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INSTRUCTOR MANUAL
INDUSTRIAL HYGIENE CHEMISTRY COURSE

LESSON NUMBER 17

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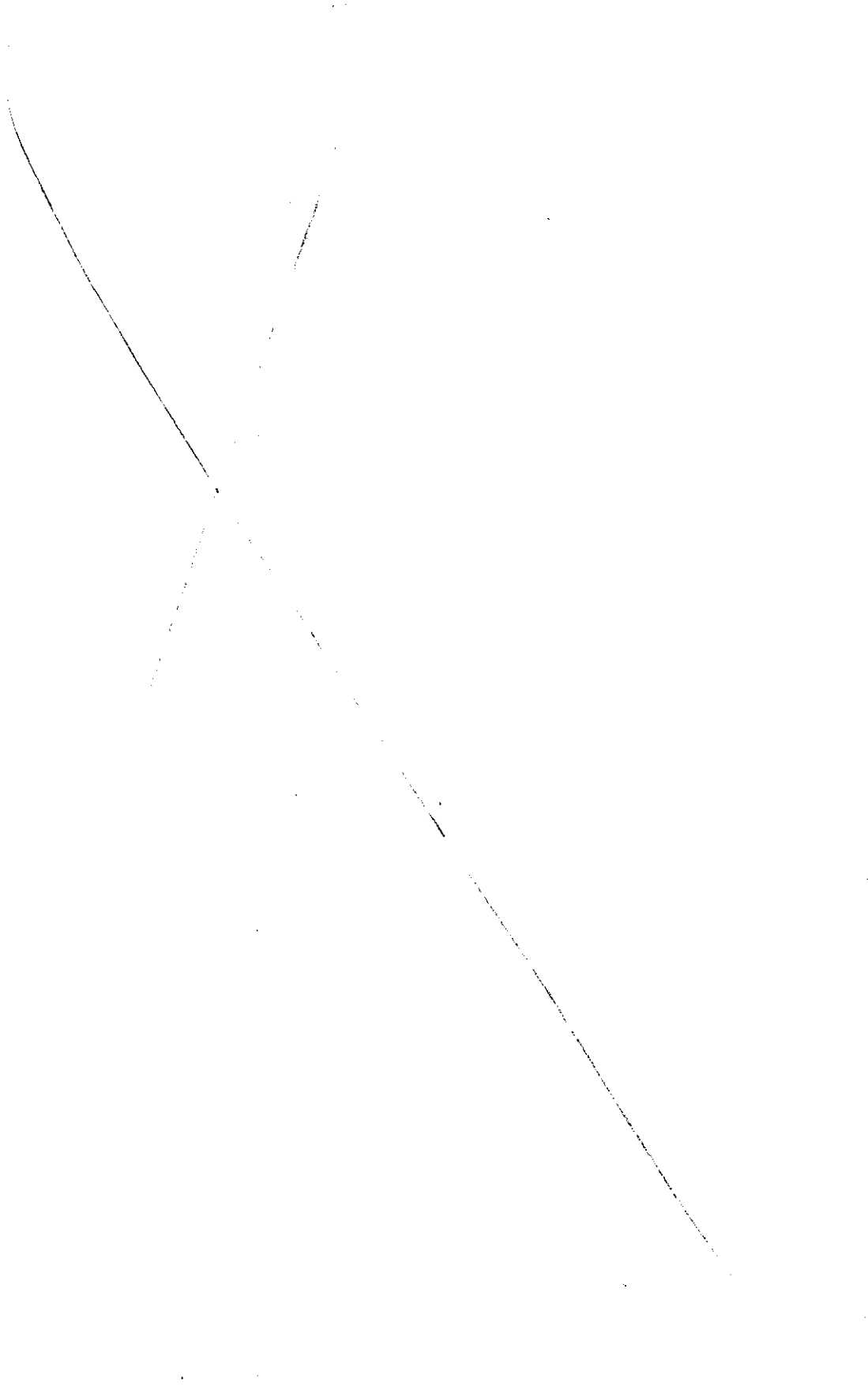


INTRODUCTION

This Instructor Manual has been prepared for industrial hygienists and analytical chemists participating in the National Institute for Occupational Safety and Health's Regional Training Program. The purpose of this Manual is to assist these professionally qualified, but possibly inexperienced, instructors in the preparation and conduct of a one-week "Industrial Hygiene Chemistry" course. This Manual will guide instructors through both lecture and laboratory lessons. It is complemented by a matching Student Manual. The course is recommended for students having, as a minimum, an undergraduate degree in chemistry (or its equivalent) along with at least one year's experience in instrumental analysis.

It is not necessary for instructors to have had prior teaching experience although such experience would be desirable. All instructors should be thoroughly familiar with industrial hygiene chemistry procedures, instruments and equipment relevant to the subject areas they will teach. In addition, each instructor should attend the course director's orientation seminar presented before the start of each one-week "Industrial Hygiene Chemistry" course.

The remainder of this introduction describes the course objectives, lessons, and the organization and format of the documentation in each lesson, including lecture and laboratory lesson plans.

Course Objectives

The following course objectives will be attained by graduates of this program:

- Given a particular chemical health hazard commonly found in the occupational environment, the trainee will be able to select an appropriate sampling strategy using available sampling techniques and to select a corresponding appropriate analytical method for quantitative characterization of the sample by using his knowledge gained from the course and technical information referenced in the course.

- Trainee will be able to apply his knowledge of wet chemical and/or instrumental analysis in employment of current methodologies for evaluating the typical work environment.

- Trainee will be able to perform and evaluate quantitative analytical determinations for four classes (types) of hazardous substances using a correspondingly different method for each class or type.

- Given the analytical results obtained through proper measurement procedures, the trainee will be able to define the data in terms of actual environmental concentration levels and to interpret the results in light of existing exposure standards.

