These liquid bulk samples should not be transported in the same container as the silica gel tubes.

8.4 **Analysis of Samples**

8.4.1 Preparation of Samples. In preparation for analysis, each silica gel tube is scored with a file in front of the first section of silica gel and broken open. The teflon plug is removed and discarded. The 700 mg of silica gel in the first section is transferred to a 5-ml stoppered sample container or automatic sample injector vial. The second and third sections are transferred to separate sample containers or vials. These 3 sections are analyzed separately.

8.4.2 Desorption of Samples. Prior to analysis, 3.0 ml of distilled water is pipetted into each sample container. Desorption should be done for 4 hours. Tests indicate that this is adequate if the sample is agitated occasionally during this period. The sample vials should be capped as soon as the water is added to minimize evaporation.

8.4.3 GC Conditions. The typical operating conditions for the gas chromatograph are:

1. 30 ml/min (80 psig) nitrogen carrier gas flow.
2. 30 ml/min (50 psig) hydrogen gas flow to detector.
3. 300 ml/min (50 psig) air flow to detector.

403

4. 200°C injector temperature.
5. 300°C manifold temperature (detector).
6. 80°C column temperature.

8.4.4 Injection. The first step in the analysis is the injection of the sample into the gas chromatograph. To eliminate difficulties arising from blow back or distillation within the syringe needle, one should employ the solvent flush injection technique. The 10-μl syringe is first flushed with solvent several times to wet the barrel and plunger. Three microliters of solvent are drawn into the syringe to increase the accuracy and reproducibility of the injected sample volume. The needle is removed from the solvent, and the plunger is pulled back about 0.2 μl to separate the solvent flush from the sample with a pocket of air to be used as a marker. The needle is then immersed in the sample, and a 5 μl aliquot is withdrawn, taking into consideration the volume of the needle, since the sample in the needle will be completely injected. After the needle is removed from the sample and prior to injection, the plunger is pulled back 1.2 μl to minimize evaporation of the sample from the tip of the needle. Observe that the sample occupies 4.9-5.0 μl in the barrel of the syringe. Duplicate injections of each sample and standard should be made. No more than a 3% difference in area is to be expected.

An automatic sample injector can be used if it is shown to give reproducibility at least as good as the solvent flush technique.

8.4.5 Measurement of area. The area of the sample peak is measured by an electronic integrator or some other suitable form of area measurement, and preliminary results are read from a standard curve prepared as discussed below (see Section 9).

8.5 **Determination of Desorption Efficiency**

8.5.1 Importance of determination. The desorption efficiency of a particular compound can vary from one laboratory to another and also from one batch of silica gel to another. Thus, it is necessary to determine at least once the percentage of the specific compound that is removed in the desorption process.

8.5.2 Procedure for determining desorption efficiency. Silica gel equivalent to the amount in the first section of the sampling tube (700 mg) is measured into a 5.0-ml sample container. This silica gel must be the same type as that used in obtaining the samples and can be obtained from unused silica gel tubes. A known amount of the analyte is injected directly into the silica gel with a 10-μl syringe, and the container is capped. The amount injected is equivalent to that present in a 5-liter sample at the selected level.

At least six tubes at each of three levels (0.5X, 1X, and 2X the standard) are prepared in this manner and allowed to stand for at least overnight to assure complete adsorption of the analyte onto the silica gel. These six tubes are referred to as the samples. A parallel blank tube should be treated in the same manner except that no sample is added to it. The sample and blank tubes are desorbed and analyzed in exactly the same manner as the sampling tube described in Section 8.4.

The weight of analyte found in each tube is determined from the standard curve (Section 9). Desorption efficiency is determined by the following equation:

$$\text{D.E.} = \frac{\text{Average Weight (mg) recovered}}{\text{Weight (mg) added}}$$

The desorption efficiency is dependent on the amount of analyte collected on the silica gel. Plot the desorption efficiency versus the weight of analyte found. This curve is used in Section 10.4 to correct for adsorption losses.

9. **Calibration and Standards**

It is convenient to express concentration of standards in terms of mg/3 ml of eluent. A series of standards, varying in concentration over the range of interest, is prepared and analyzed under the same GC conditions and during the same time period as the unknown samples. Curves are established by plotting concentrations in mg/3 ml versus peak area.

Note: Standard solutions should be analyzed at the same time that the sample analysis is done. This will minimize the effect of variations in FID response.

10. **Calculations**

10.1 Read the weights, in mg, corresponding to each peak area (area ratio in case of the internal standard method) from the standard curve.

10.2 Corrections for the blank must be made for each sample.

$$\text{mg} = \text{mg sample} - \text{mg blank}$$

where:

mg sample = mg found in front section of sample tube

mg blank = mg found in front section of blank tube

A similar procedure is followed for the backup sections.

10.3 Add the weights present in the front and both backup sections of the same sample tube to determine the total weight in the sample.

10.4 Read the desorption efficiency from the curve (Section 8.5.2) for the amount of analyte found in the front section. Divide the total weight by this desorption efficiency to obtain the corrected mg/sample.

$$\text{Corrected mg/sample} = \frac{\text{Total Weight}}{\text{D.E.}}$$

10.5 The concentration of analyte in the air sampled can be expressed in mg per m^3, which is numerically equal to μg per liter of air.

$$\text{mg/m}^3 = \frac{\text{Corrected mg (Section 10.4)} \times 1000\ (\text{liter/m}^3)}{\text{Air Volume Sampled (liter)}}$$

10.6 Another method of expressing concentration is ppm:

$$\text{ppm} = \text{mg/m}^3 \times \frac{24.45}{\text{MW}} \times \frac{760}{\text{P}} \times \frac{\text{T} + 273}{298}$$

where:

P = pressure (mm Hg) of air sampled

T = temperature (C) of air sampled

24.45 = molar volume (liter/mole) at 25°C and 760 mm Hg

MW = molecular weight (g/mole) of analyte

760 = standard pressure (mm Hg)

298 = standard temperature (K)

11. **References**

11.1 White, L. D., et al., "A Convenient Optimized Method for the Analysis of Selected Solvent Vapors in the Industrial Atmosphere", Am Ind Hyg Assoc J 31:225, 1970.

11.2 "Documentation of NIOSH Validation Tests", Contract No. CDC-99-74-45.

11.3 Final Report of NIOSH Contract No. HSM-99-71-31, "Personal Sampler Pump for Charcoal Tubes", September 15, 1972.

11.4 Willey, Maurine, DHEW Memorandum, "Breakthrough Tests for Methyl Alcohol", January 26, 1976.

HYDRAZINE COMPOUNDS IN AIR

Measurements Research Branch

Analytical Method

Analyte:	Hydrazine Methylhydrazine 1,1-Dimethylhydrazine Phenylhydrazine	**Method No.:**	P&CAM 248
Matrix:	Air	**Range:**	See Paragraph 2.1
Procedure:	Adsorption on sulfuric acid-coated silica gel, elution with water, reaction with 2-furaldehyde, extraction, GC analysis	**Precision:**	0.04 RSD for 1,1-dimethylhydrazine at 1.6 and 3.8 mg/m^3
Date Issued:	11/1/76		
Date Revised:		**Classification:**	E (Proposed)

1. **Principle of the Method**

   A measured volume of air is drawn through a tube containing sulfuric acid-coated silica gel to trap the hydrazine compounds. The sorbent is treated with distilled water to desorb the hydrazines. Reagent containing sodium acetate and 2-furaldehyde is added and the resulting derivatives are extracted into ethyl acetate and analyzed by gas chromatography with flame ionization detection.

2. **Range and Sensitivity**

   2.1 The ranges of the method for the various analytes are

Hydrazine	0.2 - 6000 μg/sample	0.002 - 60 mg/m^3
Methylhydrazine	9 - 9000	0.09 - 90
1,1-Dimethylhydrazine	4 - 12000	0.04 - 120
Phenylhydrazine	0.5 - 21000	0.005 - 220

   The ranges of air concentrations are based on sampling 96 liters of air.

   2.2 The maximum amount of hydrazine compound retained prior to breakthrough of the first sorbent section is at least 2×10^{-4} moles.

   2.3 The practical upper limit for the analysis is 2×10^{-4} moles total hydrazine compounds in 2 ml of eluent. This is 25 mole % of the acid present and 20 mole % of the 2-furaldehyde reagent added. When hydrazine, methylhydrazine, or phenylhydrazine are present at such high concentrations, derivatives may not be entirely soluble in the reaction mixture. However, they will be dissolved by the ethyl acetate added for extraction.

3. **Interferences**

   3.1 Water vapor is not a sampling interference since it activates, rather than deactivates, the sulfuric acid in the sorbent.

   3.2 Any compound which has nearly the same retention time on the GC column as one of the derivatives of the hydrazines is an interferent.

3.2.1 Atmospheric contaminants. A bulk sample of liquid or solid sources of vapors should be submitted at the same time as the sample tubes so that chemical identification of possible interferents can be made. The bulk sample must not be transported in the same container as the sample tubes.

3.2.2 Reagent contaminants. Reagent grade chemicals of the highest purity available must be used. 2-Furaldehyde is unstable and must be redistilled prior to use and stored in a freezer (−20°C).

4. Precision and Accuracy

4.1 The volume of air sampled can be measured to within ±2% if a pump with a calibrated volume indicator is used. Volumes calculated from initially set flow rates may be less accurate (±5-10%) unless changes in flow rate are manually or electronically monitored and compensated.

4.2 The collection efficiency is 100% under most conditions. This is demonstrated by a negligible amount of compound measured in the backup section of the sorbent tube.

4.3 Desorption may be incomplete, particularly for methylhydrazine, near the lower limit of analytical measurement. In preparing calibration curves, desorption of standards from sorbent sections compensates for such losses, so that accuracy is increased.

4.4 No losses have been observed for 1,1-dimethylhydrazine stored on sorbent sections in sealed tubes for up to 28 days at ordinary room temperatures.

4.5 The precision of the analysis is dependent upon the precision and sensitivity of the technique used to quantitate the GC peaks of samples and standards. An electronic digital integrator with baseline correction capability is useful for this purpose. Near the lower limits of analytical measurement manual peak height measurements are more reproducible.

4.6 A relative standard derivation (RSD) of 0.04 has been determined for analyses of two sets of 6 consecutive 20-liter air samples of 1.6 and 3.8 mg/m³ 1,1-dimethylhydrazine in air.

4.7 This method and NIOSH Method S143 gave apparently equivalent results for 1,1-dimethylhydrazine at the levels 1-10 mg/m³ (11.4).

5. Advantages and Disadvantages of the Method

5.1 The method uses a small, portable sampling device involving no liquids. This is an advantage for sampling air in a worker's breathing zone without interfering with normal work activities. It also simplifies transportation to the analytical laboratory.

5.2 The sorbent tube has a high capacity. It can be used for at least 8 hours to measure a workday average concentration, or for shorter times to measure excursion concentrations.

5.3 Desorption and preparation of samples for analysis involve simple procedures and equipment.

5.4 Several hydrazine compounds can be collected and determined simultaneously. The GC analysis distinguishes which are present and at what individual concentrations they occur. Interferences by amines are less serious than in a colorimetric method.

5.5 The major disadvantage of the method is that 2 hours are required for desorption and reaction of samples and standards. Also, when methylhydrazine is present, the reaction time must be carefully controlled.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Sorbent. The silica gel substrate should be the equivalent of Silica Gel D-08, chromatographic grade, activated and fines-free, 45/60 mesh, as produced by Coast Engineering Laboratories and sold by Applied Science Laboratories, Inc. of State College, PA. The sulfuric acid coating is prepared as follows: a selected amount, W, of silica

gel is weighed in a glass bottle. Reagent grade concentrated sulfuric acid (95-98%) is added directly to the silica gel with a glass dropper until the total weight in the bottle becomes 1.25 W. The bottle is immediately sealed and shaken to uniformly distribute the sulfuric acid on the silica gel. Mixing is repeated intermittently for an hour as the mixture cools. The resulting material is quite hygroscopic and should not be exposed to air any longer than necessary.

6.1.2 Sampling Tubes. Glass tubes 8 cm long and 6 mm I.D., tapered and flame sealed at one end, are packed with two 200-mg sections of sulfuric acid-coated silica gel. Glass wool plugs are used to separate and enclose the sections. The other end of the tube is flame sealed to prevent contamination during storage prior to use. Polyethylene caps are used to seal the tubes after sampling is completed. Pressure drops across these tubes average 6 torr at 200 cm^3/min and 33 torr at 1000 cm^3/min flow rates. The primary absorbing section of the tube is that further away from either end; the backup section is butted against a taper.

6.1.3 Personal Sampling Pump. Pumps are required which are capable of operation at 200 cm^3/min for up to 8 hours. A sample volume indicator or electronic flow control is desirable for best accuracy. The pump is to be calibrated with a representative sorbent tube in line. A wet or dry test meter or a glass rotameter capable of measuring a flow rate of 200 cm^3/min to within ±2% is used in the calibration.

6.2 Gas chromatograph with a flame ionization detector. Temperature programming capability is desirable.

6.3 GC column, 1-m × 2-mm I.D. glass, silanized and packed with 10% (by weight) Silicone OV-7 on 80/100-mesh Supelcoport or equivalent support.

6.4 Strip chart recorder compatible with the GC. An electronic digital integrator is desirable.

6.5 Test tubes, 5-ml, sealed by insertion of a septum.

6.6 Syringes, 10-μl.

6.7 Pipettes, 0.5-ml and 2-ml.

6.8 Volumetric flasks, 10-ml.

6.9 File.

6.10 Forceps.

7. **Reagents**

All chemicals must be ACS Reagent Grade or better.

7.1 Hydrazine.

7.2 Methylhydrazine.

7.3 1,1-Dimethylhydrazine.

7.4 Phenylhydrazine.

7.5 Water, double distilled, aldehyde-free.

7.6 2-Furaldehyde (furfural), b.p. 39-40°C at 5 mm Hg. If this reagent is not clear, it must be redistilled prior to use to remove oxidation products. Store distillate under refrigeration.

7.7 Sodium acetate solution, 0.50 moles/liter (41 g/liter).

7.8 Reagent solution. Prepared by diluting 2 ml of 2-Furaldehyde to 50 ml with the sodium acetate solution. This should be prepared fresh daily.

7.9 GC gases. Carrier helium, Bureau of Mines Grade A. Hydrogen, prepurified. Air, compressed and filtered.

8. **Procedure**

8.1 **Cleaning of glassware.** Wash with detergent solution, rinse with tap water and distilled water, and dry in an oven.

8/13

8.2 **Collection and Shipping of Samples**

8.2.1 Immediately before beginning the collection of a sample, break each end of the sorbent tube so as to provide openings at least 2-mm diameter.

8.2.2 Attach the tubing from the calibrated sampling pump to the backup end of the sample tube. Sample air must not pass through any hose or tubing before entering the sorbent tube.

8.2.3 With the sorbent tube in a vertical position, sample the air at 200 cm^3 min for 8 hours. Record the volume of air sampled.

8.2.4 Immediately after sampling is completed, cap the sorbent tubes with the polyethylene caps. Rubber caps must not be used.

8.2.5 Obtain a blank sample by handling one tube in the same manner as the sample tubes (break, seal and ship) except that no air is pumped through it.

8.2.6 Pack the tubes tightly to minimize chances of breakage during transit. Tubes should not be subjected to extremes of high temperature or low pressure.

8.3 **Analysis of Samples**

8.3.1 Preparation of Samples. Score each tube with a file 5 mm in front of the first glass wool plug and break it open. Remove the glass wool plug that precedes the first sorbent section and transfer it along with this initial section to a 5-ml test tube that can be septum sealed. Likewise, transfer the second plug and sorbent section to another test tube. Label each appropriately for separate analysis.

8.3.2 Desorption. Desorb the hydrazine compounds from the sulfuric acid-coated silica gel by adding 2 ml of distilled water to each sorbent section. Seal the tubes. Shake the mixtures occasionally over a period of 1 hour. Tests have shown that desorption reaches a maximum within an hour.

8.3.3 Derivatization. Add 2 ml of reagent solution to each test tube containing sorbent and eluent. When methylhydrazine is expected in the sample or standard, the time of reagent addition must be exactly noted for each mixture. Mix the reagents thoroughly.

8.3.4 Extraction. After 1 hour of reaction, add 0.5 ml of ethyl acetate to each test tube, seal tightly, and shake vigorously for 1 minute. For methylhydrazine the reaction time must be *exactly* 1 hour, since a secondary reaction is occurring in the aqueous solution. Allow the ethyl acetate layer to settle out on top of the aqueous layer. Centrifuging accelerates this process. Aliquots of the ethyl acetate extracts are then analyzed by gas chromatography.

8.3.5 Gas Chromatographic Conditions. Typical operation conditions for the gas chromatograph are as follows:

Helium carrier gas flow rate, 50 ml/min.

Hydrogen gas flow rate to detector, 40 ml/min.

Air flow rate to detector, 540 ml min.

Injection port temperature, 150°C.

Detector temperature, 200°C.

Column temperature — programmed, 80°C for 12 min, to 185°C at 24°C min, and hold for 8 min. When only methylhydrazine and 1,1-dimethylhydrazine are present, isothermal analysis at 80°C is appropriate; when only hydrazine and phenylhydrazine are present, 185°C is appropriate.

8.3.6 Injection of Sample. To eliminate difficulties arising from blowback or distillation within the syringe, use the solvent flush injection technique. Flush the 10-μl syringe several times with ethyl acetate to clean the barrel and plunger. Draw in 1 μl of ethyl acetate. With the needle removed from contact with liquid, pull the plunger

back about 0.4 μl to form a pocket of air. Then immerse the tip of the needle in the ethyl acetate extract layer of a sample and withdraw a 3-μl aliquot, taking into consideration the volume in the needle. Remove the needle from the sample and pull the plunger back another 0.4 μl to minimize evaporation from the tip of the needle. Inject and analyze duplicate 3-μl aliquots of each sample and standard extract.

8.3.7 GC Peak Measurement. Measure the areas or peak heights of the hydrazine derivative peaks obtained from analyses of samples and standards. At lower concentrations, peak-height measurements may be more precise.

9. **Calibration and Standards**

9.1 Preparation of a standard solution in water. Calculate for each compound i the volume V_i (μl):

$$V_i = \frac{X_i \times V_s}{d_i \text{ (or } c_i)}$$

X_i = the anticipated average concentration (mg/m³) of compound i in air.

V_s = volume (liters) of air sampled.

d_i = density (g/ml) of a pure compound i.

c_i = concentration (g/ml) of the compound i in a known standard aqueous solution.

Add the calculated volumes of each compound or aqueous standard solution to a 10-ml volumetric flask and dilute to the mark. A 10-μl aliquot of this new standard contains amounts of compounds equal to the amounts collected from V_s of concentration X_i. Aqueous solutions of hydrazine compounds are unstable and should be prepared fresh when used.

