American Industrial Hygiene Association Journal

ISSN: 0002-8894 (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/aiha20

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To cite this article: RAUL MORALES , STEPHEN M. RAPPAPORT & ROBERT E. HERMES (1979) Air sampling and analytical procedures for benzidine, 3,3′-dichlorobenzidine and their salts, American Industrial Hygiene Association Journal, 40:11, 970-978, DOI: 10.1080/15298667991430578

To link to this article: https://doi.org/10.1080/15298667991430578

Published online: 04 Jun 2010.

The purpose of the study was the development of new air-sampling and analytical procedures for benzidine, 3,3′-dichlorobenzidine and their salts. Air is drawn through a glass-fiber filter followed by a bed of silica gel to collect these substances as either particles or vapors. The compounds are extracted from the sampler and analyzed by HPLC with sensitivities in the range of 3 $\mu g/m^3$ for 48-L air samples. The methods were evaluated in the laboratory with test aerosol atmospheres and found to be unaffected by temperature or humidity of the sampled environment. Tests of precision, sample stability and separation from interferants indicate that the method should provide reliable results for personal monitoring procedures.

Air sampling and analytical procedures for benzidine, 3,3′-dichlorobenzidine and their salts

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introduction

The Department of Labor in 1974 promulgated standards for the production and use of 14 chemicals considered to be carcinogenic.[1] Among these were benzidine and 3,3′-dichlorobenzidine (and their salts), compounds which are used primarily in the manufacture of dyestuffs. It was estimated, in 1972, that 0.68 million kg of benzidine and 1.6 million kg of 3,3′-dichlorobenzidine were produced in the United States while an additional 0.64 million kg of 3,3′-dichlorobenzidine were imported.[2] Data concerning the use, toxicology and epidemiology of these two compounds have been extensively reviewed.[3-5]

Haley, in 1975, reviewed the methodology for analysis of benzidine and 3,3′-dichlorobenzidine and their metabolites in both environmental samples and biological tissues.[5] Typically, published procedures involved the colorimetric determination of reaction products of these compounds which were formed from diazotization, oxidation or reaction with aldehydes (to produce Schiff bases). These methods tended to be nonspecific for aromatic amines though some differentiation was possible depending upon the presence of specific interfering substances.

Reported air sampling methods incorporated impingers and bubblers to concentrate the compounds.[6,7] Impingers trap by impaction particles with aerodynamic diameters larger than ~0.8 μm and retain them in a liquid medium.[8] Both impingers and bubblers collect vapors by absorption in liquid media. Neither of these samplers efficiently traps small particles; thus, methods based upon their use may be inaccurate in applications where the compounds are present as aerosols.

This paper describes the development and evaluation of new procedures for the collection and subsequent analysis of benzidine, 3, 3′-dichlorobenzidine and their salts in air. The sampling method was initially tested with a similar compound, 4,4′-methylenebis(2-chloroaniline), MOCA, as previously reported.[9] The sampler, designed for personal sampling, employs two stages. The first stage is a filter which traps the compounds which are

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Work supported by the National Institute for Occupational Safety and Health and performed by the Los Alamos Scientific Laboratory operated under the U.S. Department of Energy, Contract No. W-7405-ENG-36 (NIOSH/LASL Agreement IA-74-35).

