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R. Morales
S. M. Rappaport
R. W. Weeks, Jr.
E. E. Campbell
H. J. Ettinger

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los alamos
scientific laboratory
of the University of California
LOS ALAMOS, NEW MEXICO 87545

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DEVELOPMENT OF SAMPLING AND ANALYTICAL METHODS FOR CARCINOGENS

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by

R. Morales, S. M. Rappaport, R. W. Weeks, Jr.,
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ABSTRACT

Components used for the generation of aerosols and vapors of certain aromatic amines on the Occupational Safety and Health Administration list of carcinogens are described. A two-stage sampling tube incorporating a glass fiber filter followed by silica gel was developed for collecting aerosols of benzidine and 3,3'-dichlorobenzidine. For α- and β-naphthylamine, a similar sampler having a greater capacity for retaining vapors was developed. Efficient desorption and sensitive analytical procedures for the quantitative determination of these four amines are described. Results of respirator canisters evaluated for protection against 4,4'-methylenebis(2-chloroaniline) and the naphthylamines are given.

I. INTRODUCTION

The Los Alamos Scientific Laboratory (LASL) is conducting research on the development of analytical methods, air-sampling techniques and related procedures for compounds (Table I) designated as carcinogens in 1974 by the Occupational Safety and Health Administration (OSHA).[1] Research is also being conducted with commercial respirator canisters to determine which canisters are able to collect and retain aerosols/vapors of the compounds. The objectives of the research are to provide sampling and analytical methods that can be used to monitor worker exposure to the carcinogenic substances. In addition, the evaluation of commercial respirator canisters will determine which canisters may provide protection against the inhalation or ingestion of these compounds by personnel working in areas where aerosols/vapors of the carcinogens may exist.

TABLE I

CARCINOGENS

Compound	Physical State (25°C)	Chemical Abstracts Service Registry Number
4,4'-Methylenebis(2-chloroaniline)	solid	101144
Benzidine & Salts*	solid	92875
3,3'-Dichlorobenzidine & Salts*	solid	91941
β-Naphthylamine	solid	91598
α-Naphthylamine	solid	134327
Bis(chloromethyl) ether	liquid	542881
Chloromethyl methyl ether	liquid	107302
β-Propiolactone	liquid	53578
Ethylenimine	liquid	151564
N-Nitrosodimethylamine	liquid	62759
4-Aminobiphenyl	solid	92671
4-Nitrobiphenyl	solid	92933
2-Acetylaminofluorene	solid	53963
4-Dimethylaminoazobenzene	solid	60117

*Only the most common salt will be considered.

Published work in these areas for these carcinogens appears to be somewhat limited in scope.

This research was funded by the National Institute for Occupational Safety and Health (NIOSH) under NIOSH/LASL Agreement, IA-74-35, which began in July, 1974, and terminated in September, 1976. A brief outline of the scope of the research as stated in the Agreement between NIOSH and LASL included the following phases:

1. Preparation, including laboratory modifications, equipment purchase and installation, and construction and calibration of an aerosol generation system.
2. Development and evaluation of nonspecific, nonquantitative field detection methods for the 14 specified carcinogens.
3. Development and evaluation of quantitative air-sampling methods for a minimum of 6 of the 14 specified carcinogens.
4. Evaluation of existing commercial respirator sorbents for protection against the carcinogens.

Three reports have been written describing the progress of the work since the initiation of the Agreement. The first two progress reports[2,3] described the accomplishments under Phase 1 and spot-test work with analogues and homologues of the carcinogens. The third report[4] described details of the aerosol and/or vapor generator system employed with 4,4'-methylenebis(2-chloroaniline), the two-stage sampler designed for collecting the aerosols of low vapor pressure compounds, the high-performance liquid chromatographic procedure for 4,4'-methylenebis(2-chloroaniline), and spot-test procedures for the listed carcinogenic aromatic amines.

This report covers research activities for the period January 1 through September 30, 1976. The areas of research described here are:

1. Test atmosphere generation and air-sampling systems for benzidine, 3,3'-dichlorobenzidine, and α- and β-naphthylamine (Sec. III, IV.A).
2. Development and evaluation of quantitative air-sampling and analytical methods for benzidine, 3,3'-dichlorobenzidine, and the naphthylamines (Sec. IV.B through F).
3. Evaluation of commercial respirator canisters for protection against 4,4'-methylenebis(2-chloroaniline) and the naphthylamines (Sec. V).

II. SUMMARY

Basically, the same system[3,4] used for generating and sampling test atmospheres of 4,4'-methylenebis(2-chloroaniline) was employed with benzidine, 3,3'-dichlorobenzidine, and the naphthylamines. The major departure from this system occurred at the generation stage for the naphthylamines. Because of the naphthylamines' higher vapor pressures, a diffusion-condensation generator was used instead of a nebulizer. The two-stage sampler (glass-fiber filter followed by silica gel) previously described[4] for 4,4'-methylenebis(2-chloroaniline) was also used to collect aerosols of benzidine and 3,3'-dichlorobenzidine. This sampler, however, was found to have a very limited capacity for the vapors of the naphthylamines. A three-stage sampler (consisting of a glass-fiber filter followed by two beds of silica gel) that had greater capacity was therefore adopted for the naphthylamines.

Rapid and sensitive analytical procedures were developed for benzidine, 3,3'-dichlorobenzidine, and α- and β-naphthylamine. For the benzidines, a high-performance liquid chromatograph (HPLC) coupled with a 254-nm ultraviolet detector was employed in the analysis. The analytical procedure developed for the naphthylamines was based on gas chromatography (flame ionization detector) and allowed for the simultaneous determination of both compounds.

Commercially available respirator canisters were evaluated to determine their efficiency in collecting and retaining the aerosols/vapors of 4,4'-methylenebis(2-chloroaniline) and the naphthylamines. No evidence of breakthrough of any of the compounds was detected after 7- to 8-h runs using challenge concentrations of 100 to 300 $\mu g/m^3$.

III. GENERATION OF TEST ATMOSPHERES

A flow diagram of the system used for the generation and sampling of test atmospheres of benzidine and 3,3'-dichlorobenzidine is shown in Fig. 1. A similar system was used for the naphthylamines in which the aerosol generator and charge neutralizer were replaced with an aerosol/vapor generator.

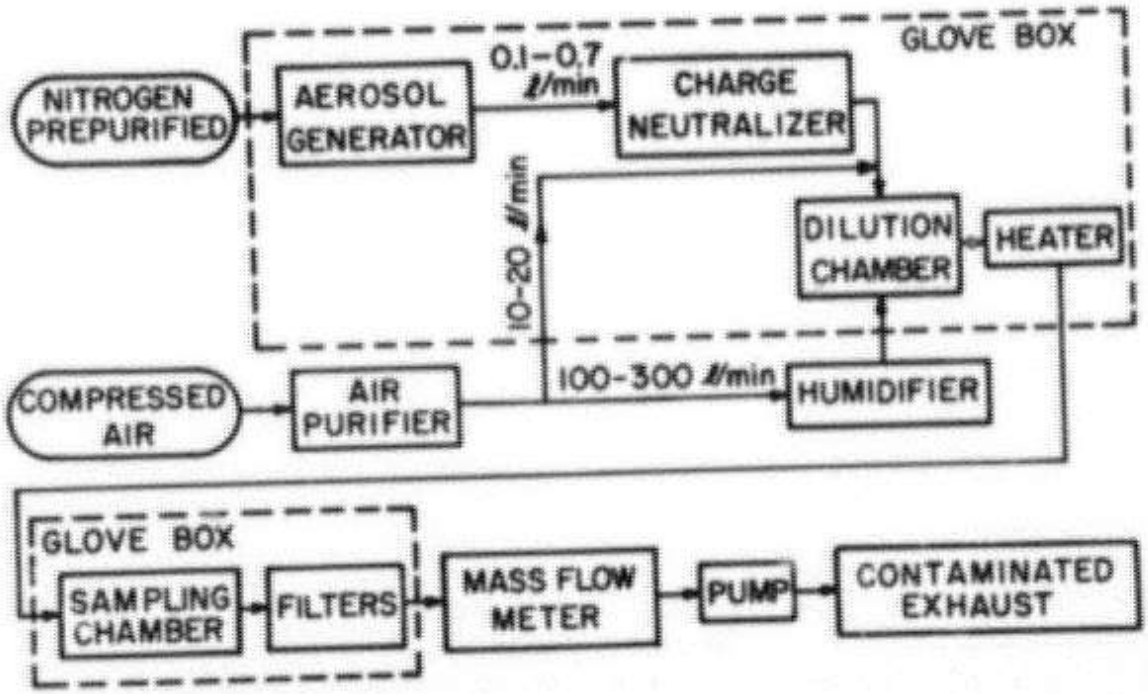

Fig. 1.
Aerosol generation and air-sampling systems.

Aerosols of the benzidines, which have negligible vapor pressures, were generated by the method previously described[4] for 4,4'-methylenebis(2-chloroaniline) using a compressed gas nebulizer similar to that shown in Fig. 2. Briefly, the system operates by passing pure dry nitrogen through the nebulizer, which is heated to a temperature above the compound's melting point. The nitrogen stream atomizes the compound into a polydisperse aerosol, and the aerosol is passed through a charge neutralizer containing 200 mCi of ^3H as titanium titride. The neutralized aerosol is then diluted with clean, dry air before entering the sampling chamber.[4] A heater and humidifier are built into the system to effect desired temperatures and relative humidity. Aerosols and vapors of α- and β-naphthylamine were produced with a specially designed vapor-diffusion generator.

A. Aerosol Generator - Benzidine and 3,3'-Dichlorobenzidine

1. Configuration. Aerosols of benzidine and 3,3'-dichlorobenzidine were generated by nebulization. Measurement of the mass flow rates of nitrogen through two identically prepared nebulizers indicated the calculated[4] internal jet diameters to be 0.112 and 0.103 mm for the benzidine and 3,3'-dichlorobenzidine nebulizer, respectively. First-order regressions of nitrogen flow rate on pressure are given in Table II.

The configuration of the nebulizer in the oven was changed slightly from that previously reported for 4,4'-methylenebis(2-chloroaniline). The distance from the nebulizer outlet to the oven wall was reduced to ~8 cm (Fig. 3), thus allowing particles to exit the hot zone more quickly. Aerosols were transported by the spent jet nitrogen after leaving the oven through the radioactive charge neutralizer and into a chamber (first dilution stage) where 20 liters/min of clean, dry air were added.

Aerosols were generated at temperatures of 20 to 30°C above the compound's melting point. The first nebulizer contained ~7 g of technical grade benzidine (Eastman Organic Chemicals, Rochester, New York) and was maintained at 156°C [benzidine m.p. = 128°C (Ref. 5)]. The second unit, containing ~8 g of technical grade 3,3'-dichlorobenzidine (ICN, K&K Laboratories, Plainview, New York), was maintained at 153°C [3,3'-dichlorobenzidine m.p. = 132-133°C (Ref. 6)]. The 7 to 8 g of initial material were sufficient to conduct all necessary runs.