Lessons

18 lessons are presented in this course:

- Introduction to Course
- Introductory Topics
- Direct Reading Instruments
- Air Flow Calibration and Sampling
- Ion Selective Electrode Laboratory
- Introduction to Spectrophotometry
- Instrumentation and Application of Spectrophotometry
- Colorimetric Determination of Free Silica (Quartz) Laboratory
- Introduction to Spectroscopy
- Atomic Absorption Spectrometry
- Atomic Absorption Spectrometry Laboratory
- Introduction to Chromatography
- Insturmentation and Application of Chromatography
- Gas Chromatography of Organic Solvents Laboratory
- Titrametric Determination of SO_2 Laboratory
- Colorimetric Determination of SO_2 Laboratory
- Biological Monitoring
- Related Topics

Lectures

Each lesson that is to be presented as a lecture is documented in a standardized format.

A. Lecture Cover Sheet

A cover sheet for each lecture presents the following information:

- Lesson title
- Lesson number and length
- Behavioral objective
- Scope of the lesson
- List of visuals
- List of exhibits
- List of equipment needed for the lesson

B. References

After the cover sheet, there is a list of references. These references are keyed to the paragraphs within each lesson. The number in parenthesis following each paragraph is the reference number. These references are included so that the instructor, if he wishes, may further research specific instructional subject matter.

C. Additional Readings

Following the reference list, in most lessons, is another listing called "Additional Reading." This bibliography contains books and articles which are generally pertinent to the subject area covered in this lesson. These are considered as important secondary reference sources.

D. Expanded Outline (left-hand page)

On the left-hand page, beginning after the Additional Readings section, is an expanded outline. This outline indicates the information that should be emphasized and covered during the lecture. The sequence of the outline should be followed during

teaching. The expanded outline gives sufficient information to explain the brief outline which is on the right-hand page. All test questions (both self tests and course evaluator) come from the expanded outline. Additionally, there are descriptions of the visuals within the outline.

E. Brief Outline (right-hand page)

This page consists of a notes column and the outline.

1. Notes Column - times (both elapsed and projected) are indicated in this column. The elapsed time designates the time it should take the instructor to reach this point in the lecture starting from 0 at the beginning of each lecture. The elapsed time is in parentheses. The projected time designates the time it should take the instructor to reach the next major portion of the outline. A major portion of an outline is designated by a capital letter in the outline. In addition, transitional phrases connecting the major outline portions are included in the notes column. These phrases are to assist the instructor in bridging from one section of the outline to the next. Notations of what visual, exercise, table, etc., should be introduced at a given point in a lesson and miscellaneous notes to the instructor are contained also in this column.

2. Outline - this is a brief outline corresponding to the expanded outline on the facing page. Words and phrases in the brief outline key the instructor to the lesson's subject content and to the expanded outline on the left-hand page. There is sufficient space between the key words in the brief outline for the instructor to write his own additional notes when he is preparing his lecture.

F. Exercises and Problems

In some lessons, exercises and problems are included. These are given during class time. The answers to the problems are worked out with students after they have had an initial try at completing them on their own. Answers are provided in the Instructor Manual.

G. Self Tests

Self tests are included after most lessons. The Instructor Manual contains the correct answers, whereas the Student Manual does not. The students should first answer the questions, and then the instructor should review the answers, explaining fully the reasons for the correct answers. The self tests are not scored by the instructor and no records are kept of the individual student's performance. The instructor should use the information from the discussion of self tests to remove student misunderstandings or lack of understanding.

H. Copies of Visuals

Copies of visuals that are to be shown in a lecture are included at the end of that lesson documentation. These can be useful in preparing for the lecture presentation.

I. Homework

No specific homework assignments are included within the lesson documentation. However, there is a great quantity of information for the students to absorb during this one-week course. Therefore, students should be urged to review nightly all lessons covered during the day and all lessons to be presented on the following day. In particular, they should become familiar with the laboratory procedures for the following day. There is much to be accomplished in every laboratory and little time to do it. If the students are familiar with the procedure, the laboratory experiments will progress much more smoothly.

Laboratories

Each lesson that is to be presented as a laboratory is documented in standardized format consisting of four elements.

A. Laboratory Cover Sheet

A cover sheet for each laboratory presents the following information:

- Lesson title
- Lesson number and length
- Behavioral objective
- Scope of the lesson
- List of equipment, apparatus and forms

B. Special Preparation Section

This section will follow the laboratory cover sheet, and includes specific directions that must be followed prior to actual class time. These instructions are concerned with the preparation of apparatus, facilities, chemicals and materials that are necessary for the laboratory session.

C. Laboratory Procedures

The procedures for performing each laboratory are fully documented on the left-hand page. The elapsed and projected times are indicated for some lessons with the elapsed times appearing in parentheses. The right-hand page is a blank page for notes on specifics of the laboratory to aid the individual instructor in giving an efficient lesson.

D. Figures and Forms

Equipment figures and student forms are included after the procedures. The figures are presented to aid the instructor in setting up the experimental equipment. The forms are to be used by the students during the laboratory to assist them in recording, calculating and analyzing data.

LESSON TITLE	LESSON NUMBER	LESSON LENGTH
Biological Monitoring	17	1:00

BEHAVIORAL OBJECTIVE

The student will be able to describe the purposes, uses, and techniques associated with urine, blood, and breath analysis as they apply to biological monitoring.

SCOPE

Introduction
Urine analysis
Blood analysis
Breath analysis

VISUALS	EXHIBITS
None	None

EQUIPMENT

None

REFERENCES

LESSON TITLE

Biological Monitoring

1. American Conference of Governmental Industrial Hygienists. Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1974, Cincinnati, Ohio (1974).