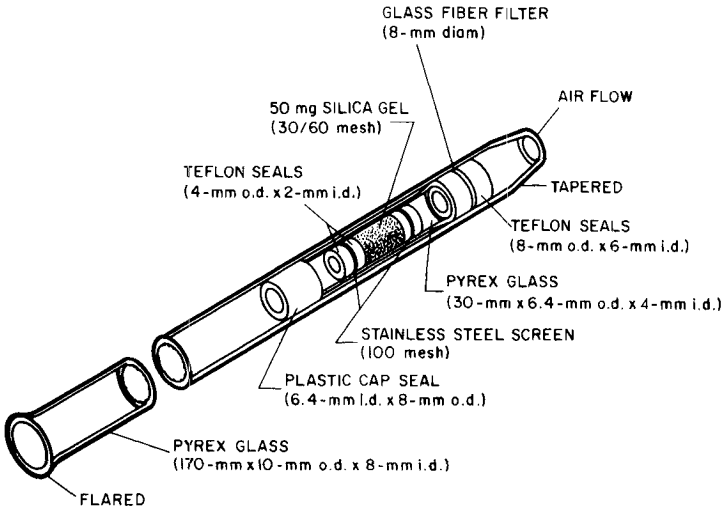


Figure 1 — Probe containing sampler stages.

present as aerosols (dusts, fumes). It is followed by a bed of silica gel which collects the vapors.

The analytical procedures utilize high performance liquid chromatography (HPLC) and provide sufficient sensitivity and resolution from interfering compounds for accurate analysis at low levels. The methods employ reverse-phase chromatography to measure the free amines. The salts are converted to the free amines prior to analysis. Young and McNair previously reported the HPLC of benzidine and many other aromatic amines.[10] Their analyses employed normal-phase (silica) columns and solvent systems.

experimental

reagents

Benzidine (technical grade) was obtained from two sources, Eastman Organic Chemicals (Rochester, NY), and as a gift from Litton Bionetics (Frederick, MD). The former was used in the generation of test atmospheres and the latter as an analytical standard. 3,3'-Dichlorobenzidine (technical grade), 3,3'-dichlorobenzidine dihydrochloride and benzidinium sulfate were obtained from ICN-K&K Laboratories (Plainview, NY). Triethylamine used in the extraction solution was obtained from Polyscience Corp. (Niles, IL). All solvents were distilled in glass as obtained from Burdick and Jackson Laboratories, Inc. (Muskegon, MI).

production of test atmospheres

Test atmospheres of benzidine and 3,3'-dichlorobenzidine were produced as described in previous reports.[9,11] Aerosols were generated by compressed-gas nebulization of melts of the free amines. Particle size distributions as determined with a cascade impactor were bimodal.[11] One distribution, accounting for 80-90% of the mass, had a mass median aerodynamic diameter (MMAD) of ~2.4 μm and a σ_g of ~1.6 while the other, accounting for 10-20% of the mass had a MMAD of ~0.1 μm and a σ_g of ~4. A series of dilution stages allowed dynamic atmospheres to be established over the concentration ranges of 20 $\mu g/m^3$ - 3.7 mg/m^3 for benzidine and 12 $\mu g/m^3$ - 3.1 mg/m^3 for 3,3'-dichlorobenzidine at a nominal flow rate of 300 L/min. All generation and sampling procedures were performed in a ventilated glove box to minimize contamination of the surrounding environment and exposures to personnel. A protocol outlining facilities and health practices for this investigation has been reported.[12]

air sampling

The sampling chamber[9] allowed as many as 10 samples to be collected simultaneously from a zone of homogeneous aerosol concentration. The temperature and humidity in the chamber could be maintained constant within the range of 25-40 ± 0.2°C and 0-95 ± 2% RH.

Samplers (contained in glass probes), were inserted through ports into the sampling

TABLE I
Challenge Aerosol Concentrations Under Various Environments of Temperature and Relative Humidity

Compound	Indicated Air Concentration, $\mu g/m^3$ ($\bar{x} \pm s$)		
	Temp. (°C)	R.H. = 20%	R.H. = 80%
Benzidine[A]	26	363±14.7	364±11.5
	35	382±20.9	365±13.9
3,3'-Dichloro-benzidine[B]	27	121±1.7	123±3.6
	37	124±2.4	123±2.5

[A]5 samples per group, sample volume = 18 L
[B]4 samples per group, sample volume = 16 L

TABLE II
Results of 2-way ANOVA to Determine Effects of Temperature, Humidity and Their Interaction on the Sampling Method