2. Aerosol Output. Generator outputs were determined over a range of nitrogen flow rates by collecting and analyzing portions of the diluted aerosol streams. Samples were collected on 25-mm polyvinyl chloride membrane filters (Metricel DM-800, 0.8-μm pore size, Gelman Instrument Company, Ann Arbor, Michigan) contained in in-line filter holders. Critical orifices controlled the sampling rate at 1 liter/min. After sampling, filters were removed from the holders and placed in 1-ml volumetric vials for analysis. The compound of interest was dissolved in 0.5 ml of a triethylamine/methyl alcohol solution and analyzed by HPLC as described in Sec. IV.C. The results, shown in Fig. 4, depict mean aerosol output (a minimum of three observations at each point) versus nitrogen pressure between 10 and 40 psig. Aerosol outputs ranged from 55.2 to 1120 μg/min for benzidine and from 32.0 to 940 μg/min for 3,3'-dichlorobenzidine.

3. Particle Size Distributions. Size distributions for benzidine and 3,3'-dichlorobenzidine aerosols were determined with the seven-stage cascade impactor previously described.[4] Representative size distributions are shown in Figs. 5 and 6. Unlike 4,4'-methylenebis(2-chloroaniline) aerosols, which conformed to a lognormal distribution, the distributions for these compounds were bimodal. Approximations of the two component size distributions were obtained by separating the impactor data into two sets, consisting of stages 1 through 4 and stages 5 through 7 plus the backup filter, respectively. Each set was treated as if it contained all of the mass of the component aerosol. The first mode, accounting for 80 to 90% of the mass, had a mass median aerodynamic diameter (MMAD) of ~2.4 μm with a geometric standard deviation (σ_g) of ~1.5; the second, accounting for 10 to 20% of the mass, had a MMAD of ~0.1 μm with a σ_g of ~4. Size distributions of aerosols generated at various operating pressures are summarized in Table III. The data indicate that the generation technique was reproducible and that particle size was generally independent of the operating pressure.

TABLE II

NEBULIZER JET DIAMETERS AND FLOW RATE EQUATIONS

Nebulizer	Compound	Jet i.d.	Flow Rate Equation[a]	r[b]
1	Benzidine	0.112 mm	$F = 52.4 + 10.8\ \Delta P$	0.9995
2	3,3'-Dichlorobenzidine	0.163 mm	$F = 73.8 + 8.35\ \Delta P$	0.9987

[a] F = Volumetric flow rate through jet, mℓ/min, at 25°C and 585-mm Hg atmospheric pressure, over nitrogen pressure range of 10 to 40 psig; ΔP = compressed nitrogen pressure, psig.

[b] r = Linear correlation coefficient.

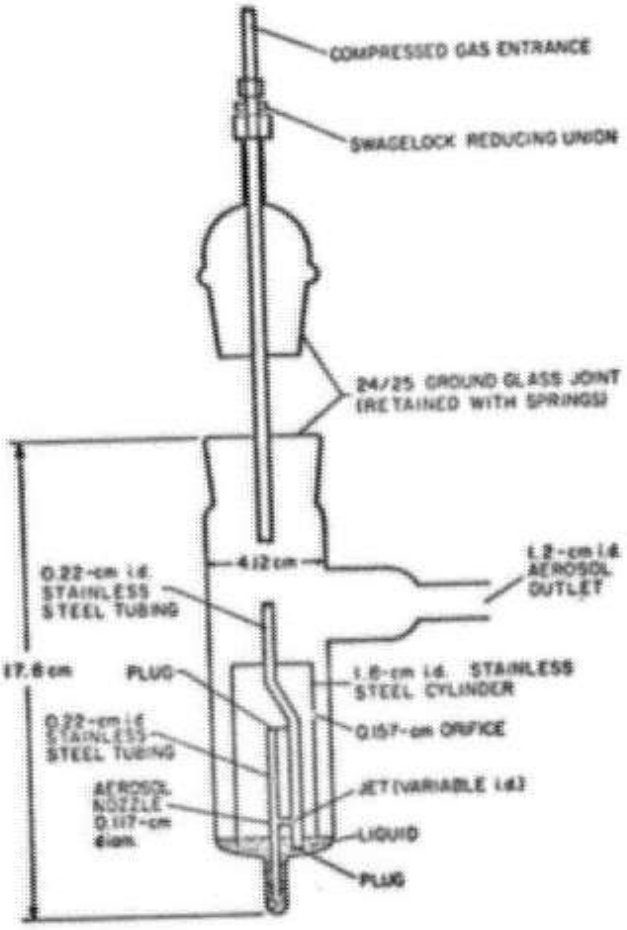

Fig. 2.
Compressed-gas nebulizer.

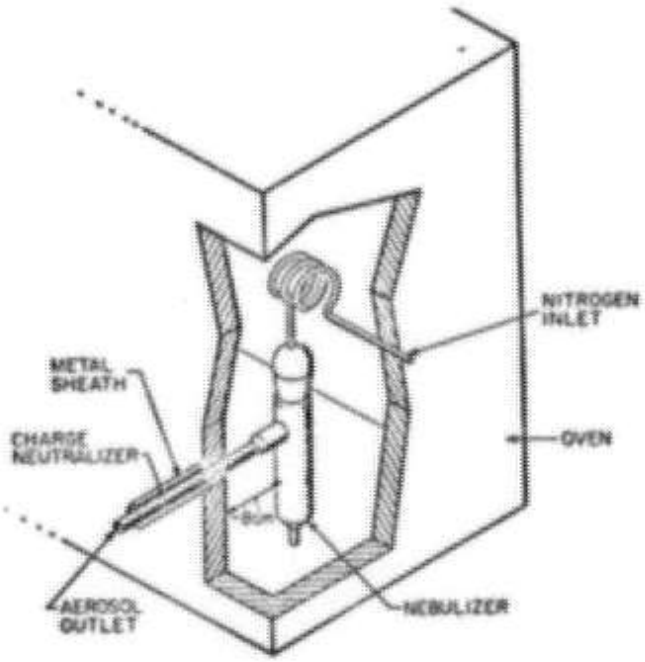

Fig. 3.
Configuration of nebulizer in the oven.

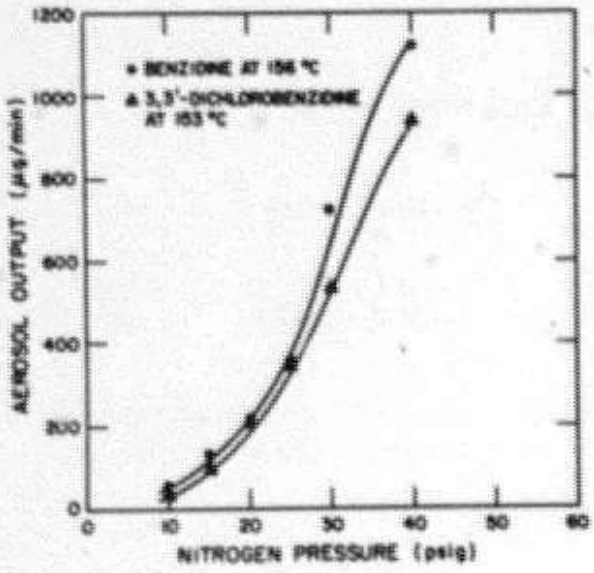

Fig. 4.
Aerosol output vs nitrogen pressure for benzidine and 3,3'-dichlorobenzidine.

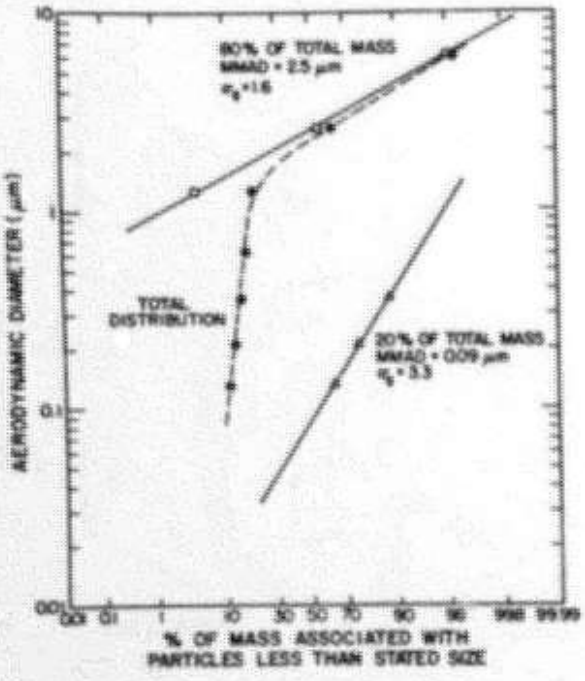

Fig. 5.
Particle size distribution for benzidine generated at 10 psig.

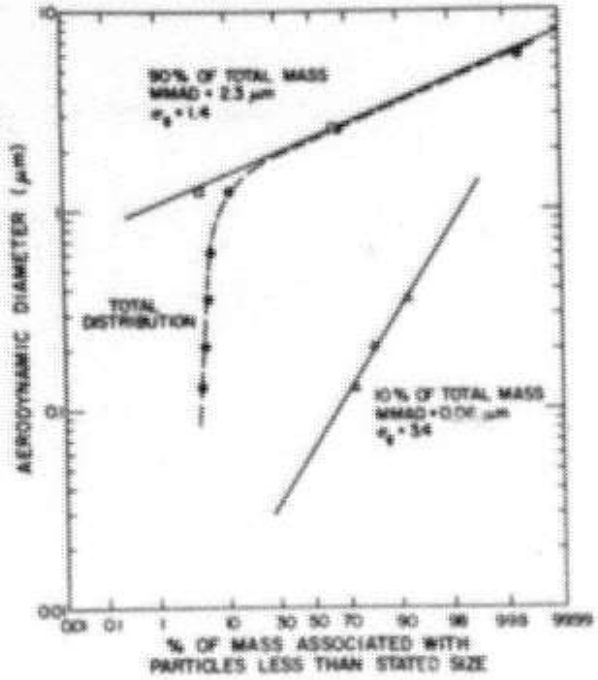

Fig. 6.
Particle size distribution for 3,3'-dichlorobenzidine generated at 30 psig.

B. Vapor Generator - Naphthylamines

Because α- and β-naphthylamine are often associated with each other in manufacture and use, it was felt that sampling and analytical techniques should be able to differentiate between them. A test atmosphere that simultaneously contained both isomers was therefore generated.