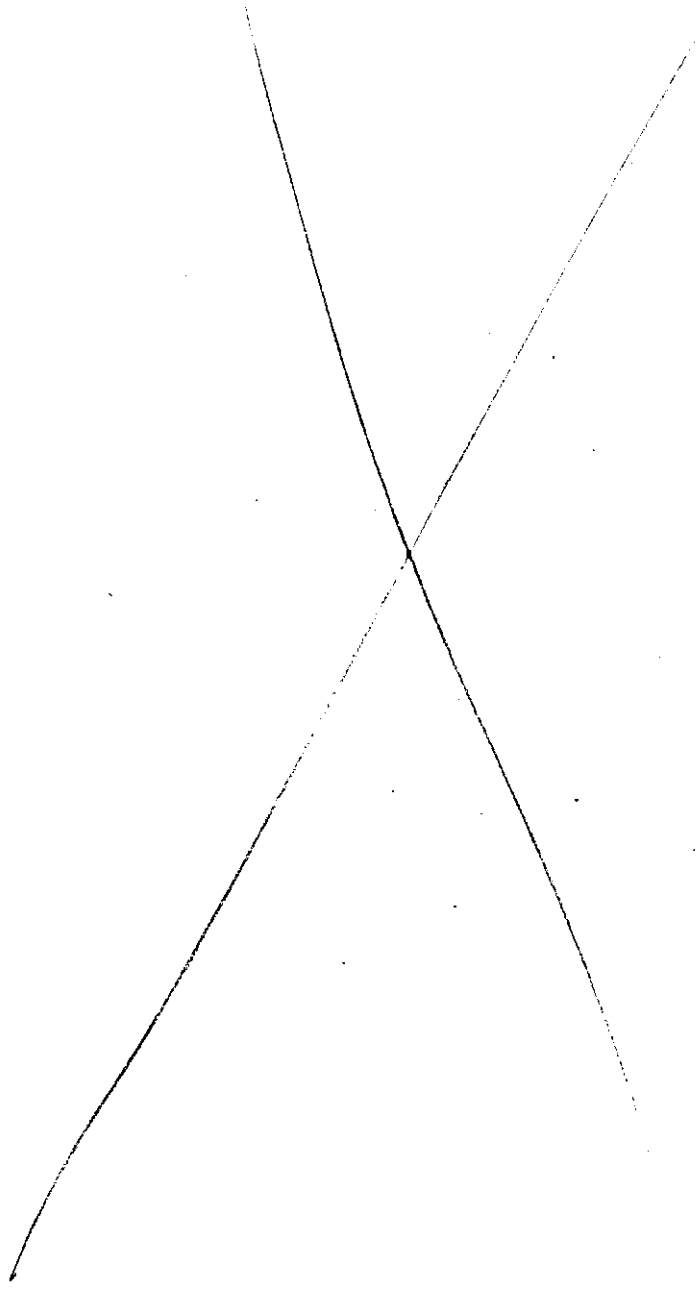
2. Linch, A. L. Biological Monitoring for Industrial Chemical Exposure Control, The Chemical Rubber Company Press, Cleveland, Ohio (1974).

3. Department of Labor, Occupational Safety and Health Administration. Subpart G--Occupational Health and Environmental Control--1910. 93, Federal Register, 23540, June 27, 1974.

4. U. S. Department of Health, Education and Welfare, Public Health Service, National Institute for Occupational Safety and Health. NIOSH Manual of Analytical Methods, U. S. Government Printing Office, Washington, D. C. (1974).

5. Koniecki, W. B., and Linch, A. L. Determination of Aromatic Nitro Compounds, Analytical Chemistry, 30, 1134, (1958).

6. Steere, N. V. Handbook of Laboratory Safety, 2nd ed., The Chemical Rubber Company Press, Cleveland, Ohio (1971).



TITLE	LESSON NUMBER
Biological Monitoring	17

A. Introduction

1. The worker himself is the ultimate sampler of his work environment. The most sophisticated sampling techniques yet developed cannot, with reasonable assurance, determine how much of a hazardous substance was absorbed and how the exposure affected the worker. The ultimate answer to the magnitude of the absorbed dose must be obtained from direct quantitative analysis of expired air, body fluids, or tissue for the hazardous substance or its metabolites, and by indirect observation of the effect on the functioning of the organs or tissues in which the toxic substance produces a reaction. With few exceptions, even the most hazardous chemicals exhibit a no-effect level below which exposure will be tolerated by most workers for an occupational lifetime without incurring any significant health impairment. This is the basis for the Threshold Limit Values (TLV's) which have been established by the American Conference of Governmental Industrial Hygienists. (1)

2. The ultimate goal in exposure control is to establish this no-effect concentration--Biological Threshold Limit Value (BTLV)--and then maintain absorption well below this level. A reasonable factor of safety is usually set at 50% of the BTLV for exposure control. The concept of materials balance based on classical engineering principles--the amount of material entering equals the amount of products plus by-products leaving the system--applies to industrial exposure control. The problem then reduces to the development and application of appropriate analytical methods. (2)

3. The three major routes of entry must be considered:

   - By mouth (oral) and absorption through the gastrointestinal (digestive) tract
   - By inhalation--absorption through the lungs
   - By skin contact--absorption directly through the epidermis. (1)

NOTES	Times (elapsed) projected	LESSON OUTLINE
	(---) 0:05	A. Introduction 1. Worker-samples of environment, TLV's 2. BTLV 3. Major routes of entry: . Oral . Inhalation . Skin contact

4. The route of entry frequently determines the rate of absorption and the toxicity potential. The route of excretion will determine the sampling technique: blood, urine or breath. In some cases, two or even all three excreting mechanisms are sampled and analyzed to provide supplementary information or to provide alternative methods. (1)

B. Urine Analysis

1. The collection of urine specimens from employees assigned to potential toxic chemical exposure areas provides the most economical, expedient and, for the individual, the least traumatic sampling method for biological threshold limit control. Almost without exception grab sampling rather than 24-hour collection provides adequate monitoring.