Compound	Variable	Degrees of Freedom	F[A]	p
Benzidine	Temperature	1,16	2.09	0.17
	Rel. Humidity	1,16	1.28	0.27
	Interaction	1,16	1.85	0.19
3,3'-Dichloro-benzidine	Temperature	1,12	2.60	0.13
	Rel. Humidity	1,12	0.810	0.39
	Interaction	1,12	1.52	0.24

[A]H_0: Variable has no significant effect upon air concentration; fixed variable model.

chamber. Each sampler (Figure 1) contained an 8-mm glass-fiber filter (Type A-E, Gelman Instrument Co., Ann Arbor, MI) followed by 50 mg of 30/60-mesh silica gel (G.C. Grade, D-08, 720-760 m^2/g, 4.3 g/cm^3, Applied Sciences Laboratories, Inc., State College, PA). Teflon rings held the filter in place. The silica gel was retained by Teflon rings supporting 100-mesh stainless steel screens. A one-hole rubber stopper connected to a plastic tube was inserted into the flared end of the probe for sampling. The flow rate was controlled by a critical orifice at 0.8 L/min.

The sampling method was evaluated to determine whether it was affected by temperature and/or humidity. The aerosol concentration was maintained constant and the temperature and relative humidity were varied in four combinations, i.e., two temperatures (~26 and 36°C) and two relative humidities, (20 and 80%) at each temperature. Groups of multiple samples (5 per group) were collected in random order at each temperature/humidity combination. By comparing the mean air-concentrations and standard deviations indicated by these sample groups, it was possible to infer whether the environmental conditions affected the accuracy and precision of the method.

analysis

Samples were stored in the dark at −15°C after collection and analyzed within a few days. Filters and silica gel sections of samplers were removed from the probes and placed in 1-mL volumetric tubes. One half mL of 0.2% triethylamine in methanol was added to each tube which was then shaken intermittently for 1 hour. This alkaline extraction solution was employed to dissolve the salts by converting them to the free amines.

Analysis was completed by injecting aliquots of the sample solutions into the HPLC. The

liquid chromatograph was a model ALC 202/401 (Waters Associates, Milford, MA) equipped with an ultraviolet detector at 254 nm. The 30-cm × 4.0-mm i.d. μBondapak C_{18} column (Waters Associates) was maintained at room temperature (23°C). Mobile phases were methanol/water (3/2, v/v) for benzidine and acetonitrile/water (7/3) for 3,3′-dichlorobenzidine. At a flow rate of 1.5 mL/min both compounds were eluted in ~3 min (k′ = 1.4). calculated efficiences were 2,800 theoretical plates for benzidine and 3,900 theoretical plates for 3,3′-dichlorobenzidine. Injection volumes were 10 μL for benzidine and 15 μL for 3,3′-dichlorobenzidine.

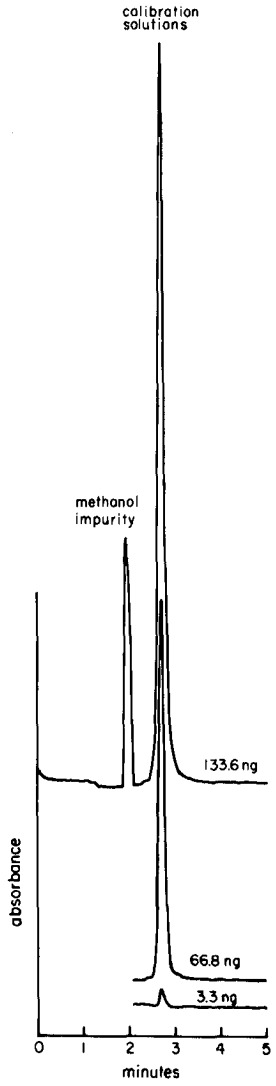


Figure 2 – Representative chromatograms of benzidine calibration solutions. Column: 30-cm × 4.0-mm i.d. μBondapak C_{18}. Mobile phase: Methanol/water (3/2) at 1.5 mL/min. Detector: UV at 254 nm and 0.04 AUFS.