Vapor pressures and corresponding saturation concentrations for these compounds, at normally encountered temperatures, are given in Table IV. Because aerosols produced at concentrations of $<100\ \mu g/m^3$ would be expected to be relatively short-lived, a generator based upon the principle of controlled vapor diffusion[7-16] was developed for these compounds. The apparatus, shown in Fig. 7, consisted of two cells (one for each compound) contained in a circulated-air oven, thermostatically controlled to ±1°C. Approximately 1.5 g of each technical grade compound was placed in an individual diffusion cell. The α-isomer was obtained from Eastman Organic Chemicals, (Rochester, New York) and the β-isomer from ICN, K&K

TABLE III

AEROSOL SIZE DISTRIBUTIONS FOR BENZIDINE AND 3,3'-DICHLOROBENZIDINE

Compound	Temp (°C)	N, Pressure (psig)	Main Distribution MMAD (μm)	σ_g	% of Mass	Minor Distribution MMAD (μm)	σ_g	% of Mass
Benzidine	156	10	2.5	1.6	80	0.09	3.3	20
		20	2.4	1.6	82	0.07	7.9	18
		20	2.4	1.5	85	0.09	4.7	15
		30	2.3	1.5	77	0.06	8.3	23
		30	2.3	1.5	80	0.10	5.5	20
		40	2.6	1.6	92	0.39	2.7	8
3,3'-Dichlorobenzidine	153	10	2.6	1.6	60	0.22	2.8	40
		20	2.5	1.4	91	0.07	5.0	9
		30	2.3	1.4	90	0.06	3.4	10
		40	1.8	1.5	89	0.16	3.2	11

Laboratories (Plainview, New York). Vapors diffused from the cell reservoirs through 5-cm lengths of Teflon bored to the selected internal diameters. Purified nitrogen flowing at 2 liters/min carried the vapors from the diffusion chamber and out of the oven. The vapors descended through an insulated condensation tube, where limited, self-nucleated aerosol formation could take place, prior to entering a glass dilution chamber. This glass vessel, similar in design and operation to the stainless steel device previously used,[4] diluted the effluent from the diffusion chamber with 20 liters/min of clean, dry air.

Theoretical outputs from the diffusion cells were calculated according to the following relationship.[9]

$$Q = (DMPA/LRT)\ (\ell n\ P/P\text{-}p)$$ where

Q = Diffusion rate, g/s,
D = Diffusion coefficient, cm^2/s,
M = Molecular weight, g/mole,
P = Total pressure in the diffusion cell, atm.,
A = Cross-sectional area of diffusion path, cm^2,
L = Length of diffusion path, cm,
R = Universal gas constant, cm^3 atm per mole K,
T = Absolute temperature, K, and
p = Vapor pressure (atm) of the compound at temperature, T.

Diffusion coefficients for the naphthylamines were approximated by the method of Gilliland,[10,11] using estimated molar volumes of 162 and 30 cm^3/mole for the amines and nitrogen, respectively. Calculations also employed diffusion path cross-sectional areas of 0.243 and 0.317 cm^2 for the cells containing the α- and β-isomers, respectively, a cell pressure of 0.77 atm. (585-mm Hg atmospheric pressure), and vapor pressures extrapolated from data given by Stull.[7] Table V lists pertinent data and calculated diffusion rates for the two compounds between 100 and 160°C.

Theoretical vapor generation rates in the 130 to 150°C temperature range were used as guides in establishing chamber air concentrations. Observed concentrations based upon collection of samples in the chamber deviated from calculated values by between 5 and 35%. The indicated air concentration of α-naphthylamine was usually higher than calculated while β-naphthylamine concentrations were lower. Obvious sources of error in the theoretical values include estimation of diffusion coefficients and extrapolation of vapor pressures to the temperatures used for generation. Since

TABLE IV

VAPOR PRESSURES AND CORRESPONDING SATURATION AIR CONCENTRATIONS FOR α- AND β- NAPHTHYLAMINE

Temp (°C)	Vapor Pressure[a] (mm Hg) α-	β-	Air Conc[b] (μg/m³) α-	β-
10	1.7×10^{-4}	1.5×10^{-4}	1.3×10^{2}	1.2×10^{2}
20	4.0×10^{-4}	3.6×10^{-4}	3.1×10^{2}	2.8×10^{2}
30	8.9×10^{-4}	8.0×10^{-4}	6.8×10^{2}	6.3×10^{2}
40	2.0×10^{-3}	1.8×10^{-3}	1.5×10^{3}	1.4×10^{3}

[a] Extrapolated from data given in Ref. 7.
[b] At 760-mm Hg atmospheric pressure.

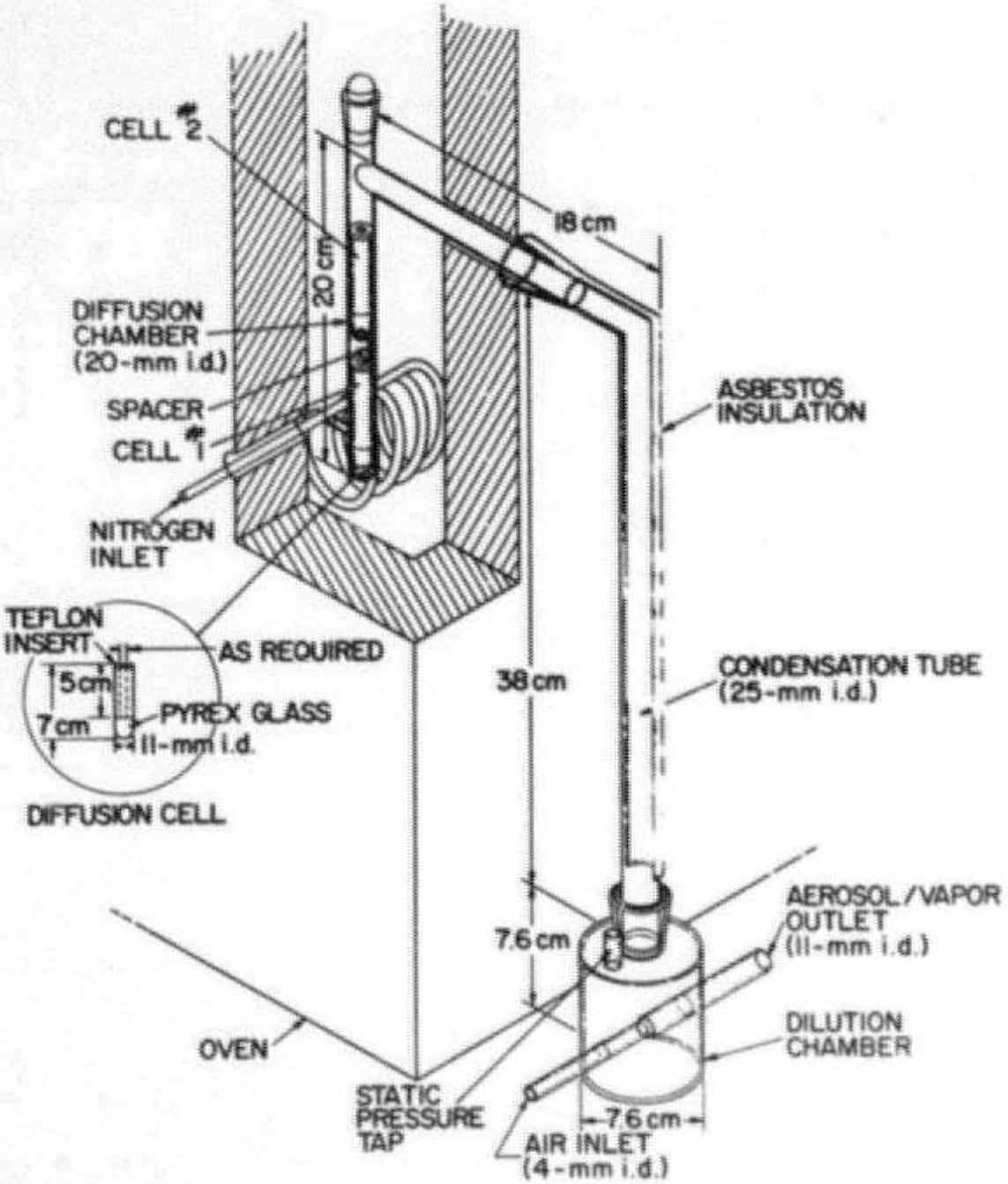

Fig. 7.
Aerosol-vapor generator for α- and β-naphthylamine.

naphthylamines used in the diffusion cells were not pure, additional deviations in diffusion rates would be expected.

Aerosol formation was also found to be unpredictable. In most instances, no detectable quantity of either compound was found on the first stage (filter) of samples collected in the chamber. This was anticipated since the vapor pressures of these compounds are high and any particles produced would be expected to be short-lived. As much as one half of the collected material was found on the filter on a number of occasions, however. The amount of the β-isomer on the filter on those occasions was generally greater than that of the α-isomer. Aerosol was never observed at generation temperatures of <140°C and was found, most often, when fresh compounds were used in the diffusion cells.

TABLE V

THEORETICAL GENERATION RATES FOR NAPHTHYLAMINES FROM DIFFUSION CELLS

Temp (°C)	D (cm²/s)	p (atm)		Q (μg/s)	
		α-	β-	α-	β-
100	0.114	1.1×10^{-4}	9.6×10^{-4}	2.8×10^{-3}	3.2×10^{-3}
110	0.118	1.8×10^{-4}	1.6×10^{-4}	4.7×10^{-3}	5.4×10^{-3}
120	0.123	3.0×10^{-4}	2.6×10^{-4}	8.0×10^{-3}	9.0×10^{-3}
130	0.128	4.6×10^{-4}	4.1×10^{-4}	0.12	0.14
140	0.133	7.1×10^{-4}	6.4×10^{-4}	0.20	0.23
150	0.137	1.1×10^{-3}	9.4×10^{-4}	0.30	0.34
160	0.142	1.6×10^{-3}	1.4×10^{-3}	0.45	0.51

Size distributions of aerosols produced at 160 and 140°C were determined by collecting samples with the cascade impactor immediately downstream from the first dilution stage. Samples were collected at 1.43 liters/min for 20 min and analyzed as described in Sec. IV.E. A representative size distribution (generated at 140°C) shows the major portion of the aerosol conforms to a lognormal distribution (Fig. 8). Aerosols generated at 160°C had MMAD values of 1.9 μm and σ_g values of 5.3 while those produced at 140°C had corresponding values of 1.5 μm and 4.5, respectively.

As the aerosols were diluted and carried through the system, sublimation was undoubtedly occurring, especially for the smaller diameter particles. Thus, particles collected in the chamber were probably distributed somewhat differently from those collected immediately downstream from the point of generation.

C. Dilution and Air Sampling Systems

Aerosols of benzidine and 3,3'-dichlorobenzidine were diluted in the same manner as were those of 4,4'-methylenebis(2-chloroaniline), that is, by employing up to three dilution stages. After leaving the oven, the aerosols were transported by the spent jet nitrogen through the radioactive charge neutralizer and into a chamber (first dilution stage) where 20 liters/min of clean, dry air were added. The second stage, consisting of an 8- by 20- by 20-cm HEPA filter with a 0.53-cm i.d. tube through its center, diluted the aerosol by ~9 times. The third dilution stage added up to 300 liters/min of air to the aerosol stream prior to its transport into the chamber. With all three stages in place and a total airflow rate of 320 liters/min, minimum obtainable chamber concentrations were 19 and 11 μg/m³ for benzidine and dichlorobenzidine, respectively. The second aerosol dilution stage was not needed with the naphthylamines. With the diffusion generator, virtually any air concentration of the naphthylamines could be obtained by varying the oven temperature and making the desired dilutions.

Several parameters were continuously monitored during sampling runs as previously described.[4] These included temperature, relative humidity, airflow rate, and aerosol concentration (benzidine and 3,3'-dichlorobenzidine only). No concentration monitor was used for the naphthylamine vapor atmospheres.