2. An example of a primary aromatic amino compound is "MOCA®" * "MOCA" over the past twelve years has been established as a commercially important curing agent for iso-cyanate containing polymers and epoxy resin systems. "MOCA" exhibits the general toxicity characteristics of the cyanogenic aromatic amines and can produce cyanosis when absorbed into the blood stream in sufficient quantity. In addition, "MOCA" has produced cancers in the rat for which assignment to the cancer-suspect agent list was made by the Federal Government in 1974. (3)

3. Urine for biological monitoring of "MOCA" should be collected in borasilicate bottles which are cleaned by soaking in 1% aqueous Na_3 PO4. Detergents must be avoided as the absorbed film on the glass which is not removed by rinsing will produce interferences in the subsequent analysis. Plain milk dilution bottles capped with snap-on gum rubber caps are recommended. To each bottle add 1 ml. of aqueous citric acid to stabilize the urine and prevent decomposition of any "MOCA" which may be present. (2)

*"MOCA" is DuPont's registered trademark for 4, 4' methylene bis (2-chloraniline) a diamine curing agent for isocynate-containing polymers.

NOTES	Times (elapsed) projected	LESSON OUTLINE
		4. Route of entry--rate of absorption and sampling techniques
(Transition A.-B.) From a very general discussion to the specific procedure of urine analysis.	(0:05) 0:20	B. Urine Analysis 1. Urine analysis in general
		2. Primary aromatic amino acid--"MOCA"
		3. Urine collection for "MOCA"

4. A 5 ml. aliquot of the urine specimen is acidified with 1 ml. concentrated HCl and heated at 80°C for 30 minutes to hydrolyze the acetyl amino derivatives. The mixture is diluted to 20 ml. and aliquoted to 2-25 ml. volumetric flasks. After cooling to 0°-5° C both aliquots are diazotized by adding 1 ml. $NaNO_2$ reagent. After one minute the excess HNO_2 is destroyed by adding 1 ml. sulfamic acid reagent, let stand 20 minutes in the ice packs, and finally develop the red-blue azo dye by adding 0.5 ml."diamine " coupling reagent (N-1-naphthyl ethylenediamine) to one aliquot only. The second aliquot serves as a reagent blank. Warm to room temperature (23°-27° C) for 15 minutes, dilute to 25 ml., mix and determine color density at 540 nm. against the blank. The micrograms of "MOCA" are read from a calibration graph or chart. Multiply this result by 0.20 to convert to mg./l. urine. If a neutral coupling (sodium acetate buffer) with Chicago Acid (8 amino-1-naphthol 5, 7 disulfonic acid) is carried out, the mixture of azo dyes usually obtained from "MOCA" and its metabolites, and natural occurring aromatic amines from protein metabolism can be separated into characteristic colored bands by paper chromatography. (2)

5. "MOCA" can be quantitatively extracted from neutral urine with dichloromethane (DCM) and separated from other urinary components by thin layer chromatography (TLC) on alumina gel film with solvent development (separation). After elution, the "MOCA" is measured colorimetrically. Some of the "MOCA" is excreted as a conjugate with glucuronic acid. Enzymatic hydrolysis with B-glucuronidase which is more efficient than acid hydrolysis is necessary before extraction. The pH of 25 ml. of urine is adjusted to 7.5, add 0.1 ml. of enzyme reagent and incubate at 37°C for four hours. Adjust the pH to 10 and extract with 3-10 ml. portions of DCM. The combined extracts are evaporated to dryness, and the residue dissolved in 0.5 ml. acetone. Streak a 50 ml. aliquot on an alumina film and develop to 10 cm. with the developer (mixture of acetic acid and ethyl acetate). A "MOCA" known should be streaked along side to locate the "MOCA" position on the plate, and provide a calibration standard. The "MOCA" spots are located by observation under UV light. The spots are extracted and quantitated by the diamine coupling procedure. The major metabolite of "MOCA", with a R_f of 0.2, can be recovered and analyzed by this procedure. "MOCA" also can be extracted from alkaline urine hydrolisate with diethyl ether. (2)

NOTES	Times (elapsed) projected	LESSON OUTLINE
It is not necessary to dwell on the particulars of the procedures. The following procedures are presented to give the students examples of practical applications.		4. Analysis--total aromatic amines 5. Analysis by TLC

6. The flame ionization gas chromatography procedure is more rapid and sensitive than TLC, and yet retains specificity. Sensitivity down to 0.04 mg./l. (40 parts per billion-PPB) of "MOCA" in urine can be obtained consistently with close attention to analytical details. A 25 ml. aliquot of ether obtained from the extraction of 50 ml. of urine made alkaline by adding $NaHCO_3$ with 50 ml. of ether is evaporated to dryness. Add 1 ml. trifluoro acetic anhydride and again evaporate to dryness. The sample is not stable at this stage; therefore, proceed at once to the final steps. Each day prepare two "MOCA" standards for calibration. Add 1 ml. of internal standard reagent (triphenylamine-TPA-in anisole) and mix. Depending upon instrument sensitivity, inject 1 to 4 ml. of the final solution into the gas chromatograph (GC) and proceed with the analysis. Measure the areas of the TPA and "MOCA" peaks, and equate the ratios of areas. A plot on linear graph paper of the calibration peak areas ("MOCA"/TPA) vs. weight ratios of "MOCA"/TPA should yield a straight line with 0.50 slope:

$$\text{wg."MOCA"} = \text{wg. TPA} \times \frac{\text{"MOCA" area}}{\text{TPA area}} \times \frac{1}{\text{calibration curve slope}}$$

$$\text{mg./l.} = \frac{\text{wg."MOCA" x 1,000}}{\text{1,000 x ml. sample}} \qquad (2)$$

7. With slight modifications, these procedures can be applied directly to air analysis. For fall-out surveys, 10 ml. of di-n-butyl maleate is measured into an aluminum tray (127 x 160 x 25 mm.) and after a suitable exposure period (4 to 21 days) is mixed. A 1 ml. aliquot is removed and acetylated with trifluoracetic anhydride as described for urine analysis. From that point, the GC analysis proceeds as already described. Meta-tolylenediamine and ortho-chloroaniline also can be analyzed by this method. (2)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		6. Flame ionization gas chromatography
		7. Similar procedure for fall-out surveys