9.2 Preparation of standards on sorbent sections. Add 200 mg of sulfuric acid-coated silica gel to each of 10 test tubes which can be tightly sealed with a septum. These may be weighed from bulk sorbent or obtained from unused tubes. Add 2.5 μl, 5.0 μl, 10.0 μl, 15.0 μl, and 20.0 μl of the prepared standard to pairs of silica gel sections in the tubes. Use a 10-μl syringe and inject through the septum onto the walls of the tube containing sorbent. Withdraw the syringe and shake the tube to distribute the hydrazines. These standards correspond to 0.25, 0.5, 1, 1.5, and 2 times X_i in V_s, respectively. Other standards may be similarly prepared, if desired. Analyze these standards along with samples in Section 8.3.

9.3 If samples are to be stored for longer than one week before analysis, prepare standards on sulfuric acid-coated silica gel soon after receipt of the samples in the laboratory. Store samples and standards at the same conditions so that storage losses (if any) will be similar.

10. **Calculations**

10.1 Prepare a calibration curve for each hydrazine compound by plotting peak areas (or heights) obtained from the analyses of standards (from section 9.2) against nominal amounts on the standard sorbent sections (A_i, μg).

$$A_i = V_i \times d_i$$

where V_i and d_i are defined in Sec. 9.1.

10.2 Read the amount of each compound from the appropriate calibration curve, using average peak heights from each sample. Correct each value for the amount found in the corresponding blank, if any. Add the amounts found in the front and backup sections (if any) of the same sample tube to obtain the total weight of compound in the air volume sampled.

10.3 Divide the total weight of each compound on each tube by the volume of air sampled (V_s) to obtain the concentration of that compound in μg/liter or mg/m³.

11. References

11.1 Campbell, E. E., G. O. Wood, and R. G. Anderson, "Development of Air Sampling Techniques", Progress Report LA-6057-PR, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, 1975.

11.2 Wood, G. O. and R. G. Anderson, "Development of Air-Monitoring Techniques Using Solid Sorbents", Progress Report LA-6216-PR, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, 1976.

11.3 Wood, G. O. and R. G. Anderson, "Development of Air-Monitoring Techniques Using Solid Sorbents", Progress Report LA-6513-PR, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, 1976.

11.4 Wood, G. O. and R. G. Anderson, "Development of Air-Monitoring Techniques Using Solid Sorbents", Progress Report LA-6738-PR, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, 1977.

BENZO[A]PYRENE IN PARTICULATES

Measurements Research Branch

Analytical Method

Analyte:	Benzo[a]pyrene	**Method No.:**	P&CAM 251
Matrix:	Collected particulate	**Range:**	6-240 ng/sample
Procedure:	Ultrasonic extraction, HPLC	**Precision:**	0.06 RSD
Date Issued:	2/15/77	**Classification:**	E (Proposed)
Date Revised:			

1. **Principle of the Method**
   - 1.1 Airborne particulates are collected on glass fiber/membrane filters and extracted with acetonitrile and the aid of ultrasonic energy.
   - 1.2 An aliquot of the filtered extract is injected into a high performance liquid chromatograph (HPLC) and the separated benzo[a]pyrene is quantitated with fluorescence detection at wavelengths selective for benzo[a]pyrene.
2. **Range and Sensitivity**
   - 2.1 The range of this method is 6-240 ng/sample when quantitation is done using peak height with the photomultiplier setting such that 1 ng of benzo[a]pyrene gives a full scale deflection for a recorder voltage of 5 mv. The range may be extended by changing the photomultiplier setting and recalibrating the detector or by quantitatively diluting an aliquot of the extract.
   - 2.2 The limit of detection is 10 pg/injection. For this level of sensitivity, 4-mm cell slit widths and deoxygenated mobile phase solvents are required.
3. **Interferences**

   Any compound that elutes with the same retention volume as benzo[a]pyrene and fluoresces at the prescribed excitation and emission wavelengths will be an interference.
4. **Precision and Accuracy**
   - 4.1 The average relative standard deviation of the analytical method is 0.06. This figure is based on the values obtained from 6 sets of replicate coke oven field samples. Each set consisted of 6 samples taken with glass fiber filters or silver membrane filters. The concentrations ranged from 14 to 2,280 ng/sample.
   - 4.2 The accuracy of this method has not been determined. However, recovery of benzo[a]-pyrene from glass fiber, silver membrane, and polyperfluoroethylene filters was at least 90%. Other experiments have shown that the filtration and transfer of the sample extract does not detectably affect the concentration. Also samples may be stored for one month if refrigerated and kept away from light.
5. **Advantages and Disadvantages of the Method**
   - 5.1 The method requires a minimum amount of sample preparation. Total extraction and analysis time is less than thirty minutes.

407

5.2 A spectrophotofluorometer is required for maximum sensitivity and selectivity for benzo[a]pyrene. A filter fluorescence HPLC detector can be used if suitable filters are available and maximum sensitivity is not required.

6. **Apparatus**

6.1 Ultrasonic bath, frequency 55kHz, power output 100 watts (Ultramet III sonic cleaner, Buehler LTD).

6.2 Liquid chromatograph, (2 Waters Associates Model 6000A pumps and U6K injector).

6.3 Column, Vydac 201-TP reverse phase-ODS (octadecylsilylated microporous silica), particle size 10 μm, length 25 cm, internal diameter 3.2 mm.

6.4 Pre-column, Vydac reverse phase, particle size 35-44 μm, length 5 cm, internal diameter 4.2 mm.

6.5 Spectrophotofluorometer, (Aminco-Bowman, equipped with a 70-μl flow cell).

6.6 Recorder.

6.7 Test tubes, 100-mm × 13-mm, with screw caps.

6.8 Delivery pipets, 3-ml.

6.9 Microanalysis disc filter holder with frit support, 25-mm, Pyrex, Millipore.

6.10 Filters, 25-mm, Millipore Duralon (nylon), 1.0-μm pore size.

6.11 Glass vials, approximate volume 20 ml, with teflon lined screw caps.

6.12 Test tube support for use in ultrasonic bath, 135-mm × 135-mm × 25-mm made from styrofoam. 15-mm diameter holes may be cut out using a rubber stopper hole borer.

6.13 Nitrogen delivery system. One arm of a T-type connector is inserted into a #4 1-hole neoprene stopper. Another arm is connected via clean tygon tubing to a tank of nitrogen.

6.14 Syringes, 10-μl and convenient sizes for making standards.

6.15 Volumetric flasks, 5-ml or convenient sizes for making standard solutions.

6.16 Forceps.

6.17 Glass fiber filters, 37 mm, free of organic binder, with an efficiency of at least 99% as determined by the DOP penetration test.

6.18 Silver membrane filters, 37 mm, 0.8 μm pore size, Selas Corporation of America, No. FM-37-(0.8), or equivalent.

6.19 Disposable plastic filter holder (cassette) for 37-mm filters, Millipore MAWP 037 AO, or equivalent. In order to accommodate the two filters, the cassette must be held together with plastic tape or a shrinkable cellulose band.

6.20 Personal sampling pump capable of operating for 4 hr at 2.0 l/min or of sampling about 0.5 m^3 of air. The pump should be calibrated with a representative filter assembly in the line. A wet or dry test meter or a glass rotameter capable of measuring the flow rate to within ±5% may be used for the calibration.

7. **Reagents**

7.1 LC mobile phase: 80% UV-grade acetonitrile, 20% distilled water (v/v). The solvents are deoxygenated by purging with nitrogen for 30 min. and then degassed under vacuum with stirring.

7.2 Nitrogen, prepurified.

7.3 Benzo[a]pyrene, analytical reagent quality.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware used for the analysis should be washed with detergent and then thoroughly rinsed with tap and distilled water.

8.2 **Collection and Shipping of Samples.** The method is for samples of airborne particulate collected at 2 lpm on glass fiber and silver membrane filters, but may be adapted for use with other samples. Blank, unused filters should be available for analysis with the samples.

8.3 **Analysis of Samples**

8.3.1 The sample filter is removed from the cassette holder and folded or rolled with the aid of tweezers such that it will slide into a small screw cap test tube.

8.3.2 Acetonitrile (3 ml) is pipetted into the test tube. This amount of solvent will cover a 37-mm diameter filter. Care should be taken during the following extraction and filtration procedure to avoid evaporation of acetonitrile.

8.3.3 The sample is then suspended in an ultrasonic bath and sonified for 5 min. using a test tube support (see 6.12).
Note: The test tube should not be hand-held during sonification.

8.3.4 The solvent extract is decanted into a microfiltration apparatus and filtered under positive nitrogen pressure. This is done by placing the stopper of the nitrogen delivery system over the micro-filter with the nitrogen flowing slowly. The nitrogen pressure is applied to acetonitrile solution by placing one's finger over the open arm of the T. The filtrate is collected in a small vial and tightly sealed with a teflon-lined screw cap after filtration is completed.

8.3.5 The samples are analyzed by liquid chromatography using the following conditions:

Mobile phase flow rate: 1.0 ml/min

Injection volume: 5 μl

Pressure: approximately 1200 lb/in^2

Temperature: ambient; if room air is not at a constant temperature and draft-free, column and pre-column should be jacketed and kept at 25°C.

Detector: spectrophotofluorometer wavelengths, excitation, 368 nm, emission 409 nm; cell slit widths, 4 mm; photomultiplier tube slit width, 5 mm.

Capacity ratio, k′: 5.8

8.3.6 Measurement of benzo[a]pyrene response. The height of the benzo[a]pyrene peak is measured with a ruler.

9. **Calibration and Standards**

Standards of various concentrations are prepared by diluting aliquots of a concentrated stock solution of benzo[a]pyrene in acetonitrile. The standards are analyzed with the unknown samples and under the same LC conditions. A standard curve is established by plotting concentration (ng/3 ml) versus peak height (mm).

Warning: Benzo[a]pyrene has been identified as a carcinogen. Proper procedures for handling this compound must be observed.

10. **Calculations**

Amount of benzo[a]pyrene in a sample in ng/sample is determined from the standard curve and corrected for the amount present in blank filters. The air concentration is calculated by dividing the amount of benzo[a]pyrene (ng/sample) by the total volume of air sampled.

11. **Reference**

11.1 Seymour, M. J., C. V. Cooper, and A. W. Teass, "HPLC-Fluorometric Determination of Benzo[a]pyrene in Airborne Particulate Coke-Oven Emissions", Presented at the American Chemical Society Meeting, San Francisco, California, 1976.

N-NITROSODIMETHYLAMINE IN AIR

Physical and Chemical Analysis Branch

Analytical Method

Analyte:	DMN	**Method No.:**	P&CAM 252
Matrix:	Air	**Range:**	0.5 ppt-10 ppb
Procedure:	Tenax GC adsorption, thermal desorption with He purge, capillary gas-liquid chromatography, mass spectrometry	**Precision:**	± 10% RSD (Analytical)
Date Issued:	1/1/75	**Classification:**	E (Proposed)
Date Revised:			

1. **Principle of the Method**

   N-Nitrosodimethylamine (DMN) is concentrated from ambient air on Tenax GC in a short glass tube (11.1, 11.2). It is desorbed by heating and purging with helium into a liquid nitrogen cooled nickel capillary trap and then introduced onto a high resolution gas chromatographic column where it is separated from interferences. The concentration of DMN is measured from the mass spectrometric signal at m/e 74 (11.3).

2. **Range and Sensitivity**

   2.1 The range of the mass spectrometric signal for the conditions listed corresponds to 0.5 ppt to 10 ppb.

   2.2 A concentration of 0.5 ppt of DMN can be determined in a 150-liter air sample.

3. **Interferences**

   Interferences may result from materials having background ions of m/e 74 ($C_2H_6N_2$, $C_2H_4NO_2$, $C_2H_6N_2O$, C_2H_3Cl, C_2H_6S, $C_3H_6O_2$, or $C_3H_{10}N_2$) if at the same retention time of DMN.

4. **Precision and Accuracy**

   4.1 The precision of this method has been determined to be ±10% of relative standard deviation when replicate sampling cartridges were spiked with 50 ng (corresponding to 10 ppb in 150 l of air). These data were obtained using 10.0 cm long glass tubes (1.5 cm I.D.) packed with 35/60 mesh of Tenax GC (bed dimensions: 1.5 cm × 6 cm in depth).

   4.2 The accuracy of the analysis is approximately ±10% of the amount reported as determined from repeated analysis of several standards.

5. **Advantages and Disadvantages of the Method**

   5.1 The gas chromatography-mass spectrometry technique interfaced with a Finnigan glass jet separator (Model 01512-42158 Finnigan Corp., Sunnyvale, CA) is extremely sensitive and specific for the analysis of DMN. The high resolution gas chromatographic separation yields a retention time that is characteristic for DMN, and relatively specific for positive assignment of the signal as DMN. The mass spectrometer in combination with high resolution gas chromatography yields a very high degree of specificity. The base peak of DMN

421

is at *m/e* 74 which is also the parent ion. In order to assign the signal at *m/e* 74 to DMN, it is absolutely necessary that the retention time matches with the signal.

5.2 Collected samples can be stored up to 1 month with less than 10% losses.

5.3 Because DMN is a suspected carcinogen in man, it is extremely important to exercise safety precautions in the preparation and disposal of liquid and gas standards, cleaning of used glassware, etc., and the analysis of air samples.

5.4 Since the mass spectrometer can not be conveniently mobilized, sampling must be carried out away from the instrument.

5.5 High resolution gas chromatography/low resolution mass spectrometry is not a convenient technique for handling a large number of samples (> 100/wk).

5.6 Efficiency of air sampling increases as the ambient air temperature decreases (i.e., sensitivity increases).

5.7 Ambient air sampling is limited to cases where the NO_x levels are less than 3 ppm when dimethylamine is also present.

6. Apparatus

6.1 Sampling Tubes

6.1.1 The sampling tubes are prepared by packing a 10 cm long × 1.5 cm I.D. glass tube with 6.0 cm of 35/60 mesh Tenax GC with glass wool in the ends. Cartridge samplers are conditioned at 270°C with helium flow at 30 ml/min for 20 min. The conditioned cartridges are transferred to Kimax® (2.5 cm × 150 cm) culture tubes, immediately sealed using Teflon-lined caps, and cooled.

6.1.2 Cartridge samplers with longer beds of sorbent may be prepared using a proportional amount of Tenax GC.

6.2 Gas Chromatographic Column

A 0.35 mm I.D. × 50 m glass SCOT capillary coated with DEGS stationary phase and 0.1% benzyl triphenylphosphonium chloride is used. The capillary column is conditioned (detector end disconnected) for 48 hr at 210°C at 1.5-2.0 ml/min helium flow.

6.3 A Finnigan-type glass jet separator on a magnetic or quadropole instrument is used at 200°C.

6.4 Inlet-Manifold

An inlet-manifold is employed (Figure 1, 11.1, 11.2, 11.4), available from NuTech Corp., 2806 Cheek Road, Durham, N.C. 27704.

6.5 Gas Chromatograph

A Varian 1700 gas chromatograph or equivalent. A gas chromatograph employing a single column oven and a temperature programmer is adequate.

6.6 Mass Spectrometer

A mass spectrometer with a resolution of 500-2000 equipped with single ion monitoring capabilities must be used in conjunction with a gas chromatograph. A Varian-MAT CH-7 has been found to be satisfactory for this purpose (11.2, 11.3).

6.7 Syringes

Syringes, 1-ml gas tight (Precision Sampling, Inc.) and 10 μl (The Hamilton Co., Inc.).

7. Reagents and Materials

All reagents must be analytical reagent grade.

7.1 N-nitrosodimethylamine

7.2 Acetone

7.3 Isoclean®

7.4 Tenax GC (35/60 mesh, Applied Science Laboratories, Inc.)

7.5 Two 2-liter round bottom flasks fitted with injection ports.

7.6 Soxhlet apparatus.

8. **Procedure**

8.1 **Cleaning of glassware**

All glassware, glass sampling tubes, cartridge holders, etc. should be washed in Isoclean® water, rinsed with doubly distilled water and acetone, and air dried. Glassware is heated to 450°F for 2 hrs.

8.2 **Preparation of Tenax GC**

Virgin Tenax GC is extracted in a Soxhlet apparatus overnight with acetone prior to its use.

8.3 **Collection of DMN in Ambient Air**

8.3.1 Continuous sampling of ambient air may be accomplished using a NuTech Model 221-A portable sampler (NuTech Corp., Durham, NC) or its equivalent (11.2). Flow rates are adjusted with a metering valve through a calibrated rotameter. Total flow is registered by a dry gas meter.

8.3.2 For larger sample sizes it is important to realize that a larger total volume of air may cause elution of DMN through the sampling tube. It has been demonstrated that exceeding a total of 385, 332, 280, 242, 224, 204, 163, 156, 148, 127, 107, 93, or 79 liters of air at ambient temperatures of 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105 or 110°F, respectively, will result in elution of DMN from the cartridge sampler. A flow of 10 cc/min to 30 l/min may be used with the sampler described in 6.1.

8.3.3 DMN has been found to be stable and quantitatively recoverable from cartridge samplers after 4 weeks when tightly closed in cartridge holders, protected from light, and stored at 0°C.

8.4 **Analysis of Samples**

8.4.1 Instrument Conditions and Set-up. The thermal desorption chamber and six-port valve are set to 200°C. The glass jet separator is maintained at 200°C. The mass spectrometer is set to monitor *m/e* 74 (Figure 2).

8.4.2 Adjust the He purge gas through the desorption chamber to 50 ml/min. Cool the Ni capillary trap at the inlet manifold with liquid nitrogen.

8.4.3 Place the cartridge sampler in the desorption chamber and desorb for 5 min.

8.4.4 Rotate the six-port valve on the inlet-manifold to position "B" and heat the Ni capillary trap to 180°C with a wax bath.

8.4.5 Temperature program the glass capillary column from 75 to 205°C at 4°C/min and hold at upper limit for 10 min. The retention time of DMN is approximately 26 min (Figure 3).

8.4.6 The analytical column is cooled to ambient temperature and the next sample is processed.

9. **Calibration and Standards**

9.1 **Preparation of Gas Standard**

9.1.1 Purge two 2-liter round bottom flasks with helium, warm flasks to 50°C with heating mantles, and use magnetic bar to stir vapors.

9.1.2 Inject 0.1-1 μl of DMN into flask and let stir for 30 min. Make further dilutions into second flask by transferring milliliter gas volumes as needed.

9.1.3 Purge air/vapor mixtures from second flask onto cartridge samplers.

9.2 **Calibration**

Prepare standard curve (with ten concentration points) by thermally desorbing cartridge samplers loaded with 3 ng to 30 μg of DMN. Plot m/e 74 response vs. ng of DMN. A linear response is observed.

10. **Calculations**

The total quantity of DMN in ambient air is determined by comparing m/e 74 response for samples of DMN with standard curve.

$$\text{ppb} = \frac{\text{ng DMN}}{\text{V}} \times \frac{24.45}{74}$$

where:

ng DMN = total ng concentration as determined in 9.2.1
V = volume of air in liters sampled at 25°C and 760 torr
24.45 = molar volume of an ideal gas at 25°C and 760 torr
MW = molecular weight of DMN, 74.