IV. DEVELOPMENT AND EVALUATION OF QUANTITATIVE AIR SAMPLING AND ANALYTICAL METHODS

Sampling and analytical techniques were developed for benzidine, 3,3'-dichlorobenzidine and the two naphthylamines. Since environments could conceivably contain these aromatic amines as aerosols and/or vapors, a multistage sampler employing a filter followed by one or more sorbent sections was used. Analytical methods employing

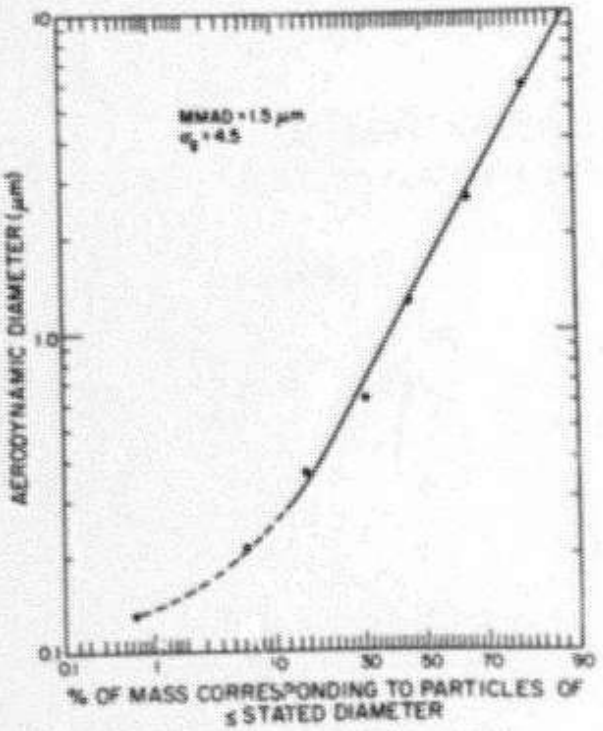

Fig. 8.
Aerosol size distribution of naphthylamines generated at 140°C.

HPLC were developed for benzidine and 3,3'-dichlorobenzine. Gas chromatography was selected for the analysis of α- and β-naphthylamine.

The combined sampling and analytical methods were evaluated as follows. Three experiments, employing dynamic test atmospheres, established the validity of the methods. First, breakthrough tests were conducted to provide an estimate of the capacity of individual sampler stages. Then, the effects of temperature and humidity on the method were evaluated. Finally, the precision of the methods was determined by collecting groups of samples containing various amounts of each compound. Concurrently, static testing established the efficiency of desorption from sampler matrices and the stability of the compounds during storage.

A. Air Sampling

1. Benzidine and 3,3'-Dichlorobenzidine. The sampler[4] originally developed for 4,4'-methylenebis(2-chloroaniline) was adopted for the benzidines. Briefly, the sampler consisted of a glass probe designed to fit snugly through special ports in the sampling chamber. As air entered the probe, it passed through an 8-mm glass-fiber filter (Type A-E, Gelman Instrument Company, Ann Arbor, Michigan) followed by 50 mg of silica gel (G. C. Grade, 30/60 mesh, 720-760 m^2/g, 4.3 g cm^3; Applied Science Laboratories, State College, Pennsylvania). The flow rate was controlled with a critical orifice at 0.8 liters/min. The filters were shown to have greater than 99.95% collection efficiency for a polydisperse NaCl aerosol (MMAD = 0.6 μm, σ_g = 2.0) over a range of relevant face velocities.[4] Vapor breakthrough determinations were conducted, as reported for 4,4'-methylenebis(2-chloroaniline),[4] and showed the 50-mg silica gel section to have sufficient capacity to remove benzidine and 3,3'-dichlorobenzidine from a volume of air consistent with personal samplers. At 180°C, vapors of these compounds carried by a stream of dry nitrogen at 0.30 liters/min did not break through the silica gel in 3 h. Experiments designed to test the effect of temperature and humidity (Sec. IV.F.) on the sampling and/or analytical methods for these compounds indicated that there was insufficient vapor present to be collected on the second stage of the sampler. Breakthrough of the benzidines through the two-stage sampler, therefore, appears remote under most sampling situations.

2. Naphthylamines. Attempts were made to apply the same two-stage sampler to α- and β-naphthylamine. However, two factors necessitated the modification of the original design. First, an impurity that had the same gas chromatographic retention time as the α-isomer was found in the Type A-E filters. Substitution with Spectro-Grade Type A filters (Gelman Instrument Co., Ann Arbor, Michigan) eliminated this problem. These filters are produced by the same manufacturing process as the Type A-E and reportedly have collection efficiencies of greater than 99.9% for a monodisperse 0.3-μm dioctyl phthalate aerosol.[13]

The second factor involved the breakthrough of naphthylamine vapors from the 50-mg silica gel section in humid air. This was unanticipated since breakthrough tests with dry nitrogen had shown the 50 mg of sorbent to have a very high capacity for either isomer. As described for 4,4'-methylenebis(2-chloroaniline),[4] this sorbent was challenged with the

vapor of either α- or β-naphthylamine at temperatures between 130 and 160°C at a flow rate of 0.2 liters/min. Mean retention volumes, Vr (corresponds to 50% breakthrough), were determined from triplicate trials and plotted against the reciprocal of absolute temperature. As shown in Fig. 9, a linear relationship exists. Extrapolation to 25°C gave expected mean retention volumes of 4.4 x 10^6 liters with a 90% confidence interval between 2.6 x 10^6 and 7.2 x 10^6 liters for α-naphthylamine, and 2.1 x 10^6 liters with a corresponding 90% confidence interval between 1.4 x 10^6 and 3.1 x 10^6 liters for β-naphthylamine. However, when preliminary samples were collected from the chamber under varied humidity environments, the amount of each compound retained by the sorbent was found to be variable. Further tests indicated that breakthrough was taking place in the high-humidity groups.

Another sampler was designed to increase the vapor-collection capacity. Similar to the original design, the sampler employed a filter followed by 100- and 50-mg silica gel sections. In order to minimize the increased pressure drop associated with the additional silica stage, 20/45-mesh silica (Grade 407, 720-760 m^2/g, 0.737 g/cc; Coast Engineering Laboratories, Gardena, California) was substituted for the 30/60-mesh silica. The modified chamber sampler used is shown in Fig. 10. The recommended personal sampler and the pressure-drop curve associated with it are shown in Figs. 11 and 12.

The capacity of the modified sampler for naphthylamine vapor was determined as follows. Two experiments were conducted in which groups of samples were collected at 30°C in both dry and humid air. Samples were drawn at a flow rate of 0.8 liters/min for periods of between 20 and 240 min from atmospheres containing both naphthylamine isomers. The first experiment (Runs A and B) employed relative humidities of 0 and 95% at mean air concentrations of 111 and 76.3 μg/m^3 for α- and β-naphthylamine, respectively. (These are the means of indicated concentrations for both runs in which there was no apparent breakthrough from the 50-mg silica gel sections.) Approximately 0.5 μg of each compound was found in the filter portion of each sampler; the remainder was found in the silica gel sections. The second experiment (Runs C and D) tested intermediate relative humidities of 20 and 80% at mean air concentrations of 85.0 and 68.2 μg/m^3 for α- and β-naphthylamine, respectively. Neither amine was found on the filters in this case. Results of the experiments are given in Table VI.

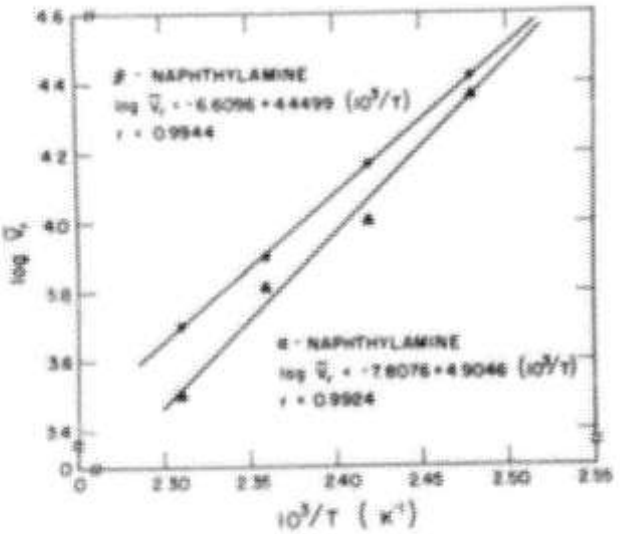

Fig. 9.
Retention volumes for α- (▲) and β-naphthylamine (●).

From Table VI, it is apparent that breakthrough is not a problem in the 0 to 20% RH groups. (Breakthrough is defined as the fraction of adsorbed sample collected by the 50-mg section.) At 0% RH, 36.4 μg of total naphthylamine vapor (22.1 μg α- and 14.3 μg β-) were removed from 184 liters of air by the 100-mg section. At 20% RH, 14.7 μg of total naphthylamine vapor (8.24 μg α- and 6.48 μg β-) from 95 liters of air was collected by the primary section with no loss. At higher relative humidities, breakthrough occurred and was always greater for the α-isomer. With the collection of 7.34 μg of total naphthylamine vapor (3.99 μg α- and 3.35 μg β-), between 1 and 2% of the individual isomers had broken through the primary section at 80% RH. As the sampled-air volume doubled from 47 to 94 liters, the breakthrough rate increased to 8.7 and 4.3% for the α- and β-isomers, respectively. At that point, more than 16 μg of vapor had been adsorbed. Breakthrough occurred even more quickly at 95% RH. Approximately 1% total naphthylamine had entered the second silica section after only 23 liters of air had been sampled and 3.67 μg of vapors collected. With a doubling of the sampling volume, more than 20% of the 7.69-μg adsorbate had entered the second section.

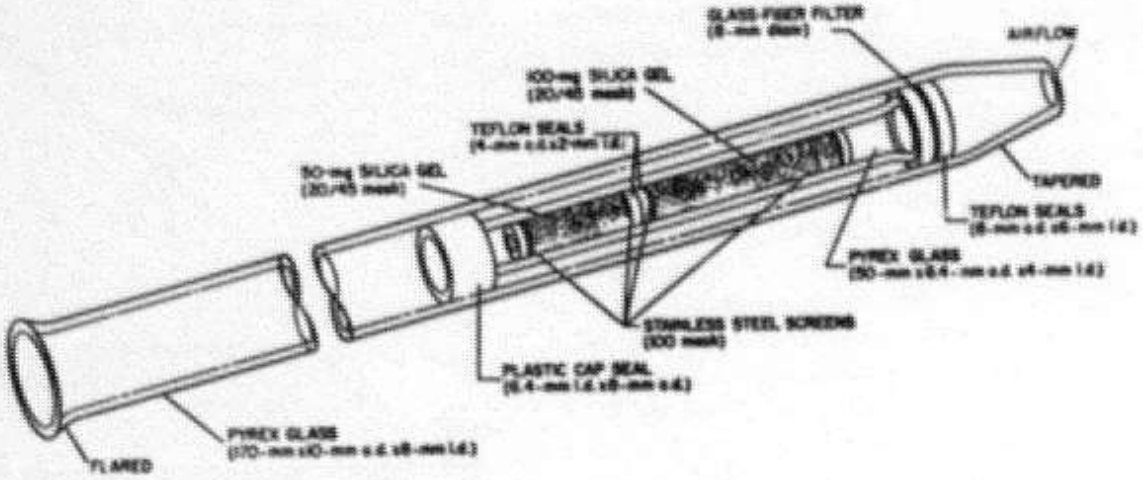

Fig. 10.