8. The filter-microimpinger assembly provides the sample collection for personnel or station monitoring. An aquous mixture of acetic and hydrochloric acids is used for gas phase aromatic amine collection. After sampling for 7 hours at 480± 25 ml. per minute, extract the filter membrane with 25 ml. diethyl ether and proceed with GC analysis as for urine. Neutralize the liquid reagent with NH_4OH, extract with 25 diethyl ether and proceed with the GC method described for the urinary ether extract. (2)

9. The procedure described in this section can be applied directly to most aromatic primary amines such as the toluidines, benzidines, chloranilines, nitroanilines, chlorotoluidines, anisidines, phenetidines, alpha-naphthylamine, tolylenediamines, etc., for both urine and air analysis. (2)

10. The dithizone method for analysis of lead will determine total lead in blood and urine but will not differentiate between lead and bismuth (positive interference). Measure the volume of the urine specimen, or weigh the blood specimen into a silica evaporating dish. Rinse the specimen container with concentrated acid which in the case of urine is added to the specimen in the graduated cylinder. If the total volume of urine plus acid is greater than 100 ml., measure some into a silica (quartz) evaporating dish, otherwise transfer the entire sample plus rinsings. Prepare blanks by aliquoting 20 ml. portions of conc. HNO_3 into silica dishes. Evaporate the contents of the evaporating dishes to dryness, and char by heating on a hot plate. Then burn to a white ash in a muffle furnace at 490± 10°C. Allow to cool on a low temperature hot plate and collect the residue in 0.5 ml. conc. HCl and 2-3 ml. distilled water. Add 20 ml. water and dissolve the ash by gentle warming, transfer to a 125 ml. calibrated squib separatory funnel and dilute to 40 ml. Then add 30 ml. "poison" buffer (ammonium cyanide-sodium sulfite-ammonium citrate in conc. NH_4OH), mix thoroughly and extract the lead into 10 ml. dithizone reagent (chloroform solution of dithizone). If the extract is noticeably red, re-extract with another 10 ml. of dithizone reagent. Combine the extracts and determine the optical density at 510 nm. From a calibration chart prepared by plotting on semi-log paper the percent transmission (linear graph paper if optical density units are used) versus known micrograms of lead read off the micrograms of lead and calculate in terms of mg./l.:

NOTES	Times (elapsed) projected	LESSON OUTLINE
		8. Procedure for personnel and station monitoring 9. Procedures applied to most aromatic primary amines 10. Dithizone method for analysis of lead

$$\text{Urine-mg./l.} = \frac{(\text{total mg. found-blanks}) \times (\text{vol. urine} + \text{vol. } HNO_3) \times 1,000}{(\text{vol. urine}) \times (\text{vol. analyzed}) \times 1,000}$$

$$\text{Blood-mg./100g.} = \frac{(\text{total mg. found-blank}) \times 100}{\text{sample wt.} \times 1,000}$$

A double extraction should be used for blood, and urine which produces a sediment in the dithizone reagent extraction step. Drain the chloroform-dithizone extract into another 125 ml. squib separatory funnel containing 40 ml. distilled water and 0.5 ml. conc. HCl to extract the lead back into an aqueous phase. Discard the chloroform phase and wash the aqueous layer with 10 ml. chloroform. Again discard the chloroform, add 30 ml. of "poison" buffer and mix thoroughly. Extract the lead into 10 ml. of dithizone reagent and determine optical density. More than 10 mg. of total lead in the 10 ml. of dithizone reagent will produce an optical density which exceeds the range of instrumental reliability. Therefore, the aqueous extract from the double extraction procedure (dilute HCl) should be made up to 50 ml. and a 10 ml. aliquot removed for the final steps in the procedure when more than 10 mg. of lead is present. (2)

11. These procedures for urine analysis are examples of a large number of available analytical methods. For example, methods for fluoride in urine have been documented by the AIHA Biochemical Assay Committee as a Biological Monitoring Guide. The dithizone method also can be modified to determine other heavy metals such as mercury, cadmium and zinc. (2)

12. The current trend in analytical methods for heavy metals in blood and urine favors atomic absorption techniques, for example, lead in blood or urine. Whole blood or urine specimens are ashed with a mixture of nitric and perchloric acids to destroy the organic matrix. The ash is dissolved in a dilute nitric acid solution and the pH adjusted to 2.6-3.0. The lead is chelated with ammonium pyrarrlidine dithiocarbamate (APLD) and extracted into methyl isobutyl ketone (MIBK). An aliquot of this extract is placed in a sample boat of an atomic absorption spectrophotometer and the absorbance determined at 217nm. (4)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		11. Other procedures are available
AA techniques are covered in depth in Lesson No. 10		12. Analysis of lead in blood or urine by AA

13. The flameless atomic absorption technique also has received wide attention for mercury analysis. The urine specimen is digested with strong nitric acid. Stannous chloride is added to reduce ionic mercury to elemental mercury which is purged from the solution in a gas stream and passed through the UV photometer unit set to detect the absorption of the 254-nanometer radiation level by the mercury vapor. The mercury concentration then is determined from a calibration chart based on known amounts of mercury. The blood procedure requires cold digestion of the specimen with sulfuric acid, then oxidation with permanganate and HNO_3 to oxidize the organic matrix before reduction to elemental mercury with stannous chloride. (4)

14. In many cases of exposure to toxic organic compounds other than aromatic nitro and amino compounds, the offending compound or its major metabolite can be detected in urine with a sensitivity which will be adequate for biological monitoring. Benzene is a typical example. Exposure to benzene will be manifested in the urine by elevated phenol and sulfate ester concentration. In fact, a BTLV for benzene has been established on the basis of phenol excretion. The ceiling TLV for benzene in air of 25 ppm (10 ppm is an intended change) corresponds to 200 mg./l. of phenol in the urine.