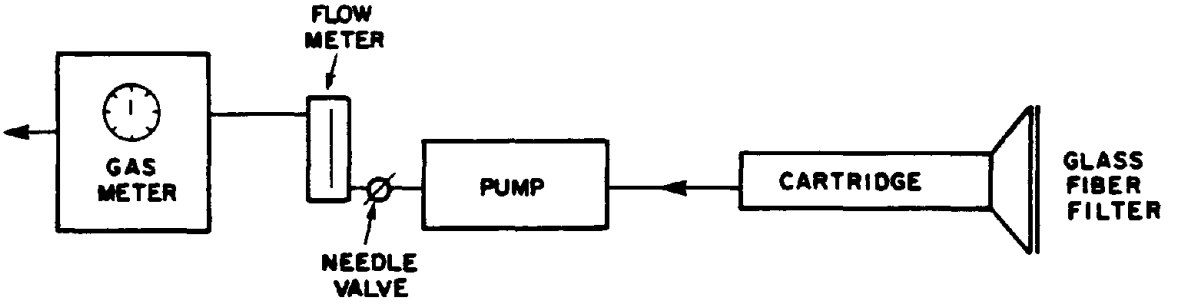
11. **References**

11.1 Pellizzari, E., Development of Method for Carcinogenic Vapor Analysis in Ambient Atmosphere, Pub. No. EPA-650/2-74-121, 1974.

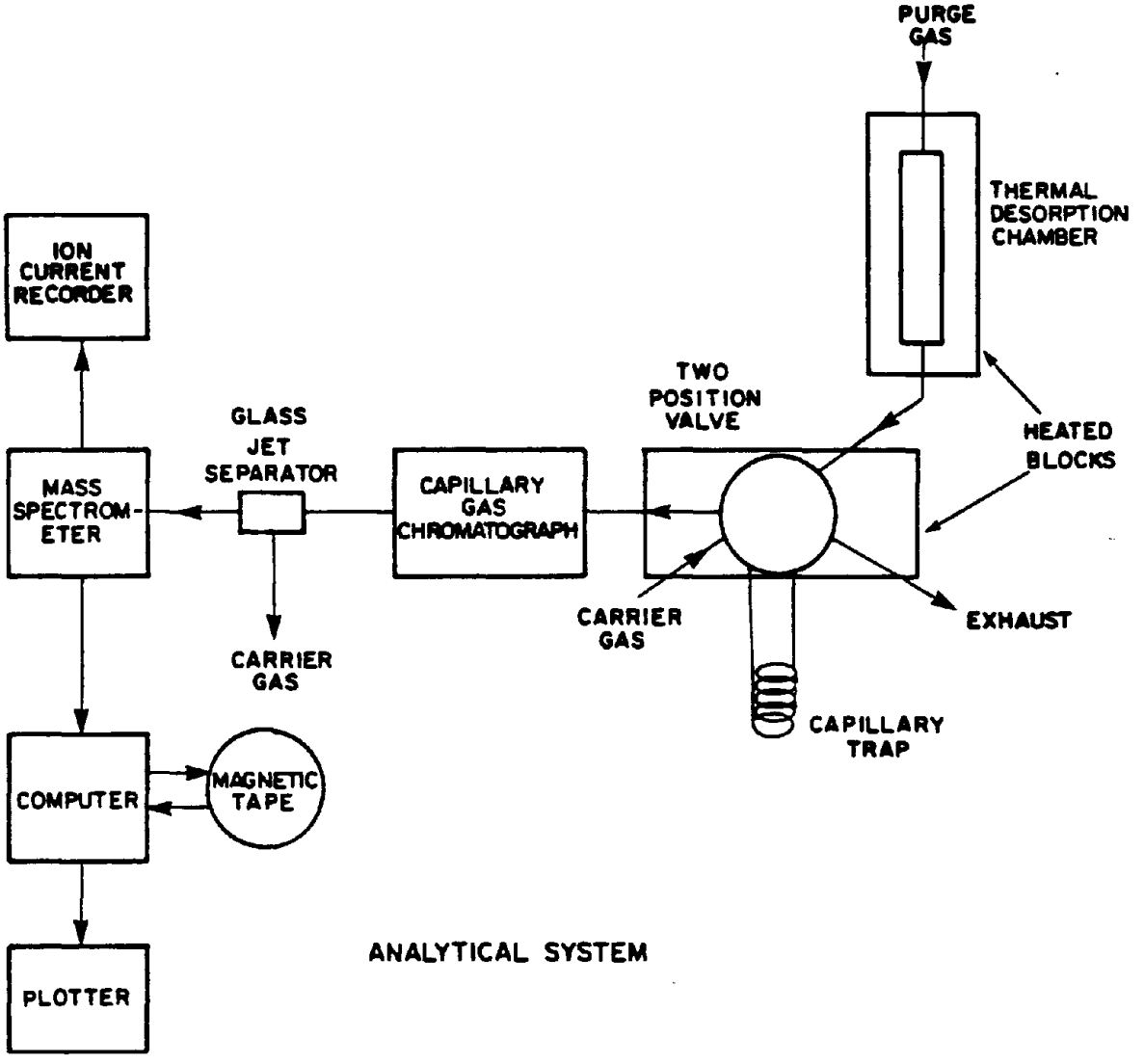
11.2 Pellizzari, E., Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors, Pub. No. EPA-600/2-75-076, 1975.

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11.4 Pellizzari, E., B. H. Carpenter, J. E. Bunch, and E. Sawicki, Collection and Analysis of Trace Organic Vapor Pollutants in Ambient Atmospheres, Environ Sci Technol 9:556, 1975.



VAPOR COLLECTION SYSTEM



ANALYTICAL SYSTEM

FIGURE 1. Vapor collection and analytical systems for analysis of hazardous vapors in ambient air

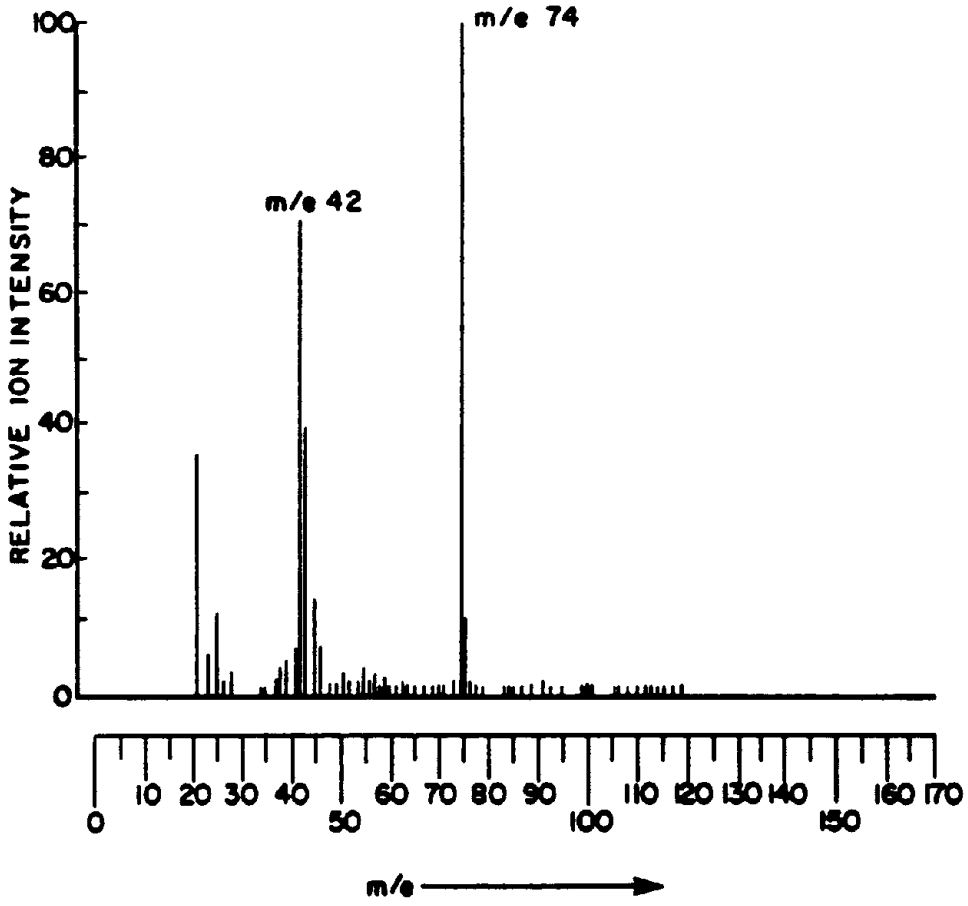


FIGURE 2. Mass spectrum of N-nitrosodimethylamine

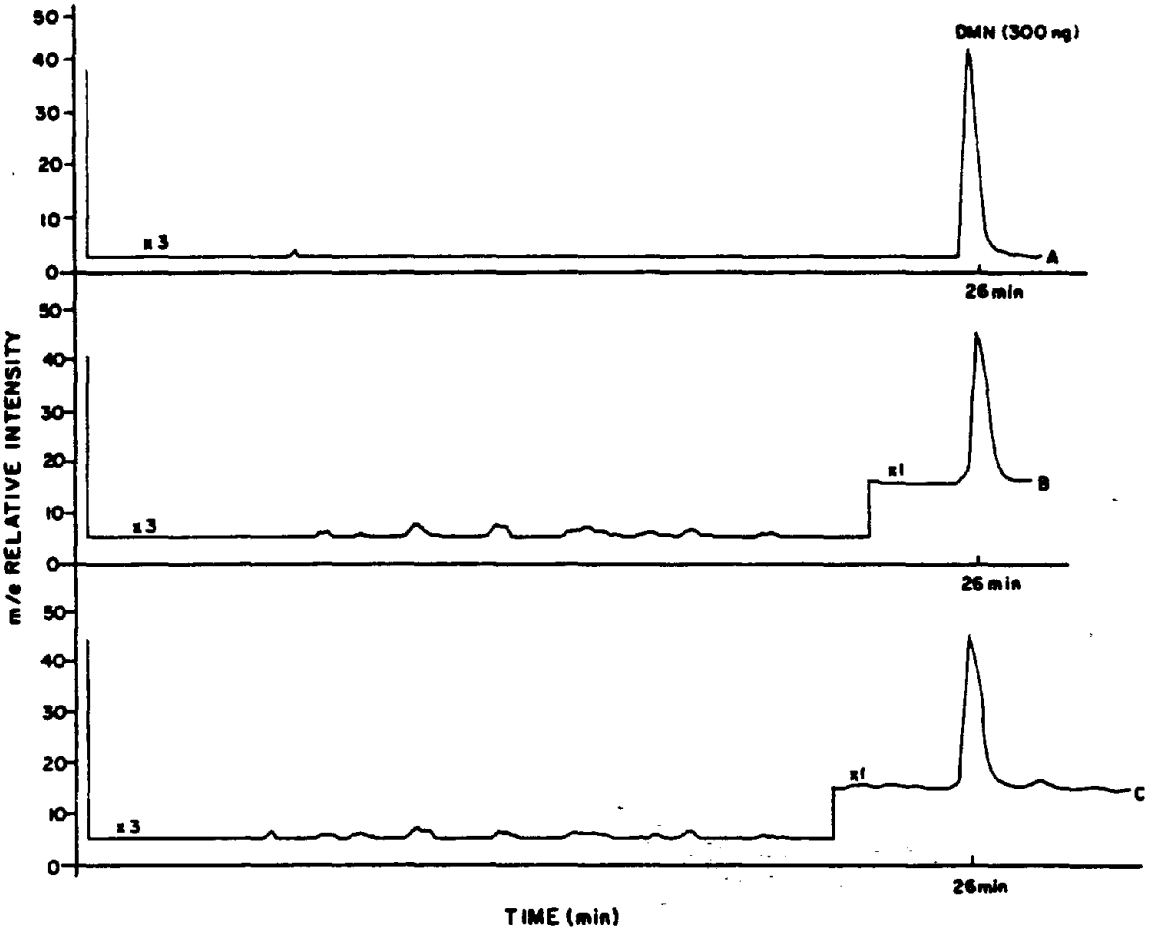


FIGURE 3. Mass (m/e 74) chromatograms (A = standard DMN; B, C = replicate air samples)

POLYCHLORINATED BIPHENYLS (PCB) IN AIR

Measurements Research Branch

Analytical Method

Analyte:	Polychlorinated biphenyls (PCB)	**Method No.:**	P&CAM 253
Matrix:	Air	**Range:**	0.01 - 10 mg/m³
Procedure:	Adsorption on Florisil®, hexane desorption, perchlorination, gas chromatography with electron capture detection	**Precision:**	4.4% RSD (Analytical) 2.8% RSD (Analytical, perchlorination)
Date Issued:	12/1/76	**Classification:**	D (Operational)
Date Revised:			

1. **Principle of the Method**

   1.1 Sampling. A measured volume of air is drawn through a small sorbent tube containing Florisil which adsorbs the PCB present in the air sample.

   1.2 Standard Analytical Procedure. The collected PCB are desorbed with hexane and the resulting solutions are analyzed using gas chromatography with electron capture detection. The concentration of PCB relative to a PCB standard (Aroclor) is read from a standard curve.

   1.3 Perchlorination Analytical Procedure. If the air sample is found to differ significantly in composition from the PCB standard, then the perchlorination analytical procedure is used. The sample solution is concentrated and reacted with antimony pentachloride. Decachlorobiphenyl, the product of perchlorination, is extracted into hexane and the resulting solution is compared with a decachlorobiphenyl standard curve. The PCB in the original solution may be calculated. This procedure is based on the work of Armour (11.2).

2. **Range and Sensitivity**

   2.1 Standard Analytical Procedure. The estimated useful range of this method is 0.01 to 10 mg/m³. The minimum detectable quantity of PCB (Aroclor 1016) from a standard curve was found to be 32 picograms per injection. In laboratory experiments no breakthrough was detected after sampling 48 liters of 10 mg/m³ of Aroclor 1016 at 75% relative humidity at a sampling rate of 200 cc/min through a 100-mg bed of Florisil. Field samples analyzed by this method ranged from 0.1 to 1.5 mg/m³.

   2.2 Perchlorination Analytical Procedure. The estimated useful range of this method is 0.01 to 10 mg/m³. The minimum detectable limit of decachlorobiphenyl, the perchlorination product, is 10 picograms per injection. This quantity is equivalent to 5 picograms of Aroclor 1016. Field samples analyzed by this method ranged from 0.1 to 1.7 mg/m³.

3. **Interferences**

   3.1 Strict measures to avoid contamination are required when using electron capture detection. Foremost, the syringe must be thoroughly cleaned after each injection. Hexane used for desorption and back flush should be injected periodically for a purity check. It should

829

show no peaks having retention times greater than 45 seconds providing that not more than 5 μl have been injected.

3.2 Any compound which has nearly the same retention time on the GC column as one of the PCB is an interference. This type of interference can often be overcome by changing the GC operating conditions or by selecting another column. Retention time data on a single column, or even on a number of columns, cannot be considered as conclusive proof of chemical identity. It is important, therefore, that a sample of the bulk mixture of PCB be submitted at the same time as the sample tubes so that chemical identification of possible interferences can be made.

3.3 Chlorinated pesticides, such as DDT, DDE, etc., have been reported as interferences which arise due to coextraction with PCB during workup of environmental samples such as water, tissue, soil, or biological fluids. In the case of personal air sampling in an industrial environment, these interferences would not be present unless these were being manufactured in the same area. Thus, unless these chlorinated pesticides are known to specifically be present, there need be no extra clean up or separation step for these materials.

3.4 Sulfur-containing compounds in petroleum products have been reported as interferences. (11.4)

3.5 Biphenyl, if present in the PCB mixture, will be an interference when samples are analyzed by perchlorination. Brominated impurities in antimony pentachloride have also been reported to lead to interferences in perchlorination procedures. Results from our NIOSH laboratories indicate, however, that these brominated impurities may be removed by vacuum distillation of the antimony pentachloride.

4. Precision and Accuracy

4.1 The volume of air sample can be measured within 1% if a pump with a calibrated volume indicator is used. Volumes calculated from initially set flow rates may be less accurate (± 5%) because of changes in flow rates during sampling.

4.2 The front section of the Florisil tube has a 100% collection efficiency for 50-l air samples at airborne concentrations up to 10 mg/m³.

4.3 No loss of PCB was observed in sealed tubes or desorbed samples stored for two months.

4.4 The precision of the analysis is dependent upon the precision and sensitivity of the technique used to quantitate the gas chromatograph peaks of samples and standards. Known quantities of PCB which were spiked on Florisil and allowed to sit overnight were quantitatively (100%) recovered. The precision of 27 spiked samples was reflected by a relative standard deviation of 4.4%.

4.5 Known quantities of PCB which were spiked on Florisil and allowed to sit overnight were desorbed in hexane and subjected to the perchlorination procedure. The average conversion of PCB to decachlorobiphenyl among 14 samples was 99.9% with a relative standard deviation of 2.1%. The recovery of decachlorobiphenyl spiked and treated in the same manner as the PCB was 99.7% with a relative standard deviation of 2.8%.

4.6 The accuracy of this method is not known. Accuracy depends upon the ability to separate and identify each compound in the PCB mixture and compare each with a known standard. Many of the isomers in commercial PCB mixtures have not been separated or identified, nor are standards of the compounds available. Therefore, the task of assessing accuracy is very difficult.

5. Advantages and Disadvantages of the Method

5.1 The sampling device is small, portable, and involves no liquids. Interferences are minimal, and most of those which do occur can be eliminated by altering chromatographic conditions. The tubes are analyzed by means of a quick instrumental method.

5.2 The capacity of the solid sorbents sampling device for PCB is large and recovery from the sorbent is quantitative.

5.3 A substantial difference in air sample composition and the commercial PCB mixture being used will result in a large error when the standard analytical procedure is used, since the standardization is based on a comparison with the PCB mixture being used. The perchlorination analytical procedure provides for qualitative confirmation of PCB as well as a quantitative measure of PCB since it is based upon measurement of the single GC-ECD response for decachlorobiphenyl.

6. **Apparatus**

6.1 A personal-sampling pump which operates in the range of 50-200 cc/min is used. These pumps should be calibrated with a representative sorbent tube in the sampling line. A wet or dry test meter or a glass rotameter capable of measuring a flow rate of 200 cc/min within 5% may be used in the calibration.

6.2 Sorbent Tubes. Each glass tube is at least 7 cm long with 4-mm inside diameter and contains two sections of 30-48 mesh deactivated Florisil. The front section is preceded by glass wool and contains 100 mg and the backup section contains 50 mg. Urethane foam is placed between the sections and after the backup section. The ends of the tube are flame sealed to prevent contamination before use.

6.3 Gas chromatograph equipped with an electron capture detector (^{63}Ni foil).

6.4 Glass column (6 ft × 2 mm I.D.) packed with 1.5% OV-17/1.95% QF-1 on 80/100 mesh Supelcoport.

6.5 Vials, 20-ml, with aluminum-lined caps.

6.6 Microliter syringe, 10-μl.

6.7 Volumetric flasks, 10-ml, with glass stoppers.

6.8 A mechanical or electronic integrator or a recorder and some method for determining peak area.

6.9 Culture tubes, 13 × 100 mm, with unlined screw top caps.

6.10 Oven, capable of maintaining 160°C ± 5°.

6.11 Sand bath.

6.12 Vortex mixer.

7. **Reagents**

ACS reagent grade or better.