Three-stage chamber sampling tube.

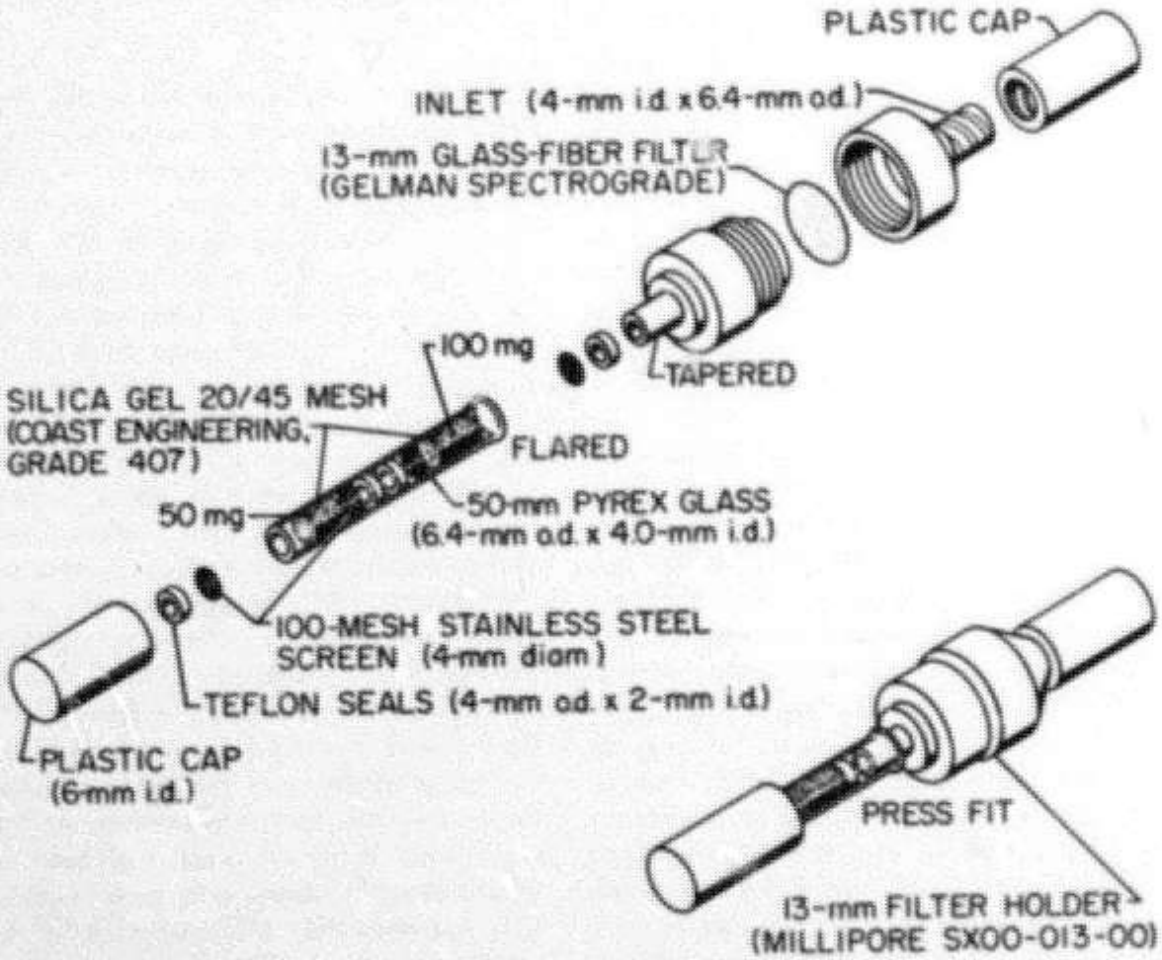

Fig. 11.

Three-stage personal sampling tube.

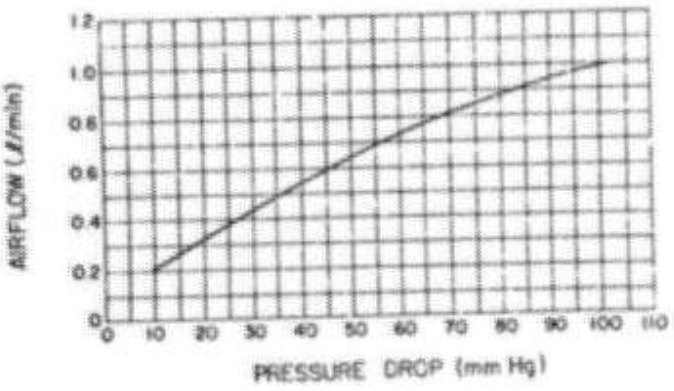

Fig. 12.
Relationship between flow rate and pressure drop for three-stage personal sampler.

Comparison of the air concentrations indicated by these samples leads to the conclusion that significant breakthrough from the back-up section had not yet occurred. Thus, the capacity of the 50-mg silica gel section must be at least 20 to 30% of that of the 100-mg section. Two additional groups of samples in which 94 and 187 liters of 95% RH air were collected are not included in Table VI. It was obvious that, in these cases, significant quantities of both compounds had broken through the sampler.

B. Desorption of Benzidine and 3,3'-Dichlorobenzidine

Filter and Silica Gel. Efficiencies of desorption for benzidine, 3,3'-dichlorobenzidine, and their salts from silica gel D-08 and glass fiber filters Type A-E were determined at various levels as shown in Tables VII-XIII. The addition and recovery of the amines were performed as follows. Ten microliters of the analyte dissolved in methanol (or in 0.17% triethylamine in methanol in the case of benzidinium sulfate and 3,3'-dichlorobenzidine dihydrochloride) were added to 50 mg of silica gel contained in a 1-ml test tube. The test tube was vibrated in a test tube vibrator for 20 s to assure a homogeneous dispersion of the spiked gel. The same procedure was followed for the filters, except that the filters were not vibrated. Some of the spiked silica gel and filters were then stored in the dark at ambient temperature (~23°C) and others were stored in a freezer at -15°C. The treated matrices were analyzed over a 2- to 4-week period to determine the efficiency of desorption. Analyses for benzidine were conducted by injecting 10 μl of the desorbed sample solution into the HPLC under the conditions described in Sec. IV.C.1. For 3,3'-dichlorobenzidine, 15 μl was injected (Sec. IV.C.2.). Desorption of the benzidines and their salts from the sampler's stages was accomplished with 0.5 ml of a 0.17% (v/v) solution of triethylamine in methyl

TABLE VI

NAPHTHYLAMINE CAPACITY AS A FUNCTION OF HUMIDITY AND SAMPLING VOLUME

Run	RH (%)	Air Vol (ℓ)	No. Samples	α-Naphthylamine			β-Naphthylamine		
				Challenge[a] Conc (μg/m³)	Amount[b] Adsorbed (μg)	Breakthrough (%)[c]	Challenge Conc (μg/m³)[a]	Amount Adsorbed (μg)[b]	Breakthrough (%)[c]
A	0	23.1	3	115. ± 5.20	2.13 ± 0.136	0	69.2 ± 0.419	1.11 ± 0.098	0
A	0	46.8	2	114. ± 5.57	4.70 ± 0.270	0	74.1 ± 3.97	2.96 ± 0.166	0
A	0	94.9	3	107. ± 4.01	9.67 ± 0.294	0	67.2 ± 3.82	5.77 ± 0.306	0
A	0	184	3	103. ± 1.52	22.1 ± 0.47	0	81.3 ± 1.95	14.3 ± 0.533	0
C	20	46.2	6	79.1 ± 3.34	1.25 ± 0.029	0	59.1 ± 4.01	0.91 ± 0.032	0
C	20	47.7	5	63.2 ± 6.78	3.07 ± 0.215	0	65.8 ± 5.68	3.03 ± 0.224	0
C	20	95.2	5	96.0 ± 3.96	8.24 ± 0.212	0	68.2 ± 4.94	6.69 ± 0.324	0
D	80	45.8	6	82.7 ± 4.54	1.71 ± 0.166	0	66.5 ± 5.16	1.05 ± 0.064	0
D	80	47.1	5	64.8 ± 3.67	3.99 ± 0.091	1.92 ± 2.35	71.2 ± 3.11	3.35 ± 0.091	1.01 ± 1.46
D	80	93.5	5	93.4 ± 3.11	8.72 ± 0.290	8.66 ± 0.868	76.7 ± 4.47	7.10 ± 0.241	4.35 ± 0.613
B	95	29.1	3	106. ± 1.29	2.05 ± 0.070	1.54 ± 2.67	85.9 ± 3.12	1.62 ± 0.056	1.86 ± 1.73
B	95	60.9	3	102. ± 6.85	4.26 ± 0.128	27.8 ± 0.22	81.2 ± 0.307	3.41 ± 0.108	19.8 ± 1.52

[a] Mean air concentration indicated for each group of samples (± standard deviation).

[b] Mean amount collected for each group of samples (± standard deviation); combined weight of compound found in both sampler sections.

[c] Fraction of total adsorbed sample collected by 50-mg sorbent section.

TABLE VII

RECOVERY OF BENZIDINE AND BENZIDINIUM SULFATE FROM SILICA GEL (-15°C)[a]

Time (days)	% Recovered[b]	
	Benzidine (0.691 μg)	Benzidinium Sulfate[c] (0.712 μg)
0	98,104,90,92 (96)[d]	106,104 (105)
1	97,96 (97)	100,103 (103)
3	97,95 (96)	87,87 (87)
4	100,93 (97)	105,116 (111)
7	90,95 (93)	96,94 (95)
11	83,92 (88)	79,84 (82)
14	75,82 (79)	74,72 (73)

[a]For all recovery data, the silica and the filters were spiked and stored at the indicated temperature until analyzed.

[b]The benzidinium sulfate (Table VII-VIII) was originally dissolved in triethylamine/methanol. See text.

[c]Mean values indicated in parentheses.

TABLE VIII

RECOVERY OF BENZIDINIUM SULFATE FROM FILTERS (-15°C)

Time (days)	% Recovered[a]		
	0.256 μg	0.712 μg	2.14 μg
0	112,102 (107)	94,97 (96)	104,100 (102)
1	94,90 (92)	102,94 (99)	98,96 (97)
5	97,-[b]	98,99 (99)	91,93 (92)
12	109,97 (100)	102,95 (99)	98,92 (95)
15	97,97 (97)	93,94 (94)	99,96 (97)
21	94,114 (104)	94,91 (93)	102,96 (99)

[a]Mean values indicated in parentheses.

[b]One sample broke.