15. Toluene can be monitored on the basis of increased hippuric acid excretion. At the 100 ppm TLV for airborne toluene, the BTLV for its metabolite--hippuric acid--is 3,000 mg./m^3. Exposure to DDT also can be controlled by limiting the urinary excretion of its metabolite-DDA. (2)

C. Blood Analysis

1. Blood analysis usually is reserved for those occupational exposure problems which cannot be monitored successfully by urine or breath analysis. There is some trauma associated with the drawing of a blood specimen, even by ear stab, which may contribute to employee morale problems if frequent sampling is attempted. However, this procedure can be justified when valuable information relative to the effects of occupational contact

NOTES	Times (elapsed) projected	LESSON OUTLINE
		13. Flameless AA for analysis of urine
		14. Benzene detected in urine
		15. Toluene monitored by increased hippuric acid excretion
	(0:25)	C. Blood Analysis
(Transition B.-C.) From analysis of urine to blood.	0:20	1. Rationale for blood analysis

with toxic chemicals is obtained for exposure control and for diagnosis of occupational disease. Observation of benign and reversible effects produced by the invasion of toxic metals, solvents, drugs, cyanogenic compounds, etc., can provide benefits for the industrial physician and hygienist which cannot be obtained in any other manner. (2)

3. A successful industrial medical surveillance program developed for monitoring the exposure of workmen to the cyanogenic aromatic nitro and amino compounds was based upon methemoglobin analysis to detect acute effects of exposure and hemoglobin analysis to detect of chemically induced anemia (loss of hemoglobin). Cyanosis is caused by tissue oxygen deficiency and occurs when the oxygenated hemoglobin is depressed below the critical oxygen demand level. (2)

4. Chemically induced anoxia can be produced by:

   - Reactive gases and vapors absorbed through the lungs (CO, H_2S, HCN, nitrobenzene and aniline)

   - Ingestion of certain nitrogenous compounds (nitrates, nitrites, sulfides, some medicinals such as the "sulfa" drugs, acetanilide, etc., and certain improperly cooked foods such as fava beans).

   - Lipoid (fat) soluble liquids and solids absorbed directly through the intact skin (dimitrobenzene, para-chloroaniline, nitroanilines, toluidines, etc.) (2)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		3. Cyanosis--anemia control program 4. Anoxia produced by: . Reactive gases and vapors . Nitrogenous compounds . Directly through skin

5. Cyanosis is not an uncommon industrial illness and a number of deaths have been reported. Most aromatic nitro and amino compounds are not in themselves cyanogenic, but the oxidation-reduction (redox system) enzyme systems induce metabolism to produce highly potent nitroso and hydroxylamine derivatives. An atom of iron in the ferrous state must be present in the hemoglobin (Hb) complex in order for the blood stream to carry oxygen. The cyanogenic aromatic metabolites interfere with yet another enzyme system which maintains iron in the reduced state. When this condition develops, iron is oxidized to the ferris state and methemoglobin (MHb), which cannot carry oxygen, is produced. Since MHb is not a normal blood constituent, except in trace concentrations, analysis for MHb provides positive diagnosis, and exposure control for cyanosis. (2)

6. Micro methods for the rapid determination not only for MHb and total hemoglobin (Hb) but also for the precusors of MHb are available. Direct instrumental analysis for hemoglobin (HbO_2) also has been developed. Direct determination of oxygen transport capacity is necessary for monitoring nitrochlorobenzenes which do not always produce MHb proportional to the amount of toxicant absorbed. (2)

7. An effective cyanosis control program can be based upon the urine analysis for the excretion of aromatic nitro and amino compounds and their metabolites on a monthly basis, and indirect blood analysis on a quarterly basis for the effects produced by contact with these cyanogenic agents, and for detection of the hypersensitive individuals in the work force. (2, 5, 6)

8. A resume of the analytical procedure for the rapid determination of MHb will serve as an example of exposure evaluation by indirect means. However, the student must understand that for purposes of exposure control, some knowledge of the magnitude of the absorbed dose must be established, (i.e., urine or breath analysis and personnel monitoring for the airborne toxic hazards) in order to establish a BTLV. One-tenth ml. of freshly drawn blood is rinsed into a spectrophotometer curvette containing 7.0 ml. of M/60 phosphate buffer. The red corpuseles are hemolyzed with one drop of saponin solution and the % transmittance (or optical density) determined at 630 nm. (%T.) against a buffer blank containing one drop of saponin. The Hb

NOTES	Times (elapsed) projected	LESSON OUTLINE
		5. Harmful effects by aromatic nitro and amino compounds 6. Micro methods for rapid determination for MHb, Hb and precursors 7. Cyanosis control program 8. Procedure for rapid determination of MHb

then is oxidized to MHb with excess potassium ferricyanide solution (1 drop of 10% reagent) and the optical transmittance again read at 630 nm. ($\%T_2$) against the original blank. From a semi-log chart on which %T (or optical density) is plotted on the logarithmic axis and %MHb is plotted on the linear X axis, read off the %MHb. The reference point on the left Y axis is obtained from the average of a number of analyses of normal blood. To determine %MHb, connect the reference point to $\%T_2$ on the right-hand Y axis by a straight line, extend %T, horizontally to the diagonal, and drop a vertical line from the intersection to the horizontal %MHb (X) axis. (6)

9. One major category which will be cited as another example of blood analysis for the detection of the effects of toxic insecticides within the human body includes the organic phosphate poisons which inhibit cholinesterase enzyme activity in the nervous system. Acetylcholine, which performs the function of a chemical mediator for nerve impulse transmission across the synaptic junctions, or from nerves to muscles and glands, is rapidly hydrolyzed to choline and acetic acid by the enzyme-cholinesterase. When this enzyme is inhibited, acetylcholine does not hydrolyze; therefore, it accumulates at the nerve junctions, and nerve impulses are not transmitted. Convulsions, muscle paralysis and death of the organism follow. Complete regeneration of the enzyme system requires up to 90 days. (2)