7.1 Hexane, Pesticide Quality.

7.2 Florisil® (registered trademark of the Floridin Co.), 30-48 mesh chromatographic adsorbent, deactivated. Florisil 30-60 mesh is sieved to the proper mesh size. Prior to packing tubes, dry a weighed amount of Florisil at 105°C for 45 minutes. After cooling to room temperature, the Florisil is added to a round bottom flask which can be attached to a rotary evaporator. Three milliliters of water per 100 gms of Florisil (i.e., 3% w/w water) are added and the mixture is turned in the rotary evaporator for one hour or until it is free flowing.

7.3 Nitrogen, prepurified.

7.4 PCB mixture under study. For example this method has been field tested only for Aroclor 1016, a PCB mixture whose chlorine content is approximately 42%. Aroclor is a registered trademark of the Monsanto Company.

7.5 Antimony Pentachloride, distilled under vacuum. (Distillation of commercial products is necessary to remove small amounts of mixed bromo-chloro antimony compounds. These compounds will produce brominated-chlorinated biphenyl derivatives which make accurate quantitation difficult, if not impossible.)

7.6 Decachlorobiphenyl.

7.7 Sodium sulfate, anhydrous.

7.8 Hydrochloric acid, 20% aqueous.

8. **Procedure**

8.1 **Cleaning of equipment.** All glassware used for the laboratory analysis is washed with detergent and thoroughly rinsed with tap water, distilled water, pesticide-grade acetone, and finally pesticide-grade hexane and dried.

8.2 **Collection and Shipping of Samples**

8.2.1 Immediately before sampling, break each end of the sorbent tube so as to provide an opening at least one-half the internal diameter of the tube (2 mm).

8.2.2 The smaller section of Florisil is used as a back-up and is positioned nearest the sampling pump.

8.2.3 The sorbent tube is vertical during sampling.

8.2.4 Air being sampled is not passed through any hose or tubing before entering the sorbent tube.

8.2.5 The air sample is taken at a flow rate of 200 cc/min or less to attain the total sample volume required. The recommended sample volume is 50 liters.

8.2.6 The sorbent tube is capped with the supplied plastic caps immediately after sampling. Under no circumstances are rubber caps to be used.

8.2.7 One tube is handled in the same manner as the sample tubes (break, seal, and ship) except that no air is pumped through it. Label this tube as a blank.

8.2.8 The tubes are packed tightly before shipping to minimize breakage in transit. The tubes are not subjected to extremes of high temperature or low pressure during shipment.

8.2.9 Samples of the PCB whose presence in the environment is suspected are submitted to the laboratory performing the analyses. These bulk materials are not transported in the same container as the samples or blank tubes. If possible, a bulk air sample is shipped for qualitative identification purposes.

8.3 **Analysis of Samples**

8.3.1 Preparation of Samples. Each tube is scored with a file and the glass wool and front section are placed in a clean, dry vial. The separating urethane foam plug, the back section of the sorbent, and the retaining urethane foam plug are placed in a second clean, dry vial.

8.3.2 Desorption of Samples. Prior to analysis, 5.0 ml of hexane is pipetted into each vial. Florisil particles should not be allowed to cling to the glass above the solvent. A minimum of 10 minutes desorption time is required before analysis.

8.3.3 Preliminary Analysis. An injection of 1 μl hexane backflush and 1 μl of sample is made at the conditions specified under 8.3.4. If the sample is too concentrated, further dilutions will be necessary to bring the concentration of the sample solution into the linear range of the electron capture detector. Once the concentration of the sample is appropriately adjusted, the chromatogram of this sample should be compared to that of a standard to determine if the air sample is qualitatively different in composition from the standard. If there is little difference in the two chromatograms, then proceed via the Standard Analytical procedure (See 8.3.5). If there is a significant difference in the two chromatograms, the Perchlorination Analytical Procedure is followed (See 8.3.6).

8.3.4 Gas Chromatographic conditions for PCB determination by the Standard Analysis Procedure are:

1. Nitrogen carrier gas flow, 60 ml/min
2. Injector temperature, 300°C
3. Interface and detector temperatures both 325°C
4. Column temperature, 180°C

8.3.5 Standard Analytical Procedure. An aliquot of the sample solution is injected under the gas chromatographic conditions in 8.3.4. The solvent flush technique is recommended for injection. The volume of back flush is 1 μl and the volume of standard or sample solution is equal to 4 μl, since larger volumes may give a solvent peak which interferes with sample components. Not less than 1 μl of back flush should be used. The syringe must be cleaned with hexane after each injection. The peak heights or areas of at least five prominent peaks in the sample chromatogram are selected and compared with those of the standard PCB mixture and the weight of PCB present is calculated.

8.3.6 Perchlorination Analytical Procedure. A 200-μl aliquot of the sample is placed in a 13-mm × 100-mm culture tube and the hexane is slowly evaporated with dry nitrogen until 10 μl or less remains. Do not allow hexane to climb the tube during evaporation and do not allow the sample to evaporate to dryness. Immediately add 0.2-0.5 ml of distilled antimony pentachloride with a disposable pipet as rapidly as possible (the antimony pentachloride decomposes rapidly in air) and cap the tube. The sample should remain light yellow after this addition, but if the sample turns dark brown or black, it must be discarded and another aliquot of that sample must be used. All samples are placed together in a sand bath. There is no liner in the cap of the tube, since most liners are attacked by the antimony pentachloride. A sample of decachlorobiphenyl is treated in a manner similar to the samples. The sand bath containing all of the treated samples is placed in a preheated oven at 160°C for at least three hours. Samples may also be treated overnight. After perchlorination, the sand bath is removed from the oven and the samples are removed from the sand and allowed to cool to room temperature. To each sample is added dropwise 0.5 ml of 20% hydrochloric acid. This mixture is then extracted four times with 1-2 ml of hexane each time. Each extract is passed through a funnel containing approximately 0.5 gram of anhydrous sodium sulfate (retained by a glass wool plug) into a 10-ml volumetric flask. The volume of the sample is brought to 10 ml by rinsing the pipet, sodium sulfate and funnel tip with hexane. The funnel should not rest on the lip of the flask during filtration. The glass wool plug and sodium sulfate in the funnel are discarded after each sample. The sample is now ready for analysis; proceed to 8.3.7.

8.3.7 Decachlorobiphenyl Analysis. An aliquot of the sample solution is injected under the gas-chromatographic conditions in 8.3.8. The solvent flush technique is recommended for injection. The volume of back flush is 2 μl and the volume of standard or sample solution is equal to 6 μl. The syringe must be cleaned with hexane after each injection. The peak heights or areas of the decachlorobiphenyl peaks are compared to that of the decachlorobiphenyl standard and weight of decachlorobiphenyl present is calculated.

8.3.8 Gas-chromatographic conditions for Decachlorobiphenyl Determination.

1. Nitrogen carrier gas flow, 90 ml/min.
2. Injector temperature, 300°C.
3. Interface and detector temperatures, 325°C.
4. Column temperature, 220°C.

8.4 Determination of Desorption Efficiency. In various experiments, 5.46 μg and 3.42 μg (3 sets) of PCB (Aroclor 1016) in hexane were spiked on 100-mg sections of Florisil and allowed

433

to sit overnight. Desorption with 5 ml of hexane the next day gave recoveries of 95.73%, 100.78%, 98.39%, and 102.12% (overall average, 99.3%). Thus, results of sample analysis need not be corrected by desorption efficiency.

9. **Calibration and Standards**

9.1 **Standard Analytical Procedure.** The particular PCB mixture being used in the work area in which air samples were taken is used as the standard. Standards are prepared in hexane at concentrations ranging from 0.008 to 0.5 ng/μl (within the linear range of the electron capture detector — upper limit is approximately 3 ng per injection). Calibration curves are established daily since the electron capture detector response may vary from day to day. The standard curve is plotted in terms of concentration (ng/ml) versus area. Since the injection volumes of the standard and the sample are identical, the concentration of the sample can be read directly from this curve.

9.2 **Perchlorination Procedure.** The product of the perchlorination of the PCB mixture with antimony pentachloride is decachlorobiphenyl. Standards of decachlorobiphenyl are prepared in hexane at concentrations ranging from 70 to 440 ng/μl. Calibration curves are established daily since the electron capture detector response may vary from day to day. The standard is plotted in terms of concentration (ng/ml) versus area. Since the injection volume of the standard and the sample are identical, the concentration of the sample can be read directly from this curve.

10. **Calculations**

10.1 **Standard Analytical Procedure.** The following are the steps in the calculation of air concentrations of PCB determined with PCB mixture as the standard.

10.1.1 The heights or areas of several selected prominent peaks (at least 5) in the chromatogram are added, compared to the total heights or areas of those same peaks in the standard, and the concentration of the sample solution (ng/ml) is read from the standard curve.

10.1.2 The concentration (ng/ml) of the sample solution is multiplied by the total volume (ml) of the sample solution and the weight of PCB in the sample is calculated. Corrections for the blank are made, if necessary.

10.1.3 The weights found on the front and the back sections of the tube are summed to find the total weight of PCB in the air sample.

10.1.4 The total weight is divided by the volume of air sampled (l) and the air concentration of PCB is reported in ng/l or the equivalent, $\mu g/m^3$.

10.2 **Perchlorination Procedure.** The following are the steps in the calculation of air concentration of PCB determined with decachlorobiphenyl as the standard.

10.2.1 The height or area of the decachlorobiphenyl peak in the chromatogram of the sample is compared to the height or area of the decachlorobiphenyl in the standard, and the concentration of decachlorobiphenyl in the sample solution is read from the standard curve.

10.2.2 The concentration of this solution (ng/ml) is multiplied by the total volume (ml) of the sample solution and the weight of decachlorobiphenyl, w_d, is calculated.

10.2.3 The weight of a specific PCB, w_s, equivalent to the weight of decachlorobiphenyl, w_d, is calculated as shown below using the appropriate value, Q, found in Table 1:

$$w_s = w_d \times Q$$

Table 1. Factors to Convert Decachlorobiphenyl to an Equivalent Amount of Aroclor (Taken from Ref. 11.2)

Aroclor	Average Molecular Weight	Q*
1221	188.5	0.38
1232	223	0.45
1242	257.5	0.52
1016	257.5	0.52
1248	292	0.59
1254	326.4	0.65

*Calculated by dividing the average molecular weight by the molecular weight of decachlorobiphenyl, 499.

10.2.4 The weight of PCB, w_t, in the original solution (from which the 200-μl aliquot was taken) is calculated.

$$w_t = w_s \times \frac{V}{a}$$

where:

V = volume of original solution in ml

a = volume of aliquot from the original solution in ml.

10.2.5 The weights of PCB on the front and back sections of the tube are summed, corrections for blanks are made, and the total weight of PCB in the air sample is calculated.

10.2.6 The total weight of PCB is divided by the volume of air sampled (l) and the air concentration is reported in ng/l or the equivalent units, $\mu g/m^3$.

11. References

11.1 Gian, C. S., H. S. Chan, and C. S. Neff, Anal Chem 47:2319, 1975.

11.2 Armour, J. A., J Assoc Off Anal Chem 56:987, 1973.

11.3 Hill, R. H., Jr., J. A. Arnold, and W. J. Woodfin, "Determination of Polychlorinated Biphenyls in Workplace Air", in preparation.

THIOPHENE IN AIR

Measurements Research Branch

Analytical Method

Analyte:	Thiophene	**Method No.:**	P&CAM 255
Matrix:	Air	**Range:**	0.05 to > 7 mg/m³
Procedure:	Adsorption on charcoal, desorption with toluene, GC analysis	**Precision (CV):**	0.032 at 5 - 500 μg (Analytical)
Date Issued:	3/25/77	**Classification:**	E (Proposed)
Date Revised:			

1. **Principle of the Method**
   - 1.1 A known volume of air is drawn through a charcoal tube to trap the thiophene vapor present.
   - 1.2 The charcoal in the tube is transferred to a small air-tight sample container, and the analyte is desorbed with toluene.
   - 1.3 An aliquot of the desorbed sample is analyzed by gas chromatography using a sulfur flame photometric detector.
2. **Range and Sensitivity**
   - 2.1 The range of the sulfur detector for the quantitation of thiophene is dependent upon the type of detector and chromatograph used. For a flame photometric detector in a Tracor MT 220 Gas Chromatograph, the useful range for quantitation was 16 to 64 ng of thiophene per aliquot injected. This range corresponds to 3.2 to 12.8 μg of thiophene per sample solution obtained in the recovery from the charcoal.
   - 2.2 For a 70-liter air sample, this method is applicable to air concentrations ranging from 0.05 mg/m³ to levels above 7 mg/m³. The upper limit depends upon the capacity of the charcoal tube and dilution of the sample solution.

     At a detection limit of 4 ng per injection (0.8 μg per sample solution), it is estimated that thiophene at a concentration of 0.01 mg/m³ could be detected in a 70-liter air sample.
3. **Interferences**
   - 3.1 When the amount of water in the air is so great that condensation actually occurs in the charcoal tube, thiophene vapors may not be trapped efficiently.
   - 3.2 Any compound which has the same retention time compared to that of thiophene at the operating conditions described in this method may interfere with the analysis. Although the sulfur flame photometric detector has a high specificity for sulfur compounds, it will respond to other types of compounds, particularly those in high concentrations.
   - 3.3 When interfering compounds are known or suspected to be present in the air, such information, including their suspected identities, should be transmitted with the sample.
4. **Precision and Accuracy**
   - 4.1 The coefficient of variation (CV) for the analytical method in the range 5 to 500 μg/charcoal tube sample is 0.032.
   - 4.2 The bias in the sampling and analytical method has not been determined.

4.3 The precision of the method is limited by the reproducibility of the pressure drop across the tubes. This drop will affect the flow rate and cause the volume to be imprecise because the pump is usually calibrated for one tube only.

5. **Advantages and Disadvantages of the Method**

5.1 The sampling device is small, portable, and involves no liquids.

5.2 A disadvantage of the method is that the capacity of the charcoal tube with respect to thiophene is limited. When the sample loading in the backup section of the tube exceeds 10% of that found in the front section, the possibility of sample loss exists.

5.3 Since the response of the sulfur flame photometric detector is proportional to the square of the quantity of thiophene, the quantitation range of thiophene is quite limited within one full scale deflection of the strip chart recorder. If the peaks are measured without the aid of an electronic integrator, solutions of thiophene in concentrations above this range must be diluted.

6. **Apparatus**

6.1 Personal sampling pump capable of maintaining a flow of air through the charcoal tube at a rate of 1 liter per min with an accuracy of ± 5%.

6.2 Charcoal tubes: glass tube with both ends flame sealed, 7 cm long with a 6-mm O.D. and a 4-mm I.D., containing 2 sections of 20/40 mesh activated charcoal separated by a 2-mm portion of urethane foam. The activated charcoal is prepared from coconut shells and is fired at 600°C prior to packing. The adsorbing section contains 100 mg of charcoal, the backup section 50 mg. A 3-mm portion of urethane foam is placed between the outlet end of the tube and the backup section. A plug of silylated glass wool is placed in front of the adsorbing section. The pressure drop must be less than one inch of mercury at a flow rate of 1 liter per min.

6.3 Gas chromatograph equipped with a flame photometric detector specific for sulfur.

6.4 Column (1.8 m × 4 mm I.D.) constructed from borosilicate glass and packed with 3% OV-210 on 80/100 Gas Chrom Q. Other columns capable of performing the required separations also may be used.

6.5 Syringes, 10-μl.

6.6 Pipets, 1-ml.

6.7 Volumetric flasks, 10-ml and other convenient sizes for preparing standard solutions.

6.8 Glass vials, 2-ml.

6.9 Parafilm.

7. **Reagents**

7.1 Toluene, chromatographic quality.

7.2 Thiophene of known purity.

8. **Procedure**

8.1 **Cleaning of equipment.** All glassware used for the laboratory analysis should be detergent washed and thoroughly rinsed with tap water and distilled water.

8.2 **Calibration of Personal Sampling Pumps.** Each personal pump must be calibrated with a representative charcoal tube in the line. This will minimize errors associated with uncertainties in the sample volume taken.

8.3 **Collection and Shipping of Samples**

8.3.1 Immediately before sampling, the ends of the tube should be broken to provide an opening at least one-half the internal diameter of the tube (2 mm).

8.3.2 The smaller section of charcoal is used as a backup and should be positioned nearest the sampling pump.

8.3.3 The charcoal tube should be vertical during sampling to minimize channeling through the charcoal.

8.3.4 Air being sampled should not be passed through any hose or tubing before entering the charcoal tube.

8.3.5 Care should be taken to measure the flow rate, time and volume as accurately as possible. The sample should be taken at a flow rate of 1.0 liter per minute or less and should have a volume of no more than 70 liters.

8.3.6 The temperature, pressure, and relative humidity of the atmosphere being sampled should be recorded.

8.3.7 The charcoal tubes should be capped with the supplied plastic caps immediately after sampling. Under no circumstances should rubber caps be used.

8.3.8 One tube should be handled in the same manner as the sample tube (break, seal, and transport), except that no air is sampled through this tube. This tube should be labeled as a blank.

8.3.9 Capped tubes should be packed tightly before they are shipped to minimize tube breakage during shipping.

8.3.10 A sample of the bulk material should be submitted to the laboratory in a glass container with a Teflon-lined cap. This sample should not be transported in the same container as the charcoal tubes.

8.4 **Analysis of Samples**

8.4.1 Preparation of Samples. In preparation for analysis, each charcoal tube is scored with a file in front of the first section of charcoal and broken open. The glass wool is removed and discarded. The charcoal in the first (larger) section is transferred to a 2-ml glass vial. The separating section of foam is removed and discarded; the second section is transferred to another vial. These two sections are analyzed separately.

8.4.2 Desorption of Samples. Prior to analysis, 1 ml of toluene is pipetted into each vial containing the charcoal. Each vial is sealed. At least 1 hour should be allowed for desorption to be complete. During this time, each sample is agitated occasionally.

8.4.3 GC Conditions. The typical operating conditions for the gas chromatograph are:

1. 40 ml/min nitrogen carrier gas flow
2. 150 ml/min hydrogen gas flow to the detector
3. 50 ml/min oxygen gas flow to the detector
4. 175°C inlet temperature
5. 190°C detector temperature
6. 50°C column temperature

8.4.4 By means of the solvent-flush injection technique, inject 5-μl aliquots of the sample solutions into the gas chromatograph. If a sample gives a peak which is larger than the largest peak, dilute an aliquot of the sample to bring the sample onto the calibration curve.

8.5 **Determination of Desorption Efficiency**

8.5.1 Activated charcoal equivalent to the amount in the front (larger) section of the sampling tube (100 mg) is poured into a 2-ml glass vial. This charcoal must be from the same batch as that used in obtaining the samples. A known amount of either neat thiophene or thiophene in toluene solution is applied directly onto the charcoal with a microliter syringe, and the vial is sealed with Parafilm.