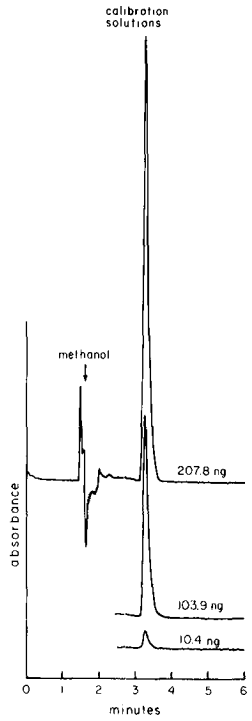


Figure 3 – Representative chromatograms of 3,3′-dichlorobenzidine calibration solutions. Column: 30-cm × 4.0-mm i.d. μBondapak C_{18}. Mobile phase: Acetonitrile/water (7/3) at 1.5 mL/min. Detector: UV at 254 nm and 0.04 AUFS.

Peaks were measured at a photometric range of 0.04 absorbance units full scale. Benzidine peak areas were quantitated manually by multiplying the peak height by its width at 1/2 the height to a minimum level of 3 ng (RSD ≤ 7%). Peak areas of 3,3′-dichlorobenzidine were quantitated with a Spectra-Physics (Santa Clara, CA) system IV-B integrator to 5 ng (RSD ≤ 7%). Below these levels of quantitation, detection was still possible but precision varied significantly with RSDs often in the range of 30-60%.

Experiments were performed to determine the stability of benzidine and 3,3′-dichlorobenzidine and their salts on the filter and silica gel components of the sampler. The most common salt of each amine was selected for testing, i.e., benzidinium sulfate and 3,3′-dichlorobenzidine dihydrochloride. Ten microliters of each analyte, dissolved in methanol (or in 0.2% triethylamine/methanol for the salts), were

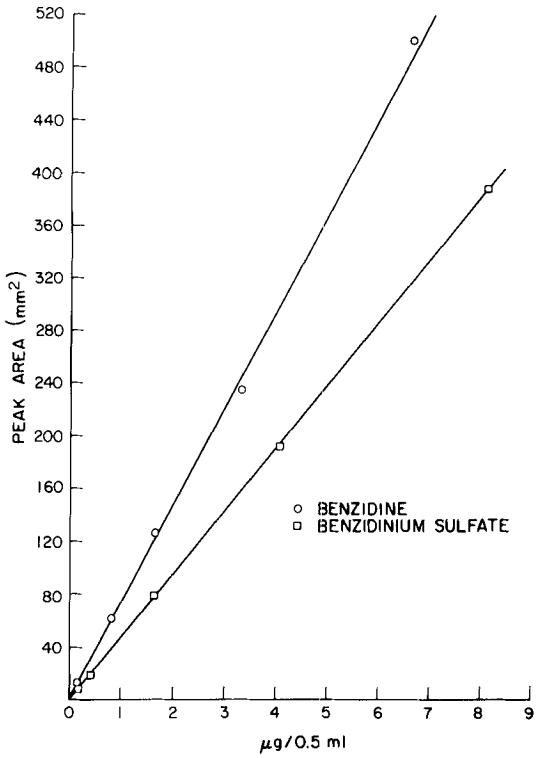


Figure 4 – Calibration curves for benzidine and its sulfate. Chromatographic conditions stated in Figure 2.

added to 50 mg of silica gel in a 1-mL volumetric tube. The tube was vibrated for 20 sec to assure homogeneous dispersion whereupon it was capped and stored in the dark at either room temperature (23°C) or in a freezer (−15°C). Filters were treated in the same manner but were not vibrated after spiking. After the necessary time interval (0-30 days) had elapsed, the spiked samples were extracted and analyzed as previously described. By comparing the recoveries of analytes it was possible to determine effects of storage conditions upon recovery of each compound.

determination of precision

The precision of the combined sampling and analytical procedures was determined over a range of sample sizes (mass analyte/sample). Three groups of 10 samples were collected in the chamber at 30°C and 80% RH. The air concentration and/or sampling volume were varied so that the amounts of compound collected covered approximately a 10-fold range.