10. Exposure control programs for manufacturing and field application usually are based on blood cholinesterase activity which can be determined on the job with available field kits. Acetylcholinesterase activity is determined in whole blood by measuring the increase in the yellow color produced when thiocholine reacts with dithiobisnitrobenzoate. The thiocholine is liberated from the substrate-acetylthiocholine by the enzyme. Chlorinated hydrocarbons have been detected directly in the blood stream by gas chromatography. Aldrin, Dieldrin, and DDT have been investigated well enough to justify this technique for exposure control within limits which are well below the health jeopardy threshold. (2)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		9. Example of insecticide exposure--organic phosphate poisons
		10. Exposure control programs

11. With slight modification, the analytical methods for lead, mercury, cadmium, arsenic, antimony, beryllium and chromium in urine can be applied to blood. The two major procedures--dithizone and atomic absorption spectroscopy--have been discussed already in this lesson. In general, urine analysis is preferred as the collection of specimens does not require intervention by medical personnel.

12. Carbon monoxide has a greater affinity for hemoglobin (Hb) than does oxygen which is displaced when CO is present in the inhaled atmosphere. The anoxia thus produced is similar in many ways to the state of cyanosis produced by the aromatic nitro and amino compounds. A number of analytical procedures for quantitating the concentration of this Hb derivative--HbCO--have been proposed. (2)

13. Oxyhemoglobin in oxalated blood is completely reduced in the presence of small amounts of sodium hydrosulfite, whereas carboxyhemoglobin is not affected. The spectral absorbance curves of oxyhemoglobin (HbO_2) and carboxyhemoglobin (HbCO) are different. Hemolysis is completed with the addition of NH_4OH. The ratio of absorbance at 555 nm. and 480 nm. is directly proportional to the percent HbCO in the blood. The difference in concentration obtained by this procedure compared to results obtained by the Van Slyke gasometric procedure are not significant at the 5% level. For 1 ml. of blood the sensitivity is 0.5% saturation. (2)

14. The classical gasometric procedure developed by Van Slyke has been reduced to easily portable microdimensions. The O_2 and CO in a 0.04 ml. specimen are swept by CO_2 into the barrel of a 1 ml. glass syringe which has been sealed to a 50 unit capillary microburett. After sequential absorption of the CO_2 and O_2, the volume of CO bubble is determined and equated to the HbCO concentration. (2)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		11. Analytical methods for heavy metals in urine applied to those in blood 12. CO combines with Hb 13. Direct determination of carboxyhemoglobin 14. Gasometric procedure for determination of CO in blood

15. The length of stain detector tube for CO has been used for the detection of CO in the gases released from a blood specimen after addition of strong acid, or after oxydation with ferricyanide. A field kit based on this principle is commercially available. However, the results will be no more than semiquantitative due to the $\pm$ 25% deviation range inherent in the response of the detector tube itself. (2)

16. The microdiffusion technique can be adapted to a field kit. A diffusion chamber is fabricated from a small petri dish by sealing in a concentric ring to form an inner chamber into which a dilute solution of palladium chloride is added. Blood and sulfuric acid are introduced into the outer chamber. The assembly is sealed with a cover plate and let stand at room temperature for an hour. CO reduces $PdCl_2$ to colloidal Pd metal:

$$CO + PdCl_2 + H_2O \longrightarrow Pd + CO_2 + 2HCl$$

The liberated HCl may be titrated or the residual $PdCl_2$ determined colorimetrically by any one of several different techniques which could be adapted easily to field kits. (2)

17. Gas chromatography is considered the best choice for CO in blood analysis when maximum reliability is required. Methods for transferring the gases liberated from blood oxidized with ferricyanide have been described in detail. For the gas chromatograph a 16 meter x 3.9 mm. OD stainless steel column packed with 60-80 mesh molecular sieve 5A followed by a katharometer detector has been recommended. (2)

18. The microdiffusion technique described for carbon monoxide can be applied to alcohols, ketones, aldehydes and other vapors which will reduce bichromates to the green chromium salts. In this method 3.0 ml. of 0.05N bichromate in 5N sulfuric acid is pipetted into the center compartment and 1.0 ml. of 20% sodium carbonate is added into the outer compartments of the Conway dish. Then 0.5 ml. of blood is added to the sodium carbonate reagent and the cover sealed to the dish with a light application of silicone grease. To three other dishes in turn 0.5 ml. of 0.075, 0.15 and 0.20% ethanol in water is added for calibration. Incubate 20 minutes at 90°C and either visually compare the bichromate solution from the blood specimen with the standards and estimate % ethanol, or measure color density in a spectrophotometer at 600 nm. (2)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		15. CO determination in blood by detector tube 16. Microdiffusion technique for determining CO in blood 17. GC for determination of CO in blood 18. Microdiffusion technique for determination of alcohol and volatile solvents

19. Pocket sized kits for drawing blood by capillary action from either a finger or an ear stab with a small lancet, and analyzing for cholinesterase activity, methemoglobin, blood sugar, hemoglobin, urea nitrogen, phosphates, etc., are commercially available. (2)

D. Breath Analysis

1. Breath analysis for the evaluation and control of exposure to the vapors from volatile solvents, carbon monoxide, aromatic hydrocarbons and certain volatile metallic compounds such as nickel carbonyl has been increasingly used over the past ten years. The concentration of volatile components in exhaled breath is directly related to the concentration in the bloodstream which, in turn, is related to the exposure concentration, duration of exposure, and time elapsed after termination of exposure (decay period). When the time factors are known, a good estimate of the exposure concentration can be estimated. After fixed station monitoring, a grab sampling survey, or even after personnel monitoring has been carried out, the ultimate question of whether or not the results actually relate to the magnitude of the absorbed dose must be answered. In many cases the relationship between expired breath, blood and urinary excretion has been studied and the results published. (2)

2. Bags fabricated from a variety of sheet plastics (polyvinyl chloride, polyethylene, polyester, polytetrafluoroethylene, polyvinyl fluoride, etc.) are commercially available and are quite well suited for collection of breath samples. Since these bags are flexible, but not elastic, a predetermined volume can be obtained in the field. Sizes of 6 to 10 liters capacity are the most useful. For convenience in transporting and storage, silica gel or activated charcoal can be added and after the target components have been absorbed, the bag can be deflated. (2)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		19. Field blood analysis kit
(Transition C.-D.) From blood to breath analysis.	(0:45)	D. Breath Analysis
	0:10	1. Reason and uses for breath analysis
		2. Use of plastic bags for breath samples

3. The considerations associated with collection of samples in bags are the following:

   - Type of plastic which governs diffusion rate through the film, absorption on the film and water vapor retention

   - Type of valve and sealing methods

   - Methods for introducing and removing samples

   - Half-life of the contaminant in storage, especially for those vapors which oxidize, decompose or react with other coexisting components of a mixture readily. When collecting breath samples this decay rate for the span of time between sample collection and analysis should be determined. This precaution also applies to glass containers. For example, the diffusion of CO through rubber balloons progress at approximately 2% per hour over a 48-hour period. (2)

4. When highly sensitive detection methods (e.g., a gas chromatograph fitted with an electron capture detector) are available, and the identity of the vapor has been established, breath samples can be collected in 50 ml. glass tubes fitted with screw caps on each end. This technique serves quite well for surveys and routine monitoring where large numbers of repetitive specimens are collected directly from the employees while on duty. The most expedient approach to the collection of representative samples is the total expired breath technique. The subject is instructed to exhale three times through the open pipette, then after exhaling the fourth breath through the tube immediately cap the ends to trap the specimen. The hole drilled through one cap is sealed with several layers of Saran through which a hypodermic needle can be inserted to withdraw a sample for injection into a gas chromatograph. (2)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		3. Bag considerations: . Diffusion . Sealing . Introducing and removing samples . Half-life contaminents 4. Using glass pipettes for breath sample

5. Infrared spectroscopy (IR) provides specificity, sensitivity, versatility, speed and simplicity. Solids, liquids and gases can be analyzed, with the published spectra providing the data needed for identification and quantitation of the components present. For air or breath analysis, IR radiation is projected through a long-path cell (usually 10 meters) fitted with IR transmitting windows, dispersed, then detected. Each component in the mixture absorbs the radiation in a pattern which characterizes its structure. A graph which displays absorption vs. wave length (spectrum) serves to identify each component in a mixture. The computor is a useful tool for "unscrambling" the plots since many organic compounds possess complex spectra. The amount of IR absorbed is proportional to the quantity of vapor present. The absorbance of known concentrations provides a linear plot which relates the weight of contaminant to absorbance observed for quantitative analysis. Since oxygen and nitrogen are transparent to IR radiation, folded beam cells with path lengths up to 40 meters can be used to attain high sensitivity in the trace ranges. A wide variety of solvents for which data are available, include halogenated hydrocarbons, alcohols, ethers, aldehydes, ketones, fixed gases (Co, CO_2 and NH_3) and a variety of miscellaneous vapors--CS_2, etc. (2)

6. Probably the most widely used techniques are based on gas chromatography for separation and quantitation of the vapor components in breath samples.

7. The "Drunkometer" into which drivers who may have been imbibing alcoholic beverages exhale to establish intoxication is perhaps the most familiar application. These instruments are colorimetric oxidation-reduction systems which are based on the reduction of acidic permanganate solution; or the reduction of acidic bichromate reagents to the green color of the chromic salts. Procedures for aldehydes, ketones, halogenated hydrocarbons and aromatic hydrocarbons have been described in detail. (2)

NOTES	Times (elapsed) projected	LESSON OUTLINE
Lesson #6 and #7 discuss spectroscopy in detail.		5. IR spectroscopy used for analysis of breath samples
Lessons #12 and #13 discuss chromatography in detail.		6. GC widely used
		7. Wet chemical methods for breath analysis

8. Detector tubes have been used for the estimation of ethanol in breath, but the kits are no longer available. However, the bichromate length of stain tube could be recalibrated for this application. A number of other solvents can be analyzed in breath analysis provided the identify of the solvent vapor of interest is known and no coexisting interferences are present. However, the results could be no better than semiquantitative due to the inherent accuracy and precision limitations ($\pm$ 25% deviation range). (2)

9. Detector tubes can be employed for the estimation of the carboxyhemoglobin content of the blood by breath analysis when the accuracy rquired is not greater than $\pm$ 25%. In terms of CO concentration in the blood, at 50 ppm CO in the ambient air, the carboxyhemoglobin concentration would be 8.6 $\pm$ 2%. Frequently this range of uncertainty can be tolerated. Field kits are commercially available for this purpose. If better reliability is needed, then IR or GC should be considered. (2)

10. Detector tubes for a number of the halogenated aliphatic hydrocarbons are also available and could be applied to breath analysis. However, the same range of uncertainty ($\pm$ 25%) applies to these tubes, only one of which has been certified (trichloroethylene). (2)

E. Self Test

1. Test instructions and review of questions are presented.

NOTES	Times (elapsed) projected	LESSON OUTLINE
		8. Detector tubes used for analysis of ethanol in breath
		9. Detector tubes used for determination of carboxyhemoglobin in breath sample
		10. Detector tubes used for determination of halogenated aliphatic hydrocarbons in breath samples
	(0:55) Self test 0:05	E. Self Test
		1. Instruction and review
	(1:00)	

LESSON TITLE	LESSON NUMBER
Biological Monitoring	17

1. How do Biological Threshold Limit Values differ from Threshold Limit Values? (very briefly)

see A. 1-A. 2

2. Briefly, under what conditions is blood analysis applicable?

see C. 1

3. Generally, what is the relationship between breath and blood and urinary excretion?

see D. 1