8.5.2 Six samples are prepared in the above manner and allowed to stand overnight to assure complete adsorption of the thiophene onto the charcoal. A parallel blank vial should

be treated in the same manner except that no sample is added to it. The sample and blank vials are treated with toluene according to Section 8.4.2 to allow desorption to take place.

8.5.3 Six standards are prepared by adding the same quantity of thiophene used in preparing the samples to 1-ml portions of toluene.

8.5.4 The samples and standards are analyzed in pairs according to Section 8.4.

8.5.5 The blank is analyzed according to Section 8.4.

8.5.6 The desorption efficiency, DE, of each sample equals the difference between the square root of each sample peak height and the square root of the blank peak height divided by the square root of the corresponding standard peak height or

$$DE = \frac{\sqrt{\overline{H}} - \sqrt{\overline{H_b}}}{\sqrt{\overline{H_s}}}$$

where:

H = the peak height of the sample

H_b = the peak height of the blank

H_s = the peak height of the standard

The average DE is calculated from the six DE values obtained.

8.5.7 Since the desorption efficiency can vary with the quantity of thiophene adsorbed, the DE should be determined at different levels of thiophene. The values of DE obtained can be used to construct a plot of desorption efficiency versus quantity of thiophene measured.

9. Calibration and Standards

9.1 A series of standard solutions of thiophene in toluene should be prepared. The range of concentrations of interest depends upon the useful range of the detector and may be in the vicinity of 3 to 13 ng/μl. A plot of the square root of the peak height versus the quantity of thiophene injected should produce a straight line intercepting the origin.

9.2 To compensate for drift in detector response, a standard of concentration falling in the middle of the calibration curve is selected. This standard is analyzed alternately with the samples. The average detector response for the two standard peaks bracketing a sample thiophene peak, rather than the calibration curve, is used to calculate the quantity of thiophene in the sample.

10. Calculations

10.1 Determine the quantity of thiophene in the aliquot analyzed in the gas chromatograph.

10.1.1 Average the heights of the two standard peaks which are bracketing the sample peak.

10.1.2 Determine the quantity of thiophene, N (in nanograms), in the sample injected:

$$N = N_s \sqrt{\frac{H}{\overline{H_s}}}$$

where:

N_s = the quantity of thiophene in the standard.

H = the peak height of the sample.

$\overline{H_s}$ = the average peak height of the standard.

10.2 Determine the total quantity of thiophene, Q (in micrograms), which has been desorbed from the two sections of charcoal:

$$Q = \left(\frac{N \cdot S \cdot F}{V}\right)_A + \left(\frac{N \cdot S \cdot F}{V}\right)_B$$

840

where:

S = the volume of the solution of thiophene in toluene before any dilution (expressed in milliliters and is usually 1 ml).

F = the factor by which the thiophene solution is diluted; e.g., a 1-ml sample diluted to 10 ml reflects a dilution factor of 10. If the solution is not diluted, F = 1.

V = the volume of thiophene solution which is injected into the chromatograph for analysis (expressed in microliters and is usually 5 μl).

A = the thiophene in the front section of the charcoal tube.

B = the thiophene in the back section of the charcoal tube.

10.3 If thiophene was found in the blank, make the appropriate correction.

10.4 Determine the total quantity of thiophene, Q_T, in the air sample by dividing the quantity Q (Section 10.2) by the desorption efficiency.

10.5 Determine the concentration, C, of thiophene in air:

$$C = \frac{Q_T}{V}$$

where:

C = the concentration of thiophene in mg/m^3.

Q_T = the total quantity of thiophene in the air sample as determined in Section 10.4.

V = the volume of air in liters.

11. **References**

11.1 White, L. D., D. G. Taylor, P. A. Mauer, and R. E. Kupel, "A Convenient Optimized Method for the Analysis of Selected Solvent Vapors in the Industrial Atmosphere", Am Ind Hyg Assoc J 31:225, 1970.

11.2 Brody, S. S. and J. E. Chaney, "Flame Photometric Detector. Application of a Specific Detector for Phosphorus and for Sulfur Compounds Sensitive to Subnanogram Quantities", J Gas Chromatogr 4(2):42, 1966.

11.3 Bowman, M. C. and M. Beroza, "Gas Chromatographic Detector for Simultaneous Sensing of Phosphorus- and Sulfur-Containing Compounds by Flame Photometry", Anal Chem 40 (10):1448, 1968.

441

AZELAIC ACID IN AIR

Measurements Support Branch

Analytical Method

Analyte:	Azelaic Acid (Nonanedioic Acid)	**Method No.:**	P&CAM 256
Matrix:	Air	**Range:**	1-10 mg/filter sample
Procedure:	Filter collection, ethanol extraction, derivatization, gas chromatography	**Precision:**	2% RSD (Analytical)
Date Issued:	2/17/77		
Date Revised:		**Classification:**	E (Proposed)

1. **Principle of the Method**

   Airborne azelaic acid (nonanedioic acid) samples are collected on polyvinyl chloride (PVC) filters. The organic acid is extracted from the filter with ethanol. The ethanol extract is evaporated to dryness and the residue is dissolved in pyridine. Addition of Bis(trimethylsilyl)trifluoroacetamide/ trimethylchlorosilane (BSTFA/TMCS) derivatizes the azelaic acid to form the trimethylsilyl ester. An aliquot of the mixture is injected into a gas chromatograph equipped with a flame ionization detector (FID) and the area of the resulting peak is determined and compared with areas obtained from the injection of standards.

2. **Range and Sensitivity**

   2.1 The range and sensitivity depend on the volume of air sampled, the amount of particulate matter present and the fraction of organic material in the filter. The range that the method was tested in is 1 mg-10 mg per sample. Preliminary tests indicate that the range can be lowered to 0.01 mg per sample but additional study is needed.

   2.2 The minimum detectable amount of azelaic acid was found to be 0.5 nanogram per one microliter injection on the P&E model 900 gas chromatograph at 1 × 10 attenuation. This corresponds to a concentration of 0.001 mg per filter sample.

3. **Interferences**

   3.1 Since water decomposes both TMS reagents and the corresponding derivatives, excessive moisture should be avoided. Laboratory work should *not* be carried out under highly humid conditions and sample to be investigated should be reasonably dry.

   3.2 Any compound that has approximately the same gas chromatographic retention time as that of azelaic acid will interfere. Such interference often can be overcome by changing the operating parameters of the gas chromatograph or by selecting a different column.

4. **Precision and Accuracy**

   4.1 The analytical precision has been determined from six gravimetric standards of 1, 2, 4, 6, 8, and 10 milligrams per sample. The Analytical Relative Standard Deviation (RSD) is 2%. The total RSD for the method has not been calculated since synthetic atmosphere samples were not generated.

   4.2 The average percent recovery is 94.9% calculated from five spiked samples of 2, 4, 6, 8, and 10 milligrams.

043

5. Advantages and Disadvantages of the Method

5.1 The analytical method is simple and rapid. Formation of the trimethyl silyl ester in comparison with other derivatization techniques requires no solvent extraction, filtration or transfer of the reaction mixture to other vessels. Derivatization is accomplished in one step.

5.2 Preliminary evidence indicates that the method is applicable to other than C_9 straight-chain aliphatic dicarboxylic acids.

5.3 The FID electrode tends to accumulate white flakes from the combustion of the injected samples. Thus, it is recommended that the electrode be cleaned daily.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Vinyl Metricel (VM-1) filters, 5 μm in pore size, 37 mm in diameter (Gelman Corp.) or any other equivalent filter made of polyvinyl chloride.

Plastic three-piece filter holders (cassettes) for 37-mm filters (Millipore Corp.) or equivalent.

6.1.2 A personal air sampling pump, capable of operating for 4 hours at 2.0 liters/min.

6.2 Gas Chromatograph

6.2.1 A gas chromatograph equipped with a flame ionization detector and a linear temperature programmer (latter not essential).

6.2.2 A G.C. column consisting of a 10 foot long (3 meters) silanized glass column ¼ in O.D. and 0.08 in (2 mm) I.D. packed with 6% SP 2100 on 60/80 mesh supelcoport or any equivalent stationary phase of 6% methyl silicone on chromosorb W acid washed and DMCS treated. It is recommended that silanized glass wool plugs be placed on each end of the column and it should be conditioned at 250°C for several hours before use.

6.2.3 A strip chart recorder with 1 mv full scale sensitivity and a digital integrator for measuring peak areas.

6.2.4 A molecular sieve column attached in series with the carrier gas line for removing carrier gas moisture.

6.2.5 An analytical balance capable of weighing to the nearest 0.01 mg.

6.3 Miscellaneous Apparatus

6.3.1 A vacuum oven or any other means for evaporating the solvent and drying the samples.

6.3.2 10 μl syringe.

6.3.3 A syringe cleaner (heats the needle while a vacuum is pulled on the syringe) is highly recommended.

6.3.4 Hot plate.

6.3.5 Large diameter beaker.

6.3.6 Teflon coated screw-cap glass vials (20 ml capacity).

6.3.7 Eppendorf pipette (1 ml) with disposable tips or equivalent.

6.3.8 Tweezers, a microspatula weighing paper, and a wood or plastic applicator stick.

7. Reagents

7.1 Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane(TMCS), obtainable from any chromatography products supplier.

7.2 Pyridine, spectroquality grade.

7.3 Ethanol, spectroquality grade.

7.4 Azelaic acid, highest purity available.

7.5 Purified helium.

7.6 Purified hydrogen.

7.7 Compressed air, "zero" grade.

8. **Procedure**

8.1 **Cleaning of Equipment**

8.1.1 Glass vials should be cleaned with detergent, rinsed well with tap water and distilled water, and then dried in an oven.

8.1.2 The syringe should be cleaned between samples by rinsing with pyridine in the syringe cleaner.

8.2 **Calibration of sampling pump.** The personal sampling pump should be calibrated with a representative filter assembly in the line. A wet or dry test meter or a glass rotameter capable of measuring the flow rate to within ± 5% may be used for the calibration.

8.3 **Collection and Shipping of Samples**

8.3.1 Assemble the filters in the filter holder so that the air being sampled passes first through the PVC filter and then through the filter support. Remove the small plugs from the filter holder and connect the filter holder to the sampling pump by means of an adapter and a length of tubing.

8.3.2 Sample at least 100 liters of air. The optimum sample volume will depend on the type of workroom environment being sampled. Since high concentrations of particulate material may plug the filter, the flow rate should be checked at least once every hour. On completion of sampling, reinsert the small plugs into the inlet and outlet of the filter holder.

8.3.3 With each group of samples prepare two blanks consisting of a filter holder with a representative filter that has been handled in the same manner as the sample filters, except that no air is drawn through them. The samples and the blanks should be shipped promptly in a damage-proof container that allows no filter holder movement. Samples received at the laboratory are logged in and immediately stored in a refrigerator until time for analysis.

8.4 **Analysis of Samples**

8.4.1 Extraction

1. Remove the top portion of the filter holder. Hold the bottom portion containing the filter and filter support, over a piece of weighing paper to catch any particulate material that may fall out. Remove the small plug from the bottom portion of the filter holder and insert the applicator stick through the hole. Gently raise the filter support and the filter and grasp the unexposed edge with tweezers. Very carefully pick up the filter, insert it in a glass vial and push it gently to the bottom with the applicator stick. Add to the vial any particulate material remaining in the filter holder or collected on the weighing paper.
2. Pipette 5.0 ml of ethanol into the vial. This amount should completely cover the filter. Screw the cap on the vial.
3. Place vials into a 70°C water bath (thermostatically controlled) for 20 minutes. The water bath level must be above the ethanol level. Shake each vial every 5 minutes. The use of ultrasonic bath is not recommended because it breaks up the PVC filter.
4. Lift the filter with tweezers so it is above the ethanol level in the vial and wash filter with ten 1-ml portions of ethanol.
5. Place vials in the vacuum oven at 45-50°C and evaporate to dryness.

8.4.2 Derivatization

Steps 1-3 should be carried out in the hood.

1. Add 1 ml of pyridine to the vial to dissolve the residue.
2. Add 1 ml of BSTFA mixture to the vial and shake vial for one minute. For samples larger than 10 mg, add an additional 0.1 ml BSTFA reagent per milligram of sample. An excess of reagent will not interfere. In general, the BSTFA reagent should be in excess.
3. Put the caps on the vials and place vials in a water bath maintained at 70°C for 20 minutes. Stir the contents of the vial every 5 minutes.

8.4.3 Gas Chromatographic Separation

The operating conditions for gas chromatographic analysis are as follows:

1. Helium carrier gas flow rate, 39 ml/min
2. Hydrogen flow rate, 37 ml/min (20 psi)
3. Air flow rate, 500 ml/min (30 psi)
4. G.C. oven (column) temperature, 190°-250°C programmed at 6°/min.
5. Detector (manifold) temperature, 250°C
6. Injection port temperature, 250°C
7. If no linear temperature programmer is available, the analysis can be performed isothermally at 200°C

8.4.4 Injection

1. Wash the 10-μl syringe with pyridine in the syringe cleaner. Draw up some sample and flush it out; repeat several times, in the hood.
2. Draw up a 2-μl aliquot of sample. Push plunger down until it reads 1 μl. Then pull the plunger back about 0.2 μl to minimize evaporation of the sample from the tip of the needle. Wipe the needle with a tissue paper.
3. Triplicate injections of each sample and standard should be made. The area under the sample peak is measured by an electronic integrator or any other method of peak area measurement. The azelaic acid peak is sufficiently sharp to permit quantitative determination by measurement of peak heights.

8.5 Determination of Percent Recovery

8.5.1 The efficiency of recovery of azelaic acid may vary from laboratory to laboratory. The percent recovery of the analyte in the range of 2 to 10 milligrams varied from 91% to 101.6% with an average recovery of 94.9%.

8.5.2 Procedure for determining percent recovery. Weigh six samples of 1, 2, 4, 6, 8, and 10 milligrams using the analytical balance. Samples must be of the same material used to prepare the standards. Place each sample in a glass vial with a PVC filter. Follow the procedure described in 8.4.1.2 - 8.4.4.3 to extract, derivatize, and analyze each sample. Use the average area of the peaks, resulting from the injections of each sample in the G.C., to convert to milligrams of azelaic acid recovered using the calibration curve obtained from the standards (see Section 9 - 10.2). The percent recovery is calculated by the following formula.

$$\text{Percent recovery} = \frac{\text{weight of azelaic acid recovered}}{\text{weight of azelaic acid in sample}} \times 100$$

See Section 10.1 to calculate the amount of azelaic acid in a sample.

9. Calibration and Standards

9.1 A series of standards, varying in concentration over the range of interest, are prepared

gravimetrically using the analytical balance. Weigh the exact amount of azelaic acid needed and place it in a marked vial. Treat the standards as described in Section 8.4.2.

9.2 Since standards and samples are derivatized with the same procedure, concentrations are expressed in terms of mg/2.0 ml.

9.3 Injections of 1 μl aliquots into the gas chromatograph should be carried out as described in Section 8.4.4.

9.4 Standard solutions must be analyzed under the same gas chromatographic conditions and at the same time that the unknown samples are analyzed. This will minimize the effect of day-to-day variations of the response of the flame ionization detector.

9.5 The calibration curve is established by plotting concentration in corrected mg/2.0 ml (see Section 10.2) versus the average peak area of the standard solutions.

10. **Calculations**

10.1 Since azelaic acid is produced in different degrees of purity, a purity factor must be included in the calculations.

$$W_c = W \left(\frac{P}{100}\right)$$

where:

W_c = corrected weight, mg

W = mg weight of azelaic acid weighed in the analytical balance

P = purity of azelaic acid, percent

10.2 Construct a calibration curve by plotting the corrected weights W_c versus their respective peak areas.

Convert the average peak area of each sample to mg azelaic acid using the standard curve. No volume corrections are needed because the standard curve is based on mg/2.0 ml and the volume of sample injected is identical to the volume of the standards injected.

10.3 The concentration of azelaic acid in air may be expressed in mg/m^3:

$$mg/m^3 = \frac{\text{weight of azelaic acid (in mg)}}{\text{volume of air sampled (in l)}} \times 1000$$

11. **References**

11.1 Snell, F. D. and Ettree, L. S., Encyclopedia of Industrial Chemical Analysis, vol. 8, pp. 368-409, Interscience Publishers, New York, 1969.

11.2 Handbook of Silylation, Pierce Chemical Company, 1972.

11.3 "Preparation of Silyl Ether Derivatives", Technical Bulletin No. 11B, Applied Science Laboratories, Inc.

447

PHOSPHORUS (WHITE) VAPOR IN AIR

Measurements Research Branch

Analytical Method

Analyte:	Phosphorus (white)	**Method No.:**	P&CAM 257
Matrix:	Air	**Range:**	0.01 to 1.00 mg/m³ in a 20-liter sample of air
Procedure:	Sorption on Tenax-GC, elution with xylene, GC determination	**Precision** (CV_T):	0.06 at 0.1 mg/m³
Date Issued:	2/26/77	**Classification:**	E (Proposed)
Date Revised:			

1. **Principle of the Method**

   A known volume of air is sampled through a glass tube containing Tenax-GC. White phosphorus vapor is sorbed from the air by the porous polymer. The sorbed phosphorus is extracted from the porous polymer with xylene. The resulting solution is analyzed by gas chromatography with flame photometric detection. To determine the phosphorus concentration, the GC response resulting from the analysis of the sample extract is compared to the responses of standard solutions of phosphorus in xylene.

2. **Range and Sensitivity**

   2.1 The detection limit of the GC analytical method is about 50 pg in a 5-μl aliquot of the sample solution, or 0.1 μg of phosphorus in a 10-ml xylene extract of the sorbent. Thus, in a 20-liter sample of air, the detection limit is 0.005 mg/m³. The detection limit can be lowered by extracting with a smaller volume of xylene. It has been found that 16 ng of phosphorus can be sorbed from air, extracted with 2 ml of xylene, and determined with fair precision. (The coefficient of variation of five measurements was 0.12.) In a 20-liter sample of air, 16 ng would correspond to a concentration of about 0.001 mg/m³. The practical limit of the range, for good accuracy and precision, is about 0.01 mg/m³ in a 20-liter sample of air, or ten times the detection limit.

   2.2 The upper limit of the range is determined by the capacity of a sorbent tube for white phosphorus vapor. The capacity is affected significantly by the temperature and relative humidity of the test atmosphere. At a temperature of 27°C and a relative humidity of less than 10%, about 20 μg can be collected in the sorbing section of a tube with less than 5% of the total phosphorus collected having passed into the backup layer. This establishes an upper concentration limit of about 1.0 mg/m³ in a 20-liter sample of air. At a temperature of 27°C and a relative humidity of greater than 90%, the capacity of the sorbing layer is about 8 μg and the upper limit is about 0.4 mg/m³ in a 20-liter sample. The capacity is about 2 to 3 μg at a temperature of 50°C and a relative humidity of less than 10%; the corresponding upper concentration limit is 0.10 to 0.15 mg/m³ in a 20-liter sample. The load capacity and, consequently, the upper concentration of the range can be increased by enlarging the sorbent bed.

3. **Interferences**

   3.1 The following gases at the indicated concentrations in air do not interfere significantly with

849

the determination of phosphorus vapor concentrations near 0.1 mg/m³.