results and conclusions

effects of temperature and humidity on sampling

Groups of samples were collected from environments which varied only in temperature and humidity. Any difference in challenge air concentrations indicated by these groups, more than predicted by chance, would be observed as an effect. The statistical procedure employed to determine whether there were significant effects was the 2-way analysis of variance (ANOVA) employing a fixed-variable model. This procedure allowed the simultaneous testing of 3 null hypotheses, i.e., there were no significant effects caused by temperature, humidity or an interaction of these two factors.

The challenge concentrations are given in Table I. For both compounds, all of the analyte was found on the filters. It appears, therefore, that in this experiment, there was insufficient vapor present to be detected. Mean air concentrations indicated by the samples were 368 $\mu g/m^3$ (6.6 μg/18 L sample) for benzidine and 123 $\mu g/m^3$ (2.0 μg/16 L sample) for 3,3'-dichlorobenzidine. An obvious outlier was detected in the 3,3'-dichlorobenzidine data. This

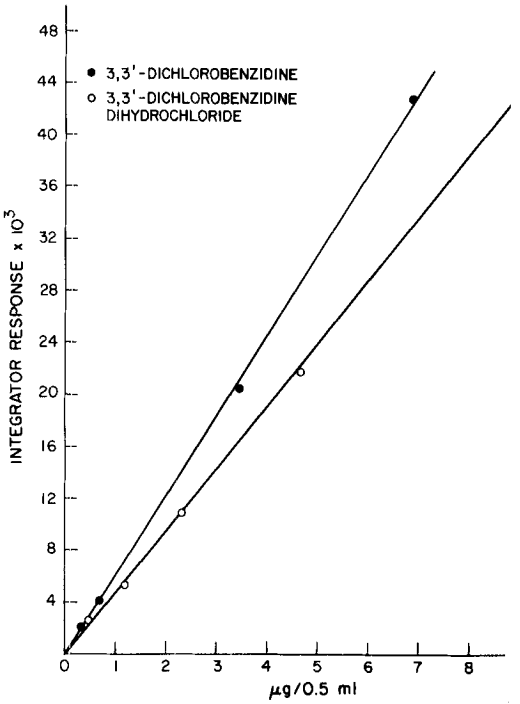


Figure 5 – Calibration curves for 3,3'-dichlorobenzidine and its dihydrochloride. Chromatographic conditions stated in Figure 3.

TABLE III
Recovery of Benzidine from Samples Stored at −15°C

Time (days)	% Recovered[A] Silica Gel 0.60 μg	Filters 0.20 μg	Filters 2.0 μg
0	98,104,90,92(96)	92,103(98)	105,98(102)
1	97,96(97)	110,107(109)	90,95(93)
3	97,95(96)	-----	----
7	90,95(93)	-----	----
11	83,92(88)	88,98(93)	98,96(97)
14	75,82(79)	-----	----
15	----	84,85(85)	91,95(93)
21	----	73,63(68)	80,85(83)

[A]Mean values indicated in parentheses.

sample and the 3 corresponding samples from the other experimental groups were eliminated from the data analysis yielding a total of 16 samples for the experiment.

An assumption of the ANOVA procedure is that there be no significant heterogeneity of variance among sample groups. Bartlett's Chi-Square Test[13] was performed for the four standard deviations in each data set and showed no significant heterogeneity to be present. The pooled estimates of the relative standard deviations (S_p) were 4.20% for benzidine and 2.14% for 3,3′-dichlorobenzidine.

Results of the 2-way ANOVAs are given in Table II which lists values of the F tests, degrees of freedom and p values for both data sets, The results indicate that none of the three variables (temperature, humidity, or interaction) significantly affected the sampling procedure. Thus, the method should be suitable for use in environments which vary considerably in these factors with no significant loss of accuracy or precision.

evaluation of analytical procedure

Figures 2 and 3 show chromatograms of benzidine and 3,3′-dichlorobenzidine, respectively, with experimental conditions as given. Figure 4 illustrates representative calibration curves for benzidine and its disulfate. Comparable curves for 3,3′-dichlorobenzidine and its dihydrochloride are shown in Figure 5.