TABLE IX

RECOVERY OF BENZIDINE FROM FILTERS (-15°C)

Time (days)	% Recovered[a]		
	0.2064 μg	0.691 μg	2.064 μg
0	92,103 (98)	89,95 (92)	105,98 (102)
1	110,107 (109)	97,95 (96)	90,96 (93)
11	86,96 (93)	100,-[b]	98,96 (97)
15	84,85 (85)	88,92 (90)	91,93 (93)
21	73,63 (68)	73,77 (75)	80,85 (83)

[a]Mean values indicated in parentheses.

[b]One sample broke.

TABLE X

RECOVERY OF 3,3'-DICHLOROBENZIDINE FROM SILICA GEL

	Time (days)	% Recovered[a]	
		0.554 μg	3.54 μg
-15°C	0	100,97,96 (98)	99,103,101 (101)
	7	110,118 (114)	104,104 (104)
	12	99,103 (101)	102,106 (104)
	21	106,100 (103)	101,101 (101)
	30	97,95 (96)	100,99 (100)
~23°C	2	99,104 (102)	110,102 (106)
	7	116,109 (113)	112,116 (114)
	12	99,95 (97)	99,104 (102)
	21	86,90 (88)	95,93 (94)
	30	90,86 (83)	90,90 (90)

[a]Mean values indicated in parentheses.

TABLE XI

RECOVERY OF 3,3'-DICHLOROBENZIDINE DIHYDROCHLORIDE[a] FROM SILICA GEL

	Time (days)	% Recovered[b]	
		0.743 μg	7.43 μg
-15°C	0	108,110 (109)	106,107 (107)
	2	107,103 (105)	104,104 (104)
	12	87,98 (93)	108,109 (109)
	21	98,101 (99)	110,110 (110)
	30	87,93 (90)	94,99 (97)
~23°C	2	104,103 (104)	112,106 (109)
	12	101,99 (100)	105,108 (107)
	21	102,113 (108)	99,102 (101)
	30	93,81 (87)	101,99 (100)

[a]The dihydrochloride (Tables XI, XIII) was originally dissolved in triethylamine/methanol. See text.

[b]Mean values indicated in parentheses.

TABLE XII

RECOVERY OF 3,3'-DICHLOROBENZIDINE FROM FILTERS

	Time (days)	% Recovered* 0.55 μg	5.54 μg
-15°C	0	92,98,99 (96)	97,99,103 (100)
	12	97,100 (99)	104,102 (103)
	21	97,97 (97)	102,107 (105)
	30	88,89 (89)	100,106 (103)
~23°C	2	97,109 (103)	105,111 (108)
	12	92,92 (92)	103,106 (105)
	21	96,96 (96)	103,97 (100)
	30	96,110 (103)	104,100 (102)

*Mean values indicated in parentheses.

TABLE XIII

RECOVERY OF 3,3'-DICHLOROBENZIDINE DIHYDROCHLORIDE FROM FILTERS

	Time (days)	% Recovered* 0.745 μg	7.45 μg
-15°C	0	95,99 (97)	103,102 (103)
	2	91,89 (90)	103,105 (104)
	21	104,107 (105)	106,109 (108)
	30	93,95,97 (95)	96,101,99 (99)
~23°C	2	111,103 (107)	114,106 (110)
	12	106,99 (103)	111,102 (107)
	30	86,87 (87)	103,107,107 (106)

*Mean values indicated in parentheses.

alcohol. This alkaline desorbing solution was employed to effect solubility of the salts and their subsequent conversion to the free amines. Because the purity of the triethylamine appears to differ from one batch to another, the desorbing solution should be chromatographed under the same conditions as the analyte to determine if it will interfere with the analysis. (The triethylamine used was obtained from Polyscience Corp., Niles, Illinois.) Filter and silica gel blanks (no analyte) provided no interference in the analyses. The efficiency of desorption (or recovery) was measured by dividing the detector's response to the treated sample by the response of an equivalent control solution that contained neither silica nor filter.

Recoveries of benzidine and benzidinium sulfate from treated filters and silica gel indicated that the compounds were unstable in these matrices at ambient temperature. Recoveries of ~70 to 80% were obtained after 8 days (data not shown) for spiked samples stored at ambient temperature. Filters and silica gel were spiked with benzidine and refrigerated (5°C) for 8 days for comparison. Recoveries averaged 79 to 84%. These low recoveries were probably a result of compound instability rather than a strong adsorption of the compound on the matrices as indicated by chromatograms exhibiting a shoulder on the benzidine peak. Even for samples stored at -15°C, losses of 12 to 18% were apparent in the treated silica gel after 11 days (Table VII). Benzidinium sulfate recoveries were ≥93% through 21 days on the glass-fiber filters (Table VIII) but a low recovery trend was already apparent for benzidine after 15 days (Table IX). The indicated instability of benzidine on the sampler's stages, therefore, suggests that the sampling tubes should be shipped and stored at approximately -15°C and that they be analyzed shortly thereafter.

Recoveries of 3,3'-dichlorobenzidine and its dihydrochloride from the sampler's stages indicated that these compounds had greater stability than benzidine or its salt. Tables X-XIII indicate that good recoveries (≥88% after 21 days) were obtained from both silica gel and filters stored at -15°C and at ambient temperature. Even after 30 days, recoveries did not average less than 83% (Table X) for silica gel spiked with 3,3'-dichlorobenzidine and stored at ambient temperatures.

C. Development of Quantitative Analytical Methods for Benzidine and 3,3'-Dichlorobenzidine

Reverse-phase chromatography was investigated for the development of analytical methods that could be employed for the analyses of benzidine and 3,3'-dichlorobenzidine. The instrumentation used consisted of a Waters ALC 202/401 (Waters Associates; Milford, Massachusetts) liquid chromatograph equipped with a 254-nm ultraviolet detector. The column employed was a 30-cm x 4.0-mm μBondapak C_{18} (Waters Associates). Distilled-in-glass methyl alcohol (UV grade) and acetonitrile (Burdick and Jackson Laboratories, Inc.; Muskegon, Michigan) were used in various ratios with distilled water to determine the best mobile phase for the given compound. The systems

described below were finally selected on the basis of efficiency, minimum tailing of peaks, and good resolution from the interferences studied. Benzidine (mp 126-127°C) was obtained from Litton Bionetics, Frederick, Maryland. 3,3'-Dichlorobenzidine (mp 130-132°C), its salt, and benzidinium sulfate were obtained from ICN-K&K Laboratories, Plainview, New York. The compounds were used as received.

1. Analytical Method for Benzidine. The pertinent parameters for the high-performance liquid chromatographic method for the analysis of benzidine were:

Column:	μBondapak C_{18} (30-cm x 4.0-mm i.d.)
Mobile Phase:	Methyl alcohol/water, 3/2 (v/v)
Flow rate:	1.5 ml/min, 1200 psig
Temperature:	~23°C
Detector:	UV (254 nm), 0.04 absorbance units full scale
Injection Volume:	10 μl
Efficiency:	2800 theoretical plates
Capacity ratio:	1.4

The retention time for benzidine with the above conditions was 2.7 min. The acid salt had the same retention time since, in both cases, the free amine was chromatographed. Quantitation was accomplished by manual area measurements with peak areas being determined by multiplying the peak height at maximum by the peak width at half height. The minimum level of quantitation was established as 0.15 μg per sample or 0.003 μg per injection (relative standard deviation of ≤7%). Below this level the precision varied significantly, and relative standard deviations of 30 to 60% were not uncommon. Calibration curves for benzidine and the sulfate are shown in Fig. 13.

Various compounds were investigated to determine if they would interfere in the analysis. Aniline was found to have the same retention time as benzidine and is therefore an interference. For solutions containing benzidine or its sulfate at concentrations of 8 ng/μl, the following compounds were found not to interfere when their concentrations were ≤16 ng/μl: o-, m-, and p-chloroaniline; 4,4'-methylenedianiline; α- and β-naphthylamine; 3-chloro-4-aminotoluene; 3,3'-dichlorobenzidine; 4,4'-methylenebis(2-chloroaniline); hydrazobenzene; 1,2- and 1,4-naphthoquinone. Figure 14 depicts the resolution capabilities of the analytical system for benzidine in the presence of other amines.

2. Analytical Method for 3,3'-Dichlorobenzidine. The pertinent parameters for the liquid chromatographic method for the analysis of 3,3'-dichlorobenzidine were:

Column:	μBondapak C_{18} (30-cm x 4.0-mm i.d.)
Mobile Phase:	Acetonitrile/water, 7/3 (v/v)
Flow Rate:	1.5 ml/min, 1200 psig
Temperature:	~23°C
Detector:	UV (254 nm), 0.04 absorbance units full scale
Injection Volume:	15 μl
Efficiency:	3900 Theoretical plates
Capacity Ratio:	1.4

The retention time for the free amine with the cited conditions was 3.4 min. Quantitation was accomplished with an Autolab System IV-B integrator (Spectral-Physics, Santa Clara, California) by setting the peak width at 10 s and the slope sensitivity at 300. Under the conditions described, the minimum level of quantitation was found to be 0.17 μg per sample or 0.005 μg per injection with a relative standard deviation of ≤7%. Below this level, as in the case with benzidine, the relative standard deviations were found to vary significantly. Calibration curves for 3,3'-dichlorobenzidine and the dihydrochloride are shown in Fig. 15.

Interference studies showed that 4,4'-methylenebis(2-chloroaniline) has a retention time close to 3,3'-dichlorobenzidine and interferes in the analysis. For solutions containing 3,3'dichlorobenzidine or its dihydrochloride at concentrations of 24 ng/μl, the following amines were found not to interfere when their concentrations were ≤60 ng/μl: o-, m-, and p-chloroaniline; benzidine; aniline; 4,4'-methylenedianiline; α- and β-naphthylamine; N-methylaniline; 2-aminotoluene (o-toluidine); and

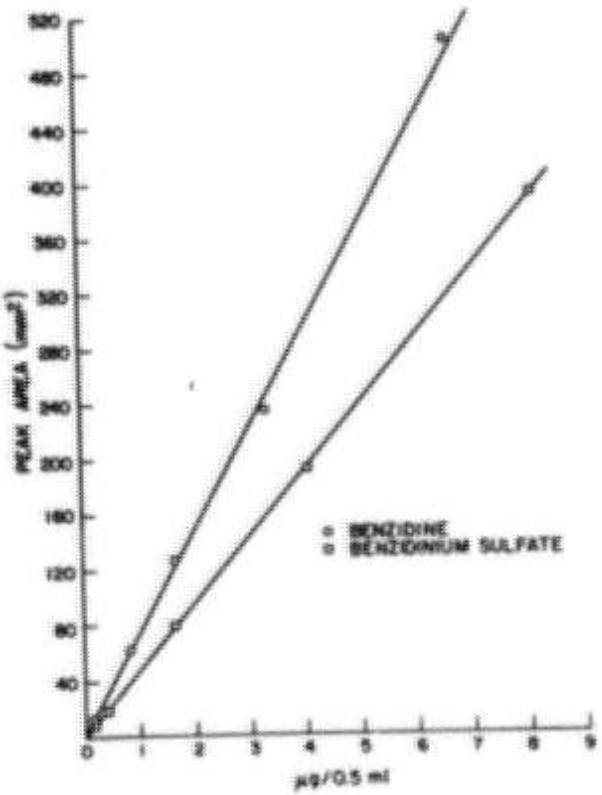

Fig. 13.
Calibration curves for benzidine and benzidinium sulfate.