Gas	Concentration, ppm
H_2S	15
SO_2	15
CO_2	9000
CO	50
PH_3	0.4

3.2 Concentrations of phosphorus pentoxide in air near 0.1 mg/m³ do not interfere with the determination of phosphorus vapor concentrations near 0.1 to 0.2 mg/m³. Higher concentrations have not been tested.

3.3 Red phosphorus is insoluble in xylene and does not interfere with the GC analysis of solutions of white phosphorus in xylene.

3.4 Water reduces the load capacity of the sorbent tube for phosphorus. (See Section 2.2)

Precision and Accuracy

4.1 The combined effect of sampling, sample preparation, and GC analysis upon the accuracy and precision of determinations has been evaluated. The error and coefficient of variation at five different phosphorus vapor concentrations are presented in the following table:

Concn. of phosphorus, mg/m³	No. of samples	Error, %	Coefficient of variation
0.010	8	+8	0.11
0.048	6	−2	0.07
0.102	12	−6	0.06
0.164	9	−3	0.05
0.370	6	−6	0.04

Most of these samples were taken from air with a relative humidity of less than 10%. The sampling rate was usually 0.2 l/min and the sample volume usually 20 liters. The sample volumes at 0.010 mg/m³ were 1 to 5 liters. The samples at 0.164 mg/m³ were taken from air with a relative humidity greater than 90%; the sampling rate was 0.4 l/min and the sample volumes ranged from 12 to 48 liters.

4.2 Variations in the sampling rate between 0.13 and 0.54 l/min and in sample volumes between 10 and 50 liters do not significantly affect accuracy and precision.

4.3 There is evidence that sampling volumes larger than 50 liters may increase the error and variability of results somewhat. Accurate and precise results can be obtained with sample volumes of 20 to 25 liters.

4.4 The temperature of the test atmosphere during sampling affects accuracy and precision. It has been found that the exposure of tubes at 50°C can reduce the recovery of phosphorus significantly; however, at sampling rates no greater than 0.2 l/min and with sample volumes of about 20 liters, the average recovery of eight samples was found to be about 94% with a coefficient of variation of about 0.12 at 50°C.

4.5 Phosphorus sorbed on Tenax-GC tubes sealed with plastic caps and stored at room temperature slowly degrades with time. The phosphorus is stabilized by refrigeration of the tubes or by extraction into xylene.

Exposed sealed tubes containing about 0.7 μg of phosphorus have been stored at 27°C for 1 week and then stored for an additional week at 0°C without significantly affecting the

accuracy or precision. The xylene extracts of exposed tubes can be stored for 2 weeks at room temperature in glass vials with aluminum-lined plastic screw caps without significantly increasing the error or variability.

4.6 The temperature at which exposed tubes are stored appears to affect the stability of phosphorus more than do other factors. The storage of tubes, each containing about 4 μg of phosphorus, at 60°C for 24 hr caused an error of about 30%. The coefficient of variation for six samples was 0.11. Other storage factors — such as the storage of sealed, exposed tubes in the light as opposed to storage in the dark, or storage at an ambient pressure of 0.7 atm as opposed to 1 atm — do not significantly affect accuracy or precision.

5. **Advantages and Disadvantages of the Method**

5.1 The solid sorbent sampler is a small portable device that involves no liquids. Desorption of the sample and its preparation for analysis are simple. The analysis is accomplished by a rapid instrumental method.

5.2 Many potential interferents are eliminated by the selectivity of the sampling device.

5.3 No compounds have been encountered that interfere with the GC analysis method. If interferents are found, changes in the GC operating conditions or the use of a different GC column may eliminate them.

5.4 The major disadvantage of the method is the deleterious effect of elevated temperatures upon the collection and storage of samples.

6. **Apparatus**

6.1 Air Sampling Equipment

6.1.1 Sorbent Tubes. Each sorbent tube is a Pyrex glass tube 7 cm long and 6 mm I.D. The sorbent material — Tenax-GC, 35/60 mesh — is divided into two layers, the sorbing section weighing 100 mg and the backup section weighing 50 mg. The layers are held in place and separated by plugs of silanized glass wool. The ends of the tube are flame-sealed to prevent contamination before use for sampling. The pressure drop across a tube is about 2.5 in of water at a sampling rate of 0.2 l/min. Polyethylene caps are used to seal the tubes after sampling has been completed.

6.1.2 Personal Sampling Pump. The personal sampling pump should be capable of operation at a constant flow rate of 200 ml/min for up to 8 hr. A sample volume indicator is desirable. The pump should be calibrated with a representative sorbent tube in the line. A wet or dry test meter or a glass rotameter capable of measuring flow rates in the range of 50 to 200 ml/min to within ± 5% may be used in the calibration.

6.2 Gas chromatograph with a flame photometric detector.

6.3 A 1-m by 4-mm I.D. glass column packed with 3% OV-1 on 100/120-mesh Gas Chrom Q (Applied Science Laboratories, Inc.), or equivalent. The column should be silanized daily with Silyl-8 (Pierce Chemical Co.), or equivalent.

6.4 Glass syringes, 10 μl.

6.5 Glass vials with aluminum-lined plastic screw caps, 20 ml.

6.6 Pipettes, volumetric flasks, and assorted laboratory glassware.

7. **Reagents**

7.1 Yellow phosphorus, purified.

7.2 Xylenes, "Baker Analyzed" Reagent (J. T. Baker Chemical Co.), or equivalent.

7.3 Helium, prepurified.

7.4 Hydrogen.

7.5 Oxygen.

7.6 Air, compressed and filtered.

8. **Procedure**

8.1 **Cleaning of Equipment.** Wash all unused glassware with detergent solution and rinse with tap and distilled water. Dry in an oven. Rinse used glassware with ethanol to remove residual xylene before rewashing.

8.2 **Collection and Shipping of Samples**

8.2.1 Immediately before beginning the collection of a sample, break each end of the sorbent tube so as to provide openings at least half the inside diameter of the tube.

8.2.2 Attach the end next to the backup section to the personal sampling pump with a piece of flexible tubing. Sample air must not pass through any hose or tubing before entering the opposite end of the sorbent tube.

8.2.3 With the sorbent tube in a vertical position and the sorbing section above the backup section, sample the air at 50 to 200 ml/min. A sample volume of 20 liters is recommended. The flow rate and sampling time, or the volume, must be measured as accurately as possible. If the temperature differs significantly from 25°C or the pressure from 1 atm, measure and record them.

8.2.4 Immediately after sampling is completed, cap the sorbent tubes with the polyethylene caps provided.

8.2.5 The exposed sorbent tubes may be handled by one of three different procedures:

1. Pack the tubes tightly to avoid breakage and transport them personally to the laboratory for storage or analysis within 24 hr.
2. Pack the tubes tightly in a refrigerated container (0°C) and ship the container by a rapid method of transportation. Refrigerate after receipt in the laboratory.
3. Desorb the phosphorus from the sorbent material with xylene as described in Sections 8.3.1 and 8.3.2 and ship the extracts in the specified tightly capped vials by air freight. (See Reference 11.1 for shipping regulations.)

Exposed, sealed tubes may be stored for at least 2 weeks at 0°C. After desorption of the phosphorus into xylene, the extracts may be stored for at least 2 weeks at 27°C.

8.2.6 Handle one tube in the same manner as the sample tubes (break, seal, and transport). This tube is the blank.

8.3 **Analysis of Samples**

8.3.1 Preparation of Sample. Place the sorbing section of a tube and the glass wool plugs surrounding it in one 20-ml vial. Place the backup sorbent section and the remaining glass wool plug in a second 20-ml vial.

8.3.2 Desorption. Add 2 to 10 ml of xylene to each vial, cap the vials, swirl the mixtures occasionally during a 5-min period. Tests have indicated that desorption is quantitative; no correction for incomplete desorption is needed. Prior to GC analysis, an aliquot of the sample extract may be diluted further with xylene.

8.3.3 Gas Chromatographic Conditions. Typical operating conditions for the gas chromatograph are as follows:

Helium carrier gas flow rate	60 ml/min
Hydrogen gas flow rate to detector	200 ml/min
Oxygen gas flow rate to detector	20 ml/min
Air flow rate to detector	60 ml/min
Column temperature	80°C
Injection port temperature	160°C
Detector temperature	220°C

8.3.4 Injection of Samples. Flush a 10-μl syringe several times with xylene. Dry the syringe by removing the plunger and drawing air through the barrel. Replace the plunger, immerse the needle into a sample extract, and withdraw a 5-μl aliquot, taking into consideration the volume in the needle. Remove the needle from the solution and pull the plunger back a short distance to minimize evaporation at the tip of the needle. Inject duplicate 5-μl aliquots of each sample and standard.

8.3.5 Measurement of the GC Responses. Measure the heights of the phosphorus peaks and estimate the concentrations of phosphorus in the sample extracts from a calibration curve prepared as described in Section 9.

9. Calibration and Standards

9.1 Prepare a standard stock solution of phosphorus in xylene at a concentration near 20 mg/ml. A convenient procedure is as follows:

9.1.1 Trim a small piece (about 200 mg) of solid white phosphorus from a bulk sample under distilled water.

9.1.2 Wash the piece with acetone, dry it with a paper towel, and transfer it quickly to a tared 10-ml volumetric flask containing several milliliters of xylene. Reweigh the flask.

9.1.3 Fill the flask to its calibration mark with xylene. Stir the contents of the flask until all of the phosphorus is dissolved.

9.2 Prepare standard solutions in the range of 0.01 to 0.50 μg/ml by serial dilutions with xylene. These standards are stable for several months when stored in airtight glass containers in the dark.

9.3 Analyze the standard solutions under the same GC conditions and during the same time period as the sample extracts. This will minimize the effect of day-to-day variations in the response of the flame photometric detector.

9.4 Prepare a calibration curve by plotting the average peak height against the corresponding concentration of a standard solution in micrograms per milliliter.

9.5 For further information concerning the preparation of standards or calibration curves, consult References 11.2, 11.3, 11.4, and 11.5.

10. Calculations

10.1 From the calibration curve, read the concentration of phosphorus in the sample extract corresponding to the average of the measured peak heights. Multiply this concentration value by the volume of the sample extract and the appropriate dilution factor if the sample extract has been diluted. The result is the weight in micrograms of phosphorus in the Tenax-GC layer analyzed. Correct this weight for the amount of phosphorus found in the corresponding blank. Add the corrected amounts of phosphorus found in the sorbing and backup sections of the sorbent tube to obtain the total corrected weight of phosphorus in the air sample.

10.2 The concentration of phosphorus in the air sample may be expressed in mg/m^3:

$$\text{Concentration of phosphorus } (mg/m^3) = \frac{W(\mu g)}{V(l)}$$

where:

W = the total corrected weight of phosphorus in the air sample.

V = the volume of air sampled.

450

10.3 The concentration may also be expressed in terms of parts per million (ppm) by volume:

$$ppm = mg/m^3 \times \frac{24.45}{M.W.} \times \frac{760}{P} \times \frac{T + 273}{298}$$

where:

24.45 = molar volume (l/mole) at 25°C and 760 mm Hg.

M.W. = molecular weight (124 g/mole).

P = pressure (mm Hg) of air sampled.

T = temperature (°C) of air sampled.

11. **References**

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11.4 Dillon, H. K., W. T., Barrett, and P. M. Eller, "Solid Sorbent Sampler for White Phosphorus in Air", accepted for presentation at the 1977 American Industrial Hygiene Conference, New Orleans, May 1977.

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854

FREE SILICA (QUARTZ, CRISTOBALITE, TRIDYMITE) IN AIRBORNE DUST

Measurements Research Branch

Analytical Method

Analyte:	Quartz, Cristobalite, Tridymite	**Method No.:**	P&CAM 259
Matrix:	Airborne dust	**Range:**	20 to 2000 μg (Quartz)
Procedure:	Filter collection, redeposition, X-ray diffraction	**Precision:**	10% RSD (Analytical) for 50 μg Quartz
Date Issued:	3/17/77	**Classification:**	D (Operational)
Date Revised:			

1. **Principle of the Method**
   - 1.1 A known volume of air is drawn through a membrane filter to trap airborne dust.
   - 1.2 The filters are ashed and the residue redeposited on silver membrane filters.
   - 1.3 The filter samples are scanned by X-ray diffraction to determine the presence of free silica polymorphs (Quartz, Cristobalite, and Tridymite) and other phases which may cause matrix interference.
   - 1.4 The mass of each free silica polymorph present is determined by measuring the diffraction peak intensity of the polymorph and of the silver filter. The mass is calculated from calibration data.
2. **Range and Sensitivity**
   - 2.1 The range of the method is 20 to 2000 μg when the sample is pure quartz. The detection limit for pure quartz is 5 μg on a 25-mm silver filter but quantitative measurements cannot be made at this level.
   - 2.2 The range of the method for pure cristobalite or tridymite is estimated to be 35 to 2000 μg. The detection limit has not been determined.
   - 2.3 The range of the method for samples containing mixtures is dependent on the amount of interfering substances and X-ray absorbing substances present.
3. **Interferences**
   - 3.1 Several compounds have diffraction peaks that interfere with the major peak for quartz; these include micas (biotite, muscovite), potash, feldspars (microcline, plagioclase), sillimanite, zircon, graphite, iron carbide, and lead sulfate. Comparable phase interference may occur for cristobalite and tridymite. The presence of phase interference can be verified by X-ray diffraction analysis. If interferences are present, analytical measurements are made using a different peak of the free silica polymorph, with a commensurate decrease in sensitivity and precision.
   - 3.2 The presence of specific elements in the sample (iron, in particular) can result in appreciable X-ray fluorescence, leading to high background intensity. This situation may be circumvented by employing a diffracted beam monochromator.

855

3.3 The interfering effects of X-ray absorption by the sample result in attenuation of the diffracted beam and corrections must be made (see Sections 10.4 and 10.5).

4. **Precision and Accuracy**

4.1 The relative standard deviation (RSD) for the analytical method was determined to be 10% over the range 50 to 250 μg pure quartz.

4.2 The accuracy of the method has not been determined.

4.3 The precision and accuracy of the method for cristobalite and tridymite have not been determined.

5. **Advantages and Disadvantages of the Method**

5.1 The X-ray diffraction method is specific and can determine the individual polymorphs of free silica. The sensitivity of the method is equivalent to or greater than that of other known methods (infrared and colorimetric). The method is non-destructive of the analyte but the ashing step helps to eliminate organic and other volatile compounds that may interfere. The method can be automated.

5.2 The equipment is relatively expensive.

6. **Apparatus**

6.1 Personal Sampling Equipment. The sampling unit for respirable dust collection has the following components:

6.1.1 Ten-mm nylon cyclone

6.1.2 Filter unit consisting of the filter media (Section 6.1.4) and appropriate three piece cassette filter holder

6.1.3 Personal sampling pump calibrated to $\pm$ 5% at the recommended flow rate. The pump must be calibrated with a representative filter holder and filter in the line.

6.1.4 Polyvinyl chloride membrane filters, 37-mm, 5-μm pore size. (Gelman VM-1 filters are unacceptable because of the high background produced by the ash.)

6.2 Area Sampling Equipment. The sampling unit for high volume respirable dust collection has the following components:

6.2.1 Calibrated Gast 1022 vacuum pump equipped with a needle valve flow controller, or equivalent

6.2.2 HASL cyclone, 1-inch (Bendix No. 240 cyclone)

6.2.3 Polyvinyl Chloride membrane filter, 47-mm, 1-μm pore size

6.2.4 Filter holder

6.3 X-ray diffraction equipment with a copper target X-ray tube. The equipment should be optimized for intensity rather than resolution.

6.4 Silver membrane filters, 25-mm diameter and 0.45-μm pore size: Selas Flotronics, Spring House, Pennsylvania 19477

6.5 Filtration apparatus (Gelman No. 1107 or equivalent) and side arm vacuum flask

6.6 Volumetric Flask, 1-liter

6.7 Reagent bottles with ground glass stoppers, 1-liter

6.8 Low temperature radiofrequency asher or muffle furnace

6.9 Porcelain crucibles with covers for muffle furnace

6.10 Pyrex beakers, 50-ml

6.11 Instrument calibration reference specimen, mica, Arkansas stone (alpha quartz), or other stable standard

6.12 Dessicator

6.13 Filter storage cassettes

6.14 Forceps

6.15 Polyethylene wash bottle

6.16 Microbalance

6.17 Ultrasonic bath or probe

7. **Reagents**

ACS Analytical Reagent Grade or Equivalent

7.1 Quartz, cristobalite, and tridymite standards

7.2 Glue, tape, or other material necessary for attaching silver filters to filter holders

7.3 Isopropanol

7.4 Dessicant (Drierite)

8. **Procedure**

8.1 **Cleaning of Equipment** All glassware should be detergent washed, thoroughly rinsed with tap water followed by distilled water, and dried.

8.2 **Calibration of Personal Pumps** Each personal pump must be calibrated with a representative filter cassette in the line. Cyclone assemblies should be cleaned before using and a visual check for scoring of the surfaces made. Replace the cyclone if it is scored.

8.3 **Collection and Shipping of Samples**

8.3.1 Attach the cassette and cyclone assembly to the worker.

8.3.2 Collect the sample at a flow rate of 1.7 liters per minute. As it is possible for filters to become plugged by heavy particulate loading or by the presence of oil mists or other liquids in the air, the pump rotameter should be observed frequently, and the sampling should be terminated at any evidence of a problem.

8.3.3 Terminate sampling after the predetermined time and note sample flow rate, collection time, date, ambient temperature and pressure. If pressure reading is not available, record the elevation.

8.3.4 After sample collection, the filter cassette should be firmly sealed with plugs in both the inlet and outlet ends.

8.3.5 Carefully record sample and identify all relevant sample data.

8.3.6 With each batch of ten samples, submit one filter from the same lot of filters that was used for sample collection and which is handled in the same way as the samples except that no air is drawn through it. Label this as a blank.

8.3.7 The filter cassettes should be shipped in a suitable container, designed to prevent damage in transit.

8.3.8 When possible, collect a bulk (settled dust) sample of the suspected parent material of the airborne dust in a suitable container and ship with the filter samples.

8.3.9 A high volume respirable dust sample should be taken using the equipment listed under 6.2. A sample of 27,000 liters should be taken at 75 liters per minute.

8.4 **Analysis of Samples**

8.4.1 Obtain a qualitative X-ray diffraction scan (broad 2θ range) of the bulk and/or high volume respirable sample to determine the presence of free silica polymorphs and any matrix interference. The expected diffraction peaks are as follows:

457

Mineral	2 Theta, Primary	2 Theta, Secondary
Quartz	26.66	20.85
Cristobalite	21.93	36.11
Tridymite	21.62	20.50
Silver	38.12	44.28

8.4.2 Ash the samples using one of the following methods:

1. Low Temperature Ashing

   Using forceps, place the filter samples in 50 ml beakers and situate within the sample compartment of the low temperature asher so that the sample exposure to the plasma is optimized. The samples should be ashed for 2 hours at 400 watts RF power and at an oxygen flow rate of 75 ml/min, using the techniques recommended in the instrument manual. After ashing, add 15 ml of isopropanol to each beaker.