Various compounds were investigated to determine if they would interfere with the analysis. One compound was found to interfere in each case, i.e., aniline in the analysis of benzidine, and 4,4′-methylenebis(2-chloroaniline) in the analysis of 3,3′-dichlorobenzidine. For solutions containing benzidine at concentrations of 8 ng/μL the following compounds did not interfere at concentrations ≤ 16 ng/μL: 2-, 3- and 4-chloroaniline, 4,4′-dichlorobenzidine, 4,4′-methylenebis(2-chloroaniline), hydrazobenzene and 1,2- and 1,4-naphthoquinone. For solutions containing 3,3′-dichlorobenzidine at concentrations of 24 ng/μL the following compounds did not

TABLE IV
Recovery of 3,3′-dichlorobenzidine from Samples Stored at 23°C

Time (days)	% Recovered[A] Silica Gel 0.55 μg	Silica Gel 5.5 μg	Filters 0.55 μg	Filters 5.5 μg
0	100,97,96(98)	99,103,101(101)	92,98,99(96)	97,99,103(100)
2	99,104(102)	110,102(106)	97,109(103)	105,111(108)
7	116,109(113)	112,116(114)	----	----
12	99,95(97)	99,104(102)	92,92(92)	103,106(105)
21	86,90(88)	95,93(94)	96,96(96)	103,97(100)
30	80,86(83)	90,90(90)	96,110(103)	104,102(103)

[A]Mean values indicated in parentheses.

TABLE V
Precision of Sampling/Analytical Method Over a Range of Sample Sizes

Compound	No. Samples	Sample[B] Mass (μg)	Sample Vol. (L)	Air Conc.[C] (μg/m³)	RSD (%)	S_p (%)[D]
Benzidine	10	3.03	48	63.2±1.43	2.26	4.19
	10	1.38	49	28.2±1.20	4.27	
	9[A]	0.34	16	21.1±1.10	5.21	
3,3'-Dichloro-benzidine	10	6.83	51	134 ±5.63	4.21	5.05
	7[A]	3.39	51	66.5±2.54	3.81	
	10	1.04	51	20.3±1.30	6.38	

[A]One or more samples were broken prior to analysis.
[B]Mean of samples within group.
[C]$\bar{x} \pm s$
[D]Pooled estimate of RSD; assumes no significant difference in sample variances.

interfere at concentrations ≤ 64 ng/μL: 2-, 3- and 4-chloroaniline, benzidine, aniline, 4,4'-methylenedianiline, 1- and 2-naphthylamine, N-methylaniline, 2-aminotoluene, and 4,4'-diamino-3,3'-dimethylbiphenyl.

stability of samples

Filters and silica gel which had been spiked with benzidine and its salt showed pronounced losses of both compounds under certain storage conditions. Recoveries of samples stored at 23°C for 8 days were only 70-80% while those of samples stored at 5°C for 8 days were between 79 and 84%. Degradation was suspected rather than irreversible adsorption because chromatograms of these samples exhibited shoulders on the benzidine peaks.

Benzidine samples stored at −15°C also showed losses, though of lesser magnitude than at the higher temperatures. As shown in Table III, within 15 days recoveries of ~80% were obtained from spiked silica gel and ≥85% from spiked filters. Recoveries of benzidinium sulfate from silica and filters were, respectively, 73% (0.71 μg spike) and 97% (0.26 - 2.1 μg spike) within 15 days. These results suggest that samples containing benzidine or its salts should be analyzed as quickly as possible and should be stored at temperatures ≤ −15°C until analyses can be conducted.