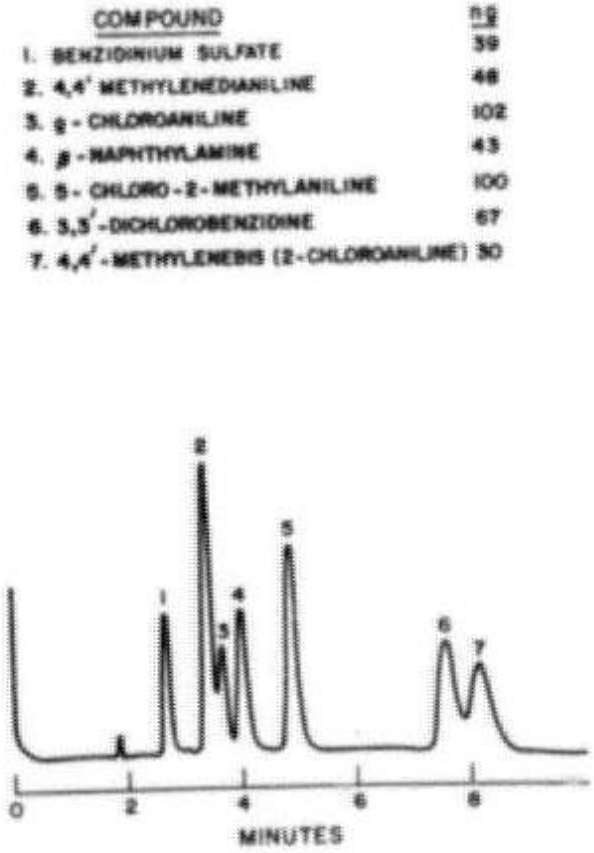

Fig. 14.
Chromatographic separation of benzidinium sulfate from other amines.

4,4'diamino-3,3'-dimethylbiphenyl (o-tolidine). The resolution capabilities of the analytical system for 3,3'-dichlorobenzidine from other amines is depicted in Fig. 16.

3. Analysis of Benzidine and 3,3'-Dichlorobenzidine Aerosols The analytical procedures (desorption a. measurement) described for these compounds were used to measure the deposition of aerosol on filters contained either on individual cascade impactor stages or on the filter sections of sampling tubes. Levels of the compounds found on individual cascade impactor filters ranged from 0.86 µg to 219 µg of benzidine and from 0.19 µg to 226 µg of dichlorobenzidine. In the sampling tube filters, the levels found for benzidine and 3,3'-dichlorobenzidine ranged from 0.31 µg to 6.5 µg and 0.39 µg to 7.33 µg, respectively.

No analyte was detected in the silica gel stage of the sampler. This suggests that very little (nondetectable) vapor phase existed for these compounds in the sampling chamber.

D. Desorption of α- and β-Naphthylamine

Filter and Silica Gel. Various solvents were investigated for the desorption of the naphthylamines from filters and silica gel considered for the sampler. Some of the solvents investigated included methylene chloride, chloroform, methyl alcohol, 2-propanol, acetonitrile, and ethyl acetate. In general, the lower polarity solvents such as chloroform produced low recoveries (70-80%) of the amines. The more polar solvents such as propanol, acetonitrile, and ethylacetate produced good recoveries (≥80%), but standard solutions in these solvents yielded erratic chromatographic results after a few days suggesting some degradation of the amines. A solution of 0.05% (v/v) acetic acid in 2-propanol yielded reproducible results with standard solutions and provided good recoveries from the matrices. Standard solutions of the amines in acidic propanol, stored in low actinic glass volumetric flasks, were stable for at least a month, if

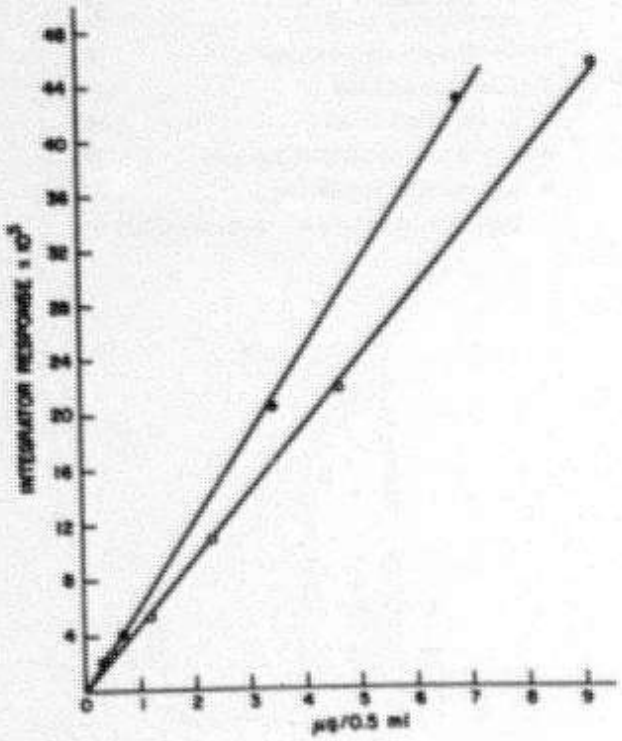

Fig. 15.
Calibration curves for 3,3'-dichlorobenzidine (•) and 3,3'-dichlorobenzidine dihydrochloride (○).

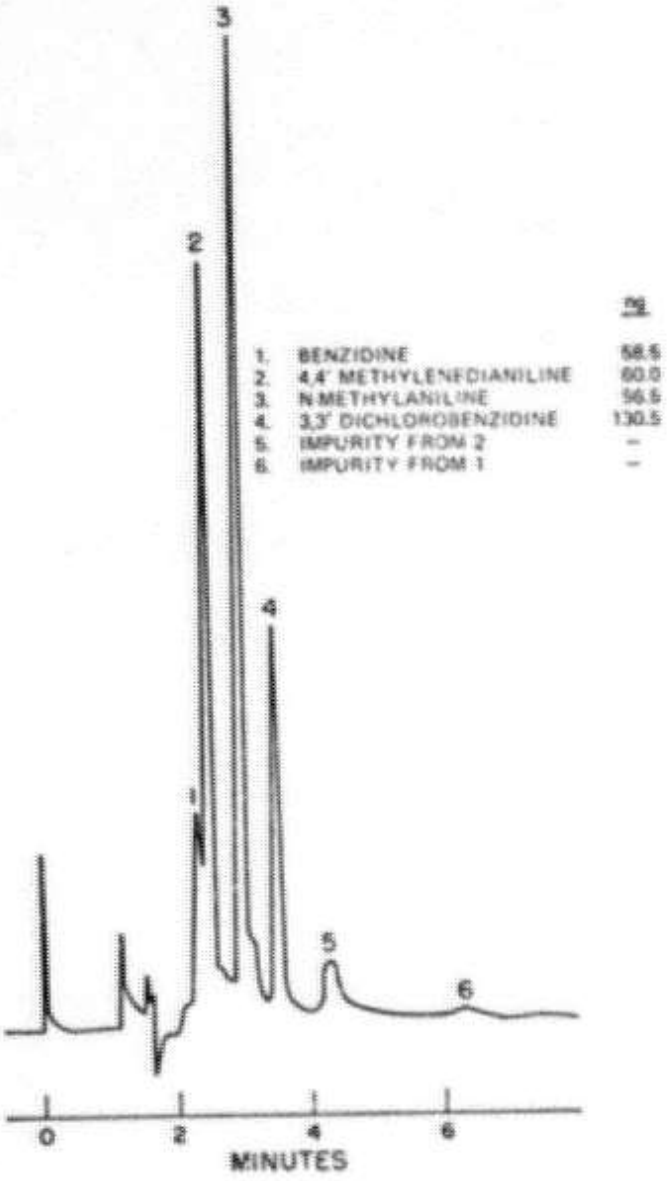

Fig. 16.
Chromatographic separation of 3,3'-dichlorobenzidine from other amines.

refrigerated. After six months, no indication of adverse effects by the acetic acid on the GC column were detected.

Nonspiked filters (blanks) were extracted with the desorbing solution to determine if the filter material could create an interference. Millipore TFE-LS, Metricel DM-800, Whatman GF/A, and Gelman Type A-E filters were found to provide peaks with the same retention times as α- or β-naphthylamine. Gelman's Type A, Type E, and Spectro-Grade filters were found to create no interference in the analysis. Spectro-Grade filters were selected for the sampler because Gelman has replaced the Types A and E with Type A-E.

Studies conducted to determine the efficiency of desorption for the naphthylamines from 30/60-mesh silica gel D-08 indicated recoveries ≥88% after 22 days for spiked samples stored at -15°C. As discussed in Sec. IV.A.2, however, two stages of 20/45-mesh silica gel were incorporated into the sampler to increase the sampler's capacity for collecting naphthylamine vapors and to reduce the pressure drop. Recovery of the amines from the 20/45-mesh silica gel proved comparable to that of the 30/60 mesh.

The addition and recovery of the naphthylamines on the Spectro-Grade filters and 20/45-mesh silica gel were performed in a manner similar to that already described for the benzidines. Exceptions, of course, included dissolution of the isomeric amines in 2-propanol for spiking purposes and desorption with the acetic acid/2-propanol solution. Both α- and β-naphthylamine were contained in the same spiking solution. The α- (mp 92-94°C) and β-naphthylamine (mp 108-110°C) were obtained from

Eastman Organic Chemicals, Rochester, New York, and ICN-K&K Laboratories, respectively.

Recovery (as a function of time) of α- and β-naphthylamine from filters and silica gel indicated the amines to be unstable in these matrices when stored at ambient temperatures. Table XIV shows that, after one week, recoveries with acidic propanol ranged from 51-73% for spiked silica gel stored at ~23°C. Recoveries of 24 to 67% were obtained within eight days for spiked filters stored at ambient temperature (filters data not shown). Evidence that the amines underwent some form of transformation on the silica gel was indicated by a pink-to-orange coloration of the matrix. The colored product was strongly adsorbed on the silica and was insoluble in such solvents as chloroform, methyl alcohol, 2-propanol, ethyl acetate, etc. Recovery of the amines from spiked silica gel and filters that were stored in a freezer at -15°C indicated the amines to be stable and recoverable (≥82% after 22 days) as shown in Tables XIV and XV. The indicated instability of the amines at ambient temperatures suggests that the sampling tubes be shipped and stored at approximately -15°C until analyses can be conducted.

E. Development of Quantitative Analytical Method for α- and β-Naphthylamine

Both HPLC and GC were employed in preliminary investigations to develop an analytical method that could be used for the simultaneous determination of α- and β-naphthylamine. Because of the similar partition coefficients that could be expected from these positional isomers, normal (adsorption) liquid chromatography rather than reverse-phase was investigated for their resolution. Partisil 10 (Whatman, Inc., Clifton, New Jersey), μPorasil (Waters Associates) and Lichrosorb 10 (Altex Scientific, Berkeley, California) all produced satisfactory resolution of the amines with a chloroform/n-hexane mobile phase. A UV detector (254 nm) employed to measure the analytes' response produced good sensitivity, about 0.6-ng absolute. It was discovered, however, that no two columns (even from the same manufacturer) produced reproducible retention times for a given mobile phase composition. For example, retention times differed by as much as 10 min for two μPorasil columns when chloroform/n-he · 2/3, v/v) was used as the mobile phase. Gas chromatography was investigated to determine if more reproducible parameters could be obtained.

Investigations with 0.9-m glass Tenax GC, 1.5-m glass 10% Apiezon L-2% KOH on Supelcon AW, 1.8-m glass 3% SP-2100 on Supelcoport, 0.9-m glass 4% Carbowax 20M + 0.8% KOH on Carbopak B, and 1.2-m stainless steel OV-25 columns provided little or no resolution between α- and β-naphthylamine. Satisfactory resolution between the amines was achieved with the SP-2100 column but the peaks exhibited significant tailing. Columns that provided excellent separation of the amines included the following glass columns (0.64-cm o.d.): 3-m 3% Dexsil 410, 1.8-m SP-2250, and 1.8-m 3% OV-225. The OV-225 column was selected for method

TABLE XIV

RECOVERY OF α- AND β-NAPHTHYLAMINE FROM SILICA GEL

		% Recovered*		% Recovered*	
	Time (days)	α-(0.436 μg)	β-(0.552 μg)	α-(1.539 μg)	β-(1.863 μg)
~23°C	0	99,95,99,100,99(98)	90,94,95,91,95(93)	89,96,92,90,96(93)	85,96,89,94(91)
	3	84,86,104(91)	84,92,88(88)	83,85,91(86)	78,78,86(81)
	7	55,59,56(57)	48,49,55(51)	73,73,73(73)	62,63,65(63)
	10	47,39,36(41)	34,37,36(36)	48,54,59(54)	40,42,42(41)
	14	10,9,22(14)	14,13,14(14)	33,35(34)	24,25(25)
-15°C	0	94,98,102,97,103(99)	100,96,102,95,103(99)	100,93,102,101,96(100)	98,99,100,95,99(98)
	7	106,96,96(99)	102,97,94(98)	101,104,100(102)	99,104,101(101)
	14	96,96,86(94)	97,94,89(93)	94,95,93(94)	92,94,93(93)
	22	93,95(94)	88,93(91)	93,95,96(95)	90,92,97(93)

*Mean values indicated in parentheses.

TABLE XV

RECOVERY OF α- AND β-NAPHTHYLAMINE FROM FILTERS (-15°C)

	% Recovered*		% Recovered*	
Time (days)	α-(0.456 μg)	β-(0.552 μg)	α-(1.539 μg)	β-(1.863 μg)
0	105,97,96,101(100)	103,104,98,101(102)	102,105,101,98(102)	103,105,102,99(102)
7	106,104,103(104)	113,109,108(110)	91,95,93(93)	91,87,85(88)
14	86,80,77(81)	102,99,98(100)	109,106,112(109)	96,96,101(98)
22	85,89,71(82)	101,102,96(100)	100,105(103)	95,100(98)

*Mean values indicated in parentheses.

development because it exhibited the best resolution and the least tailing. In addition, the Dexsil 410 column material was taken off the market by the manufacturer thus precluding its use in the future by OSHA or NIOSH.

1. Analytical Method for α- and β-Naphthylamine. The gas chromatographic procedure developed for the quantitative determination of the naphthylamines was found to be rapid and sensitive. The chromatographic conditions employed in the analysis are stated in Fig. 17, which shows the separation between the isomers for a pair of calibrating solutions. The retention times for α- and β-naphthylamine were 6 and 7 min, respectively. The minimum level of quantitation was found to be 0.3 ng for a 1-μl injection or 0.15 μg per sample. Desorption of the amines from filters and silica gel was carried out with 0.5 ml of 0.05% acetic acid in 2-propanol. Typical chromatograms for filter and silica extracts are shown in Fig. 18. Quantitation was accomplished with the Autolab System IVB integrator by setting the peak width at 18 s and the slope sensitivity at 50. Calibration curves for the amines are shown in Fig. 19.

For solutions containing α- and β-naphthylamine in concentrations of 4 ng/μl, the following compounds were found not to interfere in the analysis when their concentrations were ≤8 ng/μl: 4,4'methylenebis(2-chloroaniline), 3,3'-dichlorobenzidine, benzidine, 4,4'-methylenedianiline, o-chloroaniline, aniline, β-nitronaphthalene, α-naphthol, and β-naphthol. All the compounds listed, except for the anilines, had longer retention times than the analytes. α-Nitronaphthalene interferes with the α- and β-naphthylamine analysis. Figure 20 shows the resolution of the naphthylamines from the naphthols.

2. Results from Analyses of α- and β-Naphthylamine Aerosols and Vapors. Aerosols and vapors of the amines generated in the laboratory and collected in the sampler's matrices were analyzed by the gas chromatographic method. Levels of the compounds found on individual filters ranged from 0.3 μg to 0.99 μg and from 0.14 μg to 22.8 μg on the silica gel.

F. Evaluation of Sampling and Analytical Methods

1. Effects of Temperature and Humidity. Effects (in terms of recoverability and stability of the compounds, not capacity of the samplers) of temperature and humidity on the sampling and/or analytical method for benzidine, 3,3'-dichlorobenzidine, and the naphthylamines were tested by collecting multiple groups of samples from the chamber under various environmental conditions. While the aerosol or vapor concentration was maintained relatively constant, the temperature was adjusted to approximately 25, 30, and 35°C and the RH established at 20 and 80% at each temperature. Six groups of samples (five per group for benzidine and 3,3'-dichlorobenzidine, six per group for the naphthylamines) were collected in random order and stored in the dark at -15°C before analysis. Analyses were performed by HPLC or GC as previously described. No detectable quantities of either benzidine or 3,3'-dichlorobenzidine were found on the silica gel sections. In the case of the naphthylamines, the compounds were found in both filters and silica sections.

A two-way analysis of variance (ANOVA) was performed on each data set to determine if there was

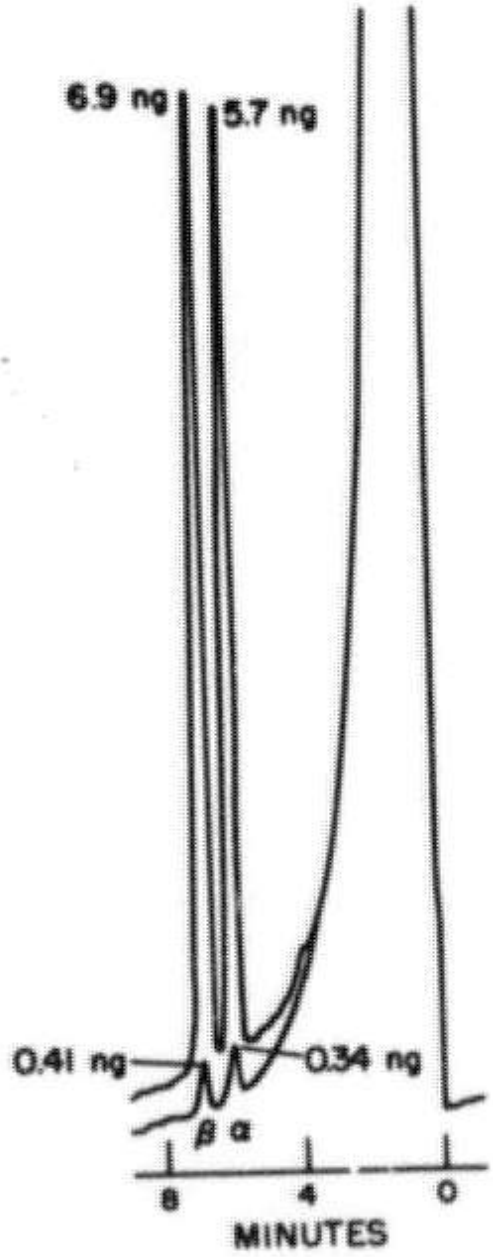

Fig. 17.
Gas chromatographic resolution of α- and β-naphthylamine. [Column: 1.8 m x 6.4 mm (glass), 3% OV-225 on, 80/100 mesh Supelcoport; Helium: 24 ml/min; Column Temp: 163°C; Detector: FID (1 x 2); Injection: 1 μl].

a difference in indicated air concentrations under the selected environments. This, in turn, indicated whether temperature, humidity, or an interaction of these two factors significantly affected the method. F values were computed according to standard procedures as discussed by Hald[13] using a random

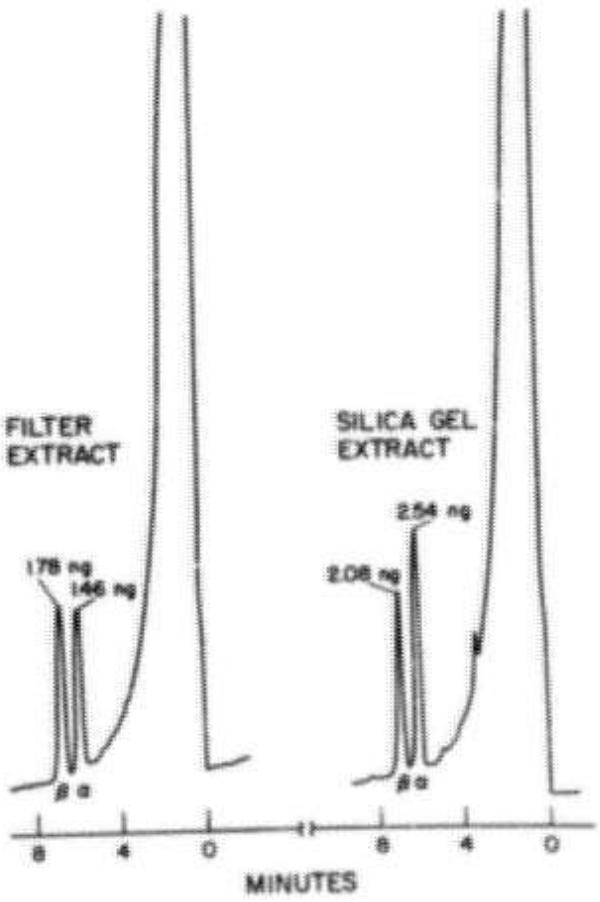

Fig. 18.
Chromatograms of filter and silica gel extracts containing α- and β-naphthylamine.

model. With respect to benzidine, 3,3'-dichlorobenzidine, and α-naphthylamine, the ANOVA indicated that neither temperature, humidity, nor an interaction of the two had a significant effect upon the method (Table XVI), although the capacity of the sampler for α-naphthylamine decreased with increasing relative humidity. This indicates that samples collected from virtually any probable environment should yield results which agree within the precision limits of the method.

Interpretation of the data for β-naphthylamine was not as straightforward as for the other compounds. Although neither temperature nor humidity independently affected the method significantly, the interaction of the two factors had a highly significant effect ($P<0.001$). This is difficult to explain because both isomers were generated, sampled, and analyzed together. Since no unusual effects were displayed for α-naphthylamine, it can be inferred that the experimental apparatus was