2. Muffle Furnace Ashing

   Using forceps, place the filter samples in porcelain crucibles, loosely cover, and place in muffle furnace. Hold for two hours at 600°C (800°C if graphite is present in the sample). After ashing, add several ml of isopropanol to the ash, scrape the crucible to loosen all particles, and transfer to a 50 ml beaker. Wash the crucible several more times and add wash to beaker. Add isopropanol to the beaker to bring the volume to about 15 ml.

8.4.3 Ultrasonically agitate the beaker contents for 3 minutes.

8.4.4 Set aside six silver filters randomly selected from the same lot of filters used for depositing the samples (See 8.4.10).

8.4.5 Place a filter in the filtration apparatus and attach the chimney. Pour several ml of isopropanol on the filter. With no suction, very little isopropanol will penetrate the filter. Pour the suspension from the beaker into the chimney, washing the beaker several times with isopropanol from the wash bottle. Apply a vacuum to the filter flask so the suspension is filtered rapidly. **Do not wash the chimney walls.** Leave the vacuum on for sufficient time to produce a dry filter. Disassemble the filter funnel, release the vacuum, and remove the filter with forceps.

8.4.6 Attach the silver filter to a sample holder and insert in the diffractometer.

8.4.7 Analyze the most intense diffraction peak of each free silica polymorph that is free from matrix interference by step scanning the peak and integrating the counts. Measure the background on each side of the peak for one half the time used for peak scanning, and add the counts from each side for a total (average) background. The position of the background must be determined for each set of samples. The net count or intensity, I_q, is the difference between the peak integrated count and the total background count.

8.4.8 Determine the net count, I_{Ag}, of the appropriate silver peak following the procedure of 8.4.7. Scan times should be shorter for silver filters but should be consistent throughout the method.

8.4.9 After each unknown is scanned, determine the net count I_r^*, of the reference specimen. Determine normalized intensities, $\hat{I}$, for each peak by dividing the peak intensity by that of the reference specimen. Examples for the quartz and silver peaks are:

$$\hat{I}_q = \frac{I_q}{I_r^*}$$

$$\hat{I}_{Ag} = \frac{I_{Ag}}{I_r^*}$$

Record the normalized intensities.

8.4.10 Mount the six clean silver filters (Section 8.4.4) and determine the net count for the silver peak, I°_{Ag}, (Section 8.4.8). Normalize the measured intensities (Section 8.4.9),

$$\hat{I}^{\circ}_{Ag} = \frac{I^{\circ}_{Ag}}{I^{\circ}_{r}}$$

and record $\hat{I}^{\circ}_{Ag}$.

9. **Calibration and Standards**

9.1 Preparation of Free Silica Standards. The following procedure for quartz is applicable to cristobalite and tridymite.

9.1.1 Wet sieve the quartz standard through a 10-μm sieve with isopropanol. Evaporate the alcohol and dry the powder in a 110°C oven. Cool the powder in a dessicator.

9.1.2 Prepare two suspensions of quartz in isopropanol by weighing approximately 10 and 50 mg of the dry quartz powder to the nearest 0.01 mg. Quantitatively transfer each to a 1-liter glass stoppered bottle using 1.00 liter of isopropanol.

9.1.3 Disperse the powder in isopropanol by using an ultrasonic probe or bath for 20 minutes.

9.1.4 Prepare a series of standard filters using the 10 and 50 mg/l suspensions. Using appropriate pipets, prepare a sufficient number of standards in triplicate to cover the analytical range.

9.1.5 Mount a filter on the filtration apparatus. Place several ml of isopropanol on the filter surface. Vigorously hand-shake the quartz suspension and immediately withdraw an aliquot from the center of the suspension. Do not adjust the volume in the pipet by expelling part of the suspension. If more than the desired aliquot is withdrawn, return all of the suspension to the bottle, rinse and dry the pipet. Resume the procedure after shaking the suspension. Transfer the aliquot from the pipet to the filter. Keep the tip of the pipet near the surface but not submerged in the suspension. As soon as the pipet has drained, apply the vacuum and rapidly filter the suspension. Leave the vacuum on for sufficient time to dry the filter. Do not wash down the sides of the chimney after the quartz deposit is in place since this will rearrange the material on the filter. Transfer the filter to the sample mount that is to be used in the diffractometer.

9.2 Perform step scans on the standards and reference specimen using the same conditions as those used for the samples. Using the procedure of Sec. 8.4.9, determine and record the normalized intensity, $\hat{I}^{\circ}_{q}$, for each peak measured.

10. **Calculations**

10.1 Calculate the exact weights of quartz deposited on each standard filter from the concentrations of the standard suspensions and aliquot volumes. Record the weight, w, of each standard. Prepare a calibration curve by plotting $\hat{I}^{\circ}_{q}$ as a function of w. Poor reproducibility (>10% RSD) at any given level indicates problems in the sample preparation technique and new standards should be made. The data should lie along a straight line for low weights and curvature at high weights should be ignored when determining the line of best fit.

10.2 Determine the initial slope, m, of the calibration curve in counts/μg. The intercept, b, of the line with the $\hat{I}^{\circ}_{q}$ axis should be approximately 0. A large negative intercept indicates an error in determining the background. This may arise from incorrectly measuring the baseline or from interference by another phase at the angle of background measurement. A large positive intercept indicates an error in determining the baseline or that an impurity is included in the measured peak.

259

10.3 Calculate and record the average normalized intensity, average $\hat{I}^{\circ}_{Ag}$ from those determined for the clean silver filters (Sec. 8.4.10).

$$\text{ave } \hat{I}^{\circ}_{Ag} = \frac{\sum_{n=1}^{6} \hat{I}^{\circ}_{Ag,\,n}}{6}$$

10.4 Using the normalized intensities, I_{Ag}, for the silver peaks of each sample (Sec. 8.4.9), and the average I_{Ag} calculated for the clean silver filters, calculate the transmittance, T, of each sample as follows:

$$T = \frac{\hat{I}_{Ag}}{\text{ave } \hat{I}^{\circ}_{Ag}}$$

10.5 Determine the correction factor, f(T), for each sample according to the formula:

$$f(T) = \frac{-R \ln T}{1 - T^R}$$

where:

$$R = \frac{\sin \theta_{Ag}}{\sin \theta_q}$$

and θ_{Ag} and θ_q are the angles θ (not 2θ) of the silver and quartz peaks. Table I lists f(T) values for T values from 0.5 to 1.0 for common $2\theta_q$ and $2\theta_{Ag}$ combinations.

10.6 Calculate the weight in micrograms of the quartz in each sample:

$$w(\mu g) = \left[\frac{\hat{I}_q - b}{m} \right] \times \left[f(T) \right]$$

10.7 If the sampling was conducted at a temperature and pressure near standard conditions (760 mm Hg pressure and 25°C), the air volume need not be corrected. If other conditions existed, correct the volume in the following manner:

$$V_s = \frac{V_i}{1000} \times \frac{P}{760} \times \frac{298}{T + 273}$$

where:

V_s = volume of air in m^3 at 25°C and 760 mm Hg
V_i = volume of air in liters as measured
P = barometric pressure in mm Hg
T = temperature of the air in °C

10.8 Calculate the airborne concentration of silica dust in micrograms per cubic meter:

$$SiO_2\ (\mu g/m^3) = \frac{w(\mu g)}{V_s(m^3)}$$

11. References

11.1 Leroux, J. and C. Powers, "Direct X-Ray Diffraction Quantitative Analysis of Quartz in Industrial Dust Films Deposited on Silver Membrane Filters", Occup Health Rev 21:26, 1970.

11.2 Bumsted, H. E., "Determination of Alpha-Quartz in the Respirable Portion of Airborne Particulates by X-Ray Diffraction", Am Ind Hyg Assoc J 34:150, 1973.

11.3 Peters, E. T., "Evaluation of the NIOSH X-Ray Diffraction Method for the Determination of Free Silica in Respirable Dust", Final Report of Contract CDC-99-74-51, May 1976.

11.4 Abell, M. T., D. D. Dollberg, J. C. Haartz, and B. A. Lange, "Evaluation of the X-Ray Diffraction Method for Free Silica Using an External Standard", To be published.

TABLE I

Commonly used correction factors
Silica/Silver peaks, degrees two theta

Silica	26.66	26.66	20.83	20.83	21.93	21.93	21.62	21.62
Silver	38.12	44.28	38.12	44.28	38.12	44.28	38.12	44.28
1.00	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.99	1.0071	1.0082	1.0091	1.0105	1.0087	1.0100	1.0088	1.0101
0.98	1.0144	1.0166	1.0184	1.0212	1.0174	1.0201	1.0177	1.0204
0.97	1.0217	1.0251	1.0278	1.0321	1.0264	1.0305	1.0268	1.0309
0.96	1.0292	1.0337	1.0373	1.0432	1.0355	1.0410	1.0360	1.0416
0.95	1.0368	1.0425	1.0470	1.0544	1.0447	1.0517	1.0453	1.0524
0.94	1.0445	1.0514	1.0569	1.0659	1.0541	1.0625	1.0548	1.0635
0.93	1.0523	1.0605	1.0670	1.0776	1.0636	1.0736	1.0645	1.0747
0.92	1.0602	1.0697	1.0772	1.0894	1.0733	1.0849	1.0743	1.0861
0.91	1.0683	1.0791	1.0876	1.1015	1.0831	1.0963	1.0844	1.0977
0.90	1.0765	1.0886	1.0982	1.1138	1.0932	1.1080	1.0945	1.1096
0.89	1.0848	1.0983	1.1089	1.1264	1.1034	1.1199	1.1049	1.1216
0.88	1.0933	1.1081	1.1199	1.1392	1.1137	1.1320	1.1154	1.1339
0.87	1.1019	1.1181	1.1311	1.1522	1.1243	1.1443	1.1261	1.1464
0.86	1.1106	1.1283	1.1424	1.1654	1.1350	1.1568	1.1370	1.1592
0.85	1.1195	1.1387	1.1540	1.1790	1.1460	1.1696	1.1481	1.1722
0.84	1.1286	1.1493	1.1657	1.1927	1.1571	1.1827	1.1595	1.1854
0.83	1.1378	1.1600	1.1777	1.2068	1.1685	1.1959	1.1710	1.1989
0.82	1.1471	1.1709	1.1899	1.2211	1.1800	1.2095	1.1827	1.2126
0.81	1.1566	1.1821	1.2024	1.2357	1.1918	1.2232	1.1946	1.2266
0.80	1.1663	1.1934	1.2150	1.2506	1.2038	1.2373	1.2068	1.2409
0.79	1.1762	1.2050	1.2280	1.2658	1.2160	1.2516	1.2192	1.2555
0.78	1.1863	1.2168	1.2411	1.2812	1.2284	1.2663	1.2319	1.2703
0.77	1.1965	1.2288	1.2546	1.2971	1.2411	1.2812	1.2447	1.2855
0.76	1.2069	1.2410	1.2683	1.3132	1.2540	1.2964	1.2579	1.3009
0.75	1.2175	1.2535	1.2822	1.3297	1.2672	1.3119	1.2713	1.3167
0.74	1.2283	1.2662	1.2965	1.3465	1.2806	1.3278	1.2849	1.3328
0.73	1.2394	1.2792	1.3110	1.3637	1.2944	1.3440	1.2989	1.3493
0.72	1.2506	1.2924	1.3259	1.3812	1.3084	1.3605	1.3131	1.3661
0.71	1.2621	1.3059	1.3410	1.3991	1.3226	1.3774	1.3276	1.3833
0.70	1.2738	1.3197	1.3565	1.4174	1.3372	1.3946	1.3424	1.4008
0.69	1.2857	1.3337	1.3723	1.4362	1.3521	1.4122	1.3576	1.4187
0.68	1.2979	1.3481	1.3885	1.4553	1.3673	1.4303	1.3730	1.4370
0.67	1.3103	1.3628	1.4050	1.4749	1.3829	1.4487	1.3888	1.4558
0.66	1.3230	1.3777	1.4218	1.4949	1.3987	1.4675	1.4050	1.4749
0.65	1.3359	1.3931	1.4390	1.5154	1.4150	1.4868	1.4215	1.4945
0.64	1.3491	1.4087	1.4567	1.5363	1.4316	1.5064	1.4383	1.5145
0.63	1.3626	1.4247	1.4747	1.5578	1.4485	1.5266	1.4556	1.5350
0.62	1.3765	1.4411	1.4931	1.5797	1.4659	1.5472	1.4732	1.5560
0.61	1.3906	1.4578	1.5120	1.6022	1.4836	1.5684	1.4913	1.5775
0.60	1.4050	1.4749	1.5314	1.6252	1.5018	1.5900	1.5098	1.5995
0.59	1.4198	1.4925	1.5511	1.6488	1.5204	1.6122	1.5287	1.6221
0.58	1.4349	1.5104	1.5714	1.6730	1.5394	1.6349	1.5481	1.6452
0.57	1.4504	1.5288	1.5922	1.6978	1.5590	1.6582	1.5679	1.6689
0.56	1.4662	1.5476	1.6135	1.7233	1.5790	1.6820	1.5883	1.6932
0.55	1.4824	1.5670	1.6353	1.7494	1.5995	1.7065	1.6092	1.7181
0.54	1.4991	1.5868	1.6577	1.7762	1.6205	1.7317	1.6306	1.7437
0.53	1.5161	1.6071	1.6807	1.8037	1.6421	1.7575	1.6525	1.7699
0.52	1.5336	1.6279	1.7043	1.8319	1.6642	1.7840	1.6751	1.7969
0.51	1.5515	1.6493	1.7285	1.8609	1.6870	1.8112	1.6982	1.8246
0.50	1.5699	1.6713	1.7534	1.8908	1.7103	1.8391	1.7220	1.8531

LEAD IN BLOOD AND URINE

Measurements Research Branch

Analytical Method

Analyte:	Lead	**Method No.:**	P&CAM 262
Matrix:	Blood, urine	**Range, Blood:**	5-250 μg/100 g
Procedure:	Chelation, extraction, AA	**Range, Urine:**	10-500 μg/liter
Date Issued:	2/25/76	**Precision:**	6.4% RSD (blood)
Date Revised:		**Classification:**	D (Operational)

1. **Principle of the Method**
   - 1.1 Whole blood or urine samples are ashed with nitric acid to destroy the organic matrix.
   - 1.2 The sample ash is dissolved by the addition of a weak nitric acid solution and the pH of the solution is adjusted to approximately 8.5.
   - 1.3 The lead is chelated with ammonium pyrrolidine dithiocarbamate (APDC) and extracted from the aqueous phase into methyl isobutyl ketone (MIBK).
   - 1.4 The MIBK containing the lead-APDC complex is aspirated into the flame of an atomic absorption spectrophotometer and its absorbance is determined at 283.3 nm.
2. **Range and Sensitivity**
   - 2.1 For a 10-ml blood sample, the analytical range extends from 5 μg to 250 μg per 100 g blood.
   - 2.2 For a 50-ml urine sample, the analytical range extends from 10 μg to 500 μg per liter of urine.
   - 2.3 The range in both blood and urine can be extended to larger amounts of lead by taking smaller aliquots.
3. **Interferences**

   There are no known interferences for this method.
4. **Precision and Accuracy**

   The accuracy and precision of this method were determined by analyzing standardized porcine blood from the National Bureau of Standards. The results were 0.99 ± 0.064 μg/g (c.f. the NBS value of 1.00 ± 0.023 μg/g) for nine samples.
5. **Advantages and Disadvantages of the Method**

   This method is fast and does not require a high level of technical skill. A trained technician can do 25-30 samples per day.
6. **Apparatus**
   - 6.1 50-ml volumetric glass-stoppered flasks.
   - 6.2 Atomic absorption spectrophotometer equipped with air-acetylene burner system.
   - 6.3 125-ml Phillips borosilicate glass beakers with watch glass covers.
   - 6.4 125-ml polyethylene bottles with Teflon lined caps.
   - 6.5 25-ml lead-free, heparinized Vacutainers.

463

7. **Reagents**

The reagents described must be made up using ACS reagent grade or a better grade of chemical.

7.1 Double distilled water.

7.2 Redistilled concentrated nitric acid (16N).

7.3 Potassium cyanide, 10% (w/v): dissolve 10 grams of KCN in a small amount of distilled water and dilute to 100 ml. (Danger — May be fatal if swallowed or absorbed through skin. Contact with acid liberates poisonous gas.)

7.4 High Purity Ammonium Hydroxide, Sp. Gr. 0.92, (G. Frederick Smith Chemical Company or equivalent).

7.5 Ammonium citrate buffer, pH 8.5: dissolve 400 g of citric acid in 100 ml of distilled water. Add 400 ml of ammonium hydroxide cautiously, cool, and adjust to pH 8.5 with phenol red indicator.

7.6 Ammonium Pyrrolidine Dithiocarbamate (APDC) (Fisher Reagent Grade or equivalent): 2% in double distilled water.

7.7 Methyl Isobutyl Ketone (MIBK; 4-Methyl-2-pentanone): water saturated.

7.8 Phenol red indicator: dissolve 0.1 g of phenol red (phenolsulfonephthalein) in 100 ml of distilled water.

7.9 Thymol — Reagent Grade.

8. **Procedure**

8.1 **Cleaning of Equipment.** All glassware must be rendered lead-free before use. This can be accomplished by using the following three-step procedure:

8.1.1 Wash in detergent-tap water and follow with tap water and distilled water rinses.

8.1.2 Soak in hot chromic acid and follow with tap and distilled water rinses.

8.1.3 Soak in 1:1 or concentrated nitric acid for 30 minutes and follow with tap, distilled, and double distilled water rinses.

This entire procedure must be used only when glassware is being initially prepared for use in the lead analysis. For each following successive use of equipment which has previously been subjected to the entire cleaning procedure, it is necessary only to use the nitric acid cleaning procedure described in step 8.1.3. Polyethylene equipment can be effectively cleaned by subjecting it to steps 8.1.1 and 8.1.3.
NOTE: Chromic acid cannot be used on polyethylene.

8.2 **Collection and Shipping of Samples**

8.2.1 Blood must be collected in lead-free heparinized vacutainers equipped with sterilized stainless steel needles. The vacutainers should be heparinized before the collection of the blood. Heparinized vacutainers are available commercially from most laboratory supply houses. The addition of a preservative after the collection of the blood can result in error because of volume changes. Caution should be exercised during handling to prevent any external contamination. The samples should be kept as cool as possible during storage and shipment (do not freeze) and should be shipped to the laboratory as quickly as possible. Heparinized, refrigerated blood samples are stable for two weeks. Ten milliliters (10 ml) of whole blood are adequate for this determination.

8.2.2 Urine samples must be collected in lead-free borosilicate or polyethylene bottles. At least 50 ml should be collected and the specific gravity measured. The sample must be preserved by the addition of 5 mg of thymol per 100 ml of urine. The urine should be kept cool during shipment and storage. Urine treated with thymol and refrigerated is stable for one week.

.8.3 **Analysis of Samples**

8.3.1 Into a clean 125-ml Phillips borosilicate glass beaker weigh 10 grams of heparinized whole blood followed by 6 ml of concentrated nitric acid. For urine, a 50-ml sample is used. A reagent blank using double distilled water in place of the blood or urine should also be started at this point. Cover with a watch glass and heat on a 120-140° hot plate overnight.