Recoveries ≥ 97% were obtained from silica and filters spiked with 3,3'-dichlorobenzidine (0.55 and 5.5 μg) or the dihydrochloride (0.7 and 7.5 μg), and stored at −15°C for up to 21 days. Table IV shows that there was relatively little loss of the compound even from samples stored at 23°C. Comparable results were obtained with the dihydrochloride. These results indicate that

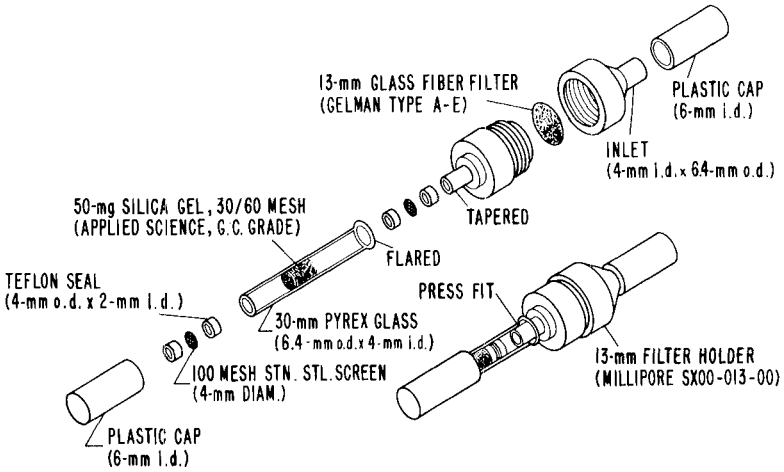


Figure 6 – Prototype personal sampler suggested for field use.

most storage conditions would not significantly affect the accuracy of this method for the analysis of 3,3'-dichlorobenzidine or its salts.

precision of method

Table V shows the precision of the methods for sampling and analysis of air samples. Conditions were varied to allow collection of between 0.34 and 3.03 μg of benzidine and between 1.04 and 6.83 μg of 3,3'-dichlorobenzidine. As in previous experiments, both free amines were found only on the filter portions of the samplers indicating the challenge atmospheres to have insufficient vapor for detection.

Relative standard deviations (RSD) of mean air concentrations were calculated for each group of samples. As shown in Table V these values varied between 2.26 and 5.21% for benzidine and between 3.81 and 6.38% for 3,3'-dichlorobenzidine. Bartlett's Chi Square Test was applied to determine if there were significant differences among each set of three RSDs. Since this test showed no heterogeneity in either case, the three values were pooled to provide a single estimate of the precision of the method (pooled estimate of RSD, S_p) for each compound. Values of S_p were 4.19% for benzidine and 5.05% for 3,3'-dichlorobenzidine. When coupled with sampling pump error of 5%, the precision of the method would be 6.5% for benzidine and 7.1% for 3,3'-dichlorobenzidine. These precision levels should approximate those encountered in field sampling situations.

discussion

Since benzidine and 3,3'-dichlorobenzidine are regulated as carcinogens, it is important that an air sampler provide an accurate assessment of exposures. In most industrial operations it is probable that these compounds would be present as aerosols though it also seems likely that vapors of the free amines would be present in certain situations, especially those that involve elevated temperatures.

The sampler described will collect either physical form (aerosol or vapor) with high efficiency and should be applicable in all situations. It is small, employs no liquids and is amenable to personal sampling with battery operated pumps. A field-sampling version of the experimental prototype is shown in Figure 6. The geometries of both units are virtually identical, the only difference being the use of a commercially available 13-mm filter holder and filter in the field device.

The analytical procedure is rapid and of sufficient resolution to allow the separation of benzidine or 3,3'-dichlorobenzidine from interfering substances. Given the levels of quantitation which were obtained, i.e., 0.15 μg/sample for benzidine and 0.17 μg/sample for 3,3'-dichlorobenzidine, and assuming that 48-L air samples were collected (0.2 L/min for 4 h), the minimum air concentrations which could be quantitated would be 3.1 $\mu g/m^3$ and 3.5 $\mu g/m^3$ for the respective compounds. These levels are the equivalent of vapor concentrations of 0.3 - 0.5 ppb at normal temperature and pressure.