8.3.2 The samples are heated until nearly dry. The color of the residue should range from yellow to white. If the residue has not reached this color, the beaker should be allowed to cool briefly, and 1 ml of nitric acid should be added and the procedure repeated. When all organic matter has been destroyed, 1 ml of HNO_3 and 5 ml of distilled H_2O are added to the beaker and heat is applied slowly. When the residue has dissolved, the solution is transferred to a clean 50-ml volumetric flask. (If the sample contains more than 100 μg Pb, an aliquot of the ash solution should be taken.)

8.3.3 Add 2 drops of phenol red indicator.

8.3.4 Add 5 ml of ammonium citrate buffer.

8.3.5 Add NH_4OH drop by drop until the solution turns red.

8.3.6 Add 1 ml of 10% (w/v) potassium cyanide solution.

8.3.7 Add 1 ml of 2% APDC solution.

8.3.8 Add 4.0 ml of water-saturated MIBK, stopper, and shake for 30 seconds.

8.3.9 Add distilled water to the flask to bring the MIBK layer into the neck of the flask.

8.3.10 Set the atomic absorption instrument to measure absorption at 283.3 nm and start the air-acetylene flame. Reduce the acetylene flow so that an oxidizing flame is obtained while aspirating water-saturated MIBK. Aspirate the standards and samples using the water-saturated MIBK to set the baseline.

9. **Calibration and Standards**

9.1 A 1 mg/ml Pb stock standard is first made up by drying lead nitrate at 100°C for two hours, cooling, weighing out 1.5980 g, dissolving in 1:10 HNO_3, and making up to a 1-liter volume with additional 1:10 HNO_3. From this 1 mg/ml standard, working standards of 1, 2, 5, and 10 μg of Pb per ml of solution can be made up by appropriate dilutions with 1:10 HNO_3. The stock standard is stable for six months. The working standards should be made up fresh weekly.

9.2 A calibration curve is constructed by pipetting 0.5 ml of each working standard into a 50-ml volumetric flask and then proceeding from step 8.3.3 of the procedure. A calibration curve of absorbance versus micrograms of Pb in the flask is then drawn up.

10. **Calculations**

10.1 The calculations for determining the concentration of Pb per 100 g of blood are as follows (the factors in parentheses are used only if an aliquot of the ashed solution was taken for extraction and analysis).

$$\left(\frac{\text{ml sample ash solution}}{\text{ml removed for extraction}}\right) \times \frac{\text{100 g blood}}{\text{g ashed}} \times \frac{\mu\text{g from}}{\text{cal. curve}} = \mu\text{g Pb/100 g blood}$$

10.2 The calculations for determining the concentration of Pb per liter of urine are similar, except that a correction for specific gravity of the urine is made.

$$\left(\frac{\text{ml sample ash solution}}{\text{ml removed for extraction}}\right) \times \frac{1000 \text{ ml urine}}{\text{ml ashed}} \times \frac{\text{microgram reading from curve}}{} \times \frac{0.024}{\left(\text{specific gravity of urine} - 1.000\right)}$$

= μg Pb/liter of urine [corrected to sp. gr. 1.024 (11.2)]

11. References

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11.2 Elkins, H. B., "Concentration Adjustments in Urinalysis", Am Ind Hyg Assoc J 35:559, 1974.

INDEX

Volume 1, Methods 102-262
Volume 2, Methods S1-S134
Volume 3, Methods S135-S391

Acetic anhydride S170
Acetone 127, S1
Acetonitrile (Methyl cyanide) S165
Acetylene dichloride (see 1,2-Dichloroethylene)
Acetylene tetrabromide (Tetrabromoethane) S117
Acrolein 118, 211
Acrylonitrile 202, S156
Aldrin S275
Allyl alcohol S52
Allyl chloride S116
Aluminum 173
Amines, aliphatic 221
Amines, aromatic 168
Ammonia 205
n-Amyl acetate S51
sec-Amyl acetate (alpha-Methyl butyl acetate) S31
Aniline 168, S310
Anisidine 168
Anthanthrene 183, 184, 206
Anthracene 206
Antimony 107, 173, 189, 193, 197, S2
Arsenic 139, 140, 173, 180, 188, 192, 196, S309
Arsine S229
Asbestos 239, 245
Azelaic acid 256
Azo dyes 234
Barium 173, S198
Benz(c)acridine 183
Benz(a)anthracene 183
Benz(a,h)anthracene 183, 184
Benz(a)anthrone 183
Benzene 127, S311
Benzene-solubles 217
Benzidine 243
Benzo(b)fluoranthene 183
Benzo(j)fluoranthene 183
Benzo(k)fluoranthene 183, 184
Benzo(g,h,i)perylene 183
Benzo(a)pyrene 183, 184, 186, 206, 251
Benzo(e)pyrene 183, 184
Benzyl chloride S115
Beryllium 121, 173, S339
Bibenzyl 183
Biphenyl-phenyl ether mixture (see Phenyl ether-biphenyl vapor mixture)

Bis(chloromethyl) ether 213, 220
Bismuth 173
2,2-Bis(p-chlorophenyl)-1,1,1-trichloroethane (see DDT)
Boron oxide S349
Bromoform S114
Butadiene (1,3-Butadiene) S91
2-Butanone (Methyl ethyl ketone or MEK) 127, S3
2-Butoxy ethanol (Butyl cellosolve) S76
sec-Butyl acetate S46
tert-Butyl acetate S32
Butyl acetate (n-Butyl acetate) S47
sec-Butyl alcohol S53
tert-Butyl alcohol S63
Butyl alcohol (n-Butyl alcohol) S66
Butyl cellosolve (see 2-Butoxy ethanol)
n-Butyl glycidyl ether S81
Butylamine 221
p-tert-Butyltoluene S22
Cadmium 173, 191, 223, 224, S312, S313
Calcium 173
Calcium arsenate 173
Calcium oxide 173, S205
Camphor S10
Carbaryl (Sevin) S273
Carbon black S262
Carbon dioxide S249
Carbon disulfide 179, S248
Carbon monoxide 112, 113
Carbon tetrachloride 127, S314
Carbonyl chloride (see Phosgene)
Chloride 115
Chlorinated camphene (Toxaphene) S67
Chlorinated diphenyl oxide S119
Chlorine 209
2-Chloro-1,3-butadiene (see Chloroprene)
1-Chloro-2,3-epoxypropane (see Epichlorohydrin)
Chlorobenzene (Monochlorobenzene) S133
Chlorobromomethane S113
Chlorodiphenyl (54% chlorine) S121
2-Chloroethanol (see Ethylene chlorohydrin)
Chloroform 127, S351
Chloromethyl methyl ether 220
Chloroprene (2-Chloro-1,3-butadiene) S112
Chromic acid 152, 169, S317
Chromium 152, 173, 182, S323, S352
Chrysene 183, 184, 206
Chrysotile 245
Coal tar naphtha (see Naphtha, coal tar)
Coal tar pitch volatiles 183, 184, 217
Cobalt 173
Copper dusts and mists 173, S186

Copper 173
Cresol, all isomers S167
Cristobalite 109, 259, S315
Cumene S23
Cyanide 116, S250
Cyclohexane S28
Cyclohexanol S54
Cyclohexanone S19
Cyclohexene S82
Cyclohexylamine 221
DBPC 226
DDT (2,2-Bis(p-chloropheny1)-1,1,1-trichloroethane) S274
Diacetone alcohol (4-Hydroxy-4-methyl-2-pentanone) S55
Diazomethane S137
Diazonium salts 234
1,2-Dibromoethane (see Ethylene dibromide)
Dibutylphthalate S33
1,1-Dichloro-1-nitroethane S213
o-Dichlorobenzene S135
p-Dichlorobenzene S281
3,3'-Dichlorobenzidine 246
Dichlorodifluoromethane (Refrigerant 12) S111
1,1-Dichloroethane (Ethylidene chloride) S123
1,2-Dichloroethane (see Ethylene dichloride)
Dichloroethyl ether S357
1,2-Dichloroethylene (Acetylene dichloride) S110
Dichloromethane (see Methylene chloride)
Dichloromonofluoromethane (Refrigerant 21) S109
Dichlorotetrafluoroethane (Refrigerant 114) S108
Dieldrin S283
Diethylamine 221, S139
Diethylene dioxide (see Dioxane)
Difluorodibromomethane S107
Difluorodichloromethane S111
Diisobutyl ketone (2,6-Dimethyl-4-heptanone) S358
Diisopropylamine 221
Dimethoxymethane (see Methylal)
Dimethyl acetamide S254
Dimethyl benzene (see Xylene)
1,3-Dimethyl butyl acetate (see sec-Hexyl acetate)
Dimethyl formamide S255
2,6-Dimethyl-4-heptanone (see Diisobutyl ketone)
Dimethylamine 221, S142
2,4-Dimethylaminobenzene (see Xylidine)
N,N-Dimethylaniline 168, S164
1,1-Dimethylhydrazine 248, S143
Dioxane 127, S360
p,p-Diphenylmethane diisocyanate 142
Dipropylene glycol methyl ether S69
2,6-Di-tert-butyl-p-cresol 226
Dowtherm A (see Phenyl ether-biphenyl vapor mixture)

262-7

Epichlorohydrin (1-Chloro-2,3-epoxypropane) S118
EPN (O-Ethyl-O-p-nitrophenyl phenylphosphonothiolate) S285
2,3-Epoxy-1-propanol (see Glycidol)
1,2-Epoxypropane (see Propylene oxide)
Ethanol (see Ethyl alcohol)
Ether S80
2-Ethoxyethylacetate S41
Ethyl acetate S49
Ethyl acrylate S35
Ethyl alcohol (Ethanol) S56
Ethyl benzene S29
Ethyl bromide S106
Ethyl butyl ketone (3-Heptanone) S16
Ethyl ether S80
Ethyl formate S36
Ethyl sec-amyl ketone (see 5-Methyl-3-heptanone)
Ethyl silicate S264
O-Ethyl-O-p-nitrophenyl thionobenzene phosphonate (see EPN)
Ethylamine 221, S144
Ethylene chloride (see Ethylene dichloride)
Ethylene chlorohydrin (2-Chloroethanol) S103
Ethylene dibromide (1,2-Dibromoethane) S104
Ethylene dichloride (1,2-Dichloroethane) 127, S122
Ethylene glycol dinitrate 203, S216
Ethylene oxide S286
di-2-Ethylhexylphthalate (see di-sec-Octyl phthalate)
Ethylidene chloride (see 1,1-Dichloroethane)
N-Ethylmorpholine S146
Fluoranthene 183, 184
Fluoride 114, 117, 212
Fluorotrichloromethane (Refrigerant 11) S102
Formaldehyde 125, 235
Formic acid 232
Gallium 177, 198
Glycidol (2,3-Epoxy-1-propanol) S70
Heptane S89
3-Heptanone (see Ethyl butyl ketone)
2-Heptanone (see Methyl (n-amyl) ketone)
Hexachloroethane S101
Hexachloronaphthalene S100
Hexane S90
2-Hexanone (Methyl butyl ketone or MBK) S178
Hexone (Methyl isobutyl ketone or MIBK) S18
sec-Hexyl acetate (1,3-Dimethyl butyl acetate) S37
Hydrazine 248, S237
Hydrogen bromide S175
Hydrogen chloride S246
Hydrogen cyanide 116
Hydrogen fluoride 117, 212, S176
Hydrogen sulfide 126, S4
Hydroquinone S57

4-Hydroxy-4-methyl-2-pentanone (see Diacetone alcohol)
Indium 173, 190, 194, 199
Iron 173
Isoamyl acetate S45
Isoamyl alcohol S58
Isobutyl acetate S44
Isobutyl alcohol S64
Isophorone S367
Isopropanol (see Isopropyl alcohol)
Isopropyl acetate S50
Isopropyl alcohol (Isopropanol) S65
Isopropyl benzene (see Cumene)
Isopropyl glycidyl ether S77
Isopropylamine 221, S147
Isopropylether S368
Kepone 225
Ketene S92
Lead 102, 173, 191, 195, 200, 208, 214, 262, S341
Lindane S290
Liquified petroleum gas (see LPG)
Lithium 173
LPG (Liquified petroleum gas) S93
Magnesium 173
Magnesium oxide fume S369
Malathion S370
Manganese 173, S5
MBK (see 2-Hexanone)
MDI (see Methylene bisphenyl isocyanate)
MEK (see 2-Butanone)
Mercury 145, 165, 167, 175
Mesityl oxide S12
Methanol (see Methyl alcohol)
2-Methoxyethanol (see Methyl cellosolve)
Methyl(n-amyl)ketone (2-Heptanone) S15
5-Methyl-3-heptanone S13
Methyl acetate S42
Methyl acrylate S38
Methyl alcohol (Methanol) 247, S59
Methyl bromide S372
alpha-Methyl butyl acetate (see sec-Amyl acetate)
Methyl butyl ketone (see 2-Hexanone)
Methyl cellosolve (2-Methoxyethanol) S79
Methyl cellosolve acetate S39
Methyl chloride 201
Methyl chloroform (1,1,1-Trichloroethane) 127, S328
Methyl cyanide (see Acetonitrile)
Methyl ethyl ketone (see 2-Butanone)
Methyl iodide S98
Methyl isoamyl acetate S37
Methyl isobutyl carbinol S60
Methyl isobutyl ketone (see Hexone)

262-9

8/11

alpha-Methyl styrene S26
Methylal (Dimethoxymethane) S71
Methylamine 221
Methylcyclohexane S94
4,4'-Methylenebis(2-chloroaniline) 236
Methylene bisphenyl isocyanate (MDI) 142
Methylene chloride (Dichloromethane) 127, S329
Methylhydrazine 248, S149
MIBK (see Hexone)
MOCA 236
Molybdenum 173
Molybdenum insoluble compounds S376
Molybdenum soluble compounds S193
Monochlorobenzene (see Chlorobenzene)
Monomethyl aniline S153
Monomethyl hydrazine 248, S149
Morpholine S150
Naphtha, coal tar (Coal tar naphtha) S86
Naphthalene S292
Nickel 173, S206
Nicotine S293
Nitric oxide 231
p-Nitroaniline 168
Nitrobenzene S217
p-Nitrochlorobenzene S218
Nitrogen dioxide 108, 231
Nitroglycerin 203, S216
Nitroglycol 203
N-Nitrosodimethylamine 252
Nitrotoluene S223
Octachloronaphthalene S97
Octane S378
di-sec-Octyl phthalate (di-2-Ethylhexylphthalate) S40
Oil mist 159, S272
Organic solvents 127
Organo(alkyl) mercury 175
Ozone 153, 154, S8
Palladium 173
Parathion 158, S295
PCB 244, 253, S121
Pentachloronapthalene S96
Pentachlorophenol 230
Pentane S379
2-Pentanone S20
Perchloroethylene (see Tetrachloroethylene)
Perylene 184, 206
Petroleum distillates (Petroleum naphtha) S380
Petroleum naphtha (See Petroleum distillates)
Phenanthene 206
Phenol S330
Phenyl ether S72

Phenyl ether-biphenyl vapor mixture (Dowtherm A) S73
Phenyl ethylene (see Styrene)
Phenyl glycidyl ether S74
Phenylhydrazine 248, S160
Phosgene (Carbonyl chloride) 219
Phosphate 215
Phosphoric acid 216, S333
Phosphorus (white, yellow) 242, 257
Phthalic anhydride S179
Platinum, soluble salts S191
PNAs 183, 184, 186, 206
Polychlorinated biphenyl (PCB) 244, 253, S121
Polymethylsiloxane 227
Polynuclear aromatic hydrocarbons (PNAs) 183, 184, 186, 206
Potassium 173
Propane S87
Propanol (see Propyl alcohol)
n-Propyl acetate S48
Propyl alcohol (Propanol) S62
n-Propyl nitrate S227
Propylene dichloride S95
Propylene oxide (1,2-Epoxypropane) S75
Pyrene 183, 184
Pyridine S161
Quartz (see Silica, crystalline)
Refrigerant 11 (see Fluorotrichloromethane)
Refrigerant 113 (see 1,1,2-Trichloro-1,2,2-trifluoroethane)
Refrigerant 114 (see Dichlorotetrafluoroethane)
Refrigerant 12 (see Dichlorodifluoromethane)
Refrigerant 21 (see Dichloromonofluoromethane)
Rhodium, metal fume and dust S188
Rhodium, soluble salts S189
Rubidium 173
Selenium 124, 181, S190
Sevin (see Carbaryl)
Silica, crystalline 106, 109, 110, 259, S315
Silicon 173
Silver 173
Sodium 173
Sodium hydroxide 241
Stoddard solvent S382
Strontium 173
Styrene (Vinyl benzene) 127, S30
Sulfur dioxide 146, 160, 163, 204
Sulfuric acid 187, S174
TDI (see Toluene-2,4-diisocyanate)
Tellurium 173, S204
Tellurium hexafluoride S187
Terphenyl 183, S27
Tetrabromoethane (see Acetylene tetrabromide)

B13

262-11

1,1,1,2-Tetrachloro-2,2-difluoroethane S131
1,1,2,2-Tetrachloro-1,2-difluoroethane S132
1,1,2,2-Tetrachloroethane S124
Tetrachloroethylene (Perchloroethylene) 127, S335
Tetrachloromethane (see Carbon tetrachloride)
Tetrachloronaphthalene S130
Tetrahydrofuran S78
Tetramethyl succinonitrile S155
Tetramethyl thiuram disulfide (see Thiram)
Tetranitromethane S224
Tetryl (2,4,6-Trinitrophenylmethylnitramine) S225
Thallium 173, S306
Thiophene 255
Thiram (Tetramethyl thiuram disulfide) 228
Tin 173, 176, S183
Tin, organic compounds 176
Tissue preparation 237
Titanium dioxide S385
Toluene 127, S343
Toluene-2,4-diisocyanate (TDI) 141
o-Toluidine 168, S168
Toxaphene (see Chlorinated camphene)
Tributyl phosphate S208
1,1,2-Trichloro-1,2,2-trifluoroethane (Refrigerant 113) S129
1,1,1-Trichloroethane (see Methyl chloroform)
1,1,2-Trichloroethane 127, S134
Trichloroethylene 127, S336
Trichloromonofluoromethane (see Fluorotrichloromethane)
Trichloronaphthalene S128
1,2,3-Trichloropropane S126
Tridymite 109, 259, S315
Triethylamine 221, S152
Trifluoromonobromomethane S125
2,4,6-Trinitrophenylmethylnitramine (see Tetryl)
Triorthocresyl phosphate S209
Triphenyl phosphate S210
Turpentine S88
Vanadium 173, S391
Vinyl benzene (see Styrene)
Vinyl chloride 178
Vinyl toluene S25
Xylene (Xylol or Dimethyl benzene) 127, S318
Xylidine (2,4-Dimethylaminobenzene) 168, S162
Xylol (see Xylene)
Yttrium S200
Zinc 173
Zinc oxide 222
Zirconium compounds S185

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262-12

874