The only apparent disadvantage of the method is the relative instability of benzidine and its salts on the sampler stages. However, by keeping samples cold ($\leqslant -15°C$) and analyzing them within a few days of collection, the procedure should be satisfactory.

acknowledgement

The authors gratefully acknowledge the assistance of Donald Gettemy and Claudine Kasunic who performed many of the sampling and analytical procedures.

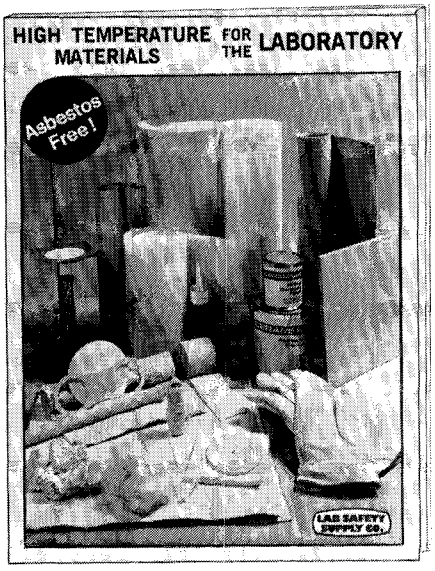
references

1. **Occupational Safety and Health Administration:** *Federal Register 39*:23551 (1974).
2. Final Rules Set for Carcinogens. *Chem. and Eng. News 52*:12 (1974).
3. *IARC Monographs on the Evaluation of Carcinogenic Risk to Man,* Vol. 1, pp. 80-86. International Agency for Research on Cancer, Lyon, France (1971).
4. *IARC Monographs on the Evaluation of Carcinogenic Risk to Man,* Vol. 4, pp. 49-55. International Agency for Research on Cancer, Lyon, France (1973).
5. **Haley, T.J.:** Benzidine Revisited - A Review of the Literature and Problems Associated With the Use of Benzidine and Its Congeners. *Clin. Toxicol. 8*:13-42 (1975).
6. **Glassman, J.M. and J.W. Meigs:** Benzidine (4,4_-diaminobiphenyl) and Substituted Benzidines - A Microchemical Screening Technique for Estimating Levels of Industrial Exposure From Urine and Air Samples. *A.M.A. Arch. Ind. Hyg. Occup. Med. 4*:519-432 (1951).

7. **Butt, L.T. and N. Strafford**: Papilloma of the Bladder in the Chemical Industry. Analytical Methods for the Determination of Benzidine and *beta*-Naphthylamine, Recommended by A.B.C.M. Sub-Committee. *J. Appl. Chem. 6*:525-539 (1956).
8. **Mercer, T.**: *Aerosol Technology in Hazard Evaluation,* pp. 151-154. Academic Press, New York (1973).
9. **Rappaport, S.M. and R. Morales:** Air-sampling and Analytical Method for 4,4'-Methylenebis(2-chloroaniline). *Anal. Chem. 51(1)*:19-23 (Jan., 1979).
10. **Young, P.R. and H.M. McNair:** High-pressure Liquid Chromatography of Aromatic Amines. *J. Chromatog. 119*:569-579 (1976).
11. **Rappaport, S.M. and D.J. Gettemy:** The Generation of Aerosols of Carcinogenic Aromatic Amines. *Am. Ind. Hyg. Assoc. J. 39*:287-294 (1978).
12. **Rappaport, S.M. and E.E. Campbell:** The Interpretation and Application of OSHA Carcinogen Standards for Laboratory Operations. *Amer. Ind. Hyg. Assoc. J. 37*:690-696 (1976).
13. **Bethea, R.B. Duran and T. Baullion:** *Statistical Methods for Scientists and Engineers,* pp. 247-251. Marcel Dekker, New York (1975).

Accepted March 19, 1979

...ASBESTOS-FREE INSULATION FOR LABORATORIES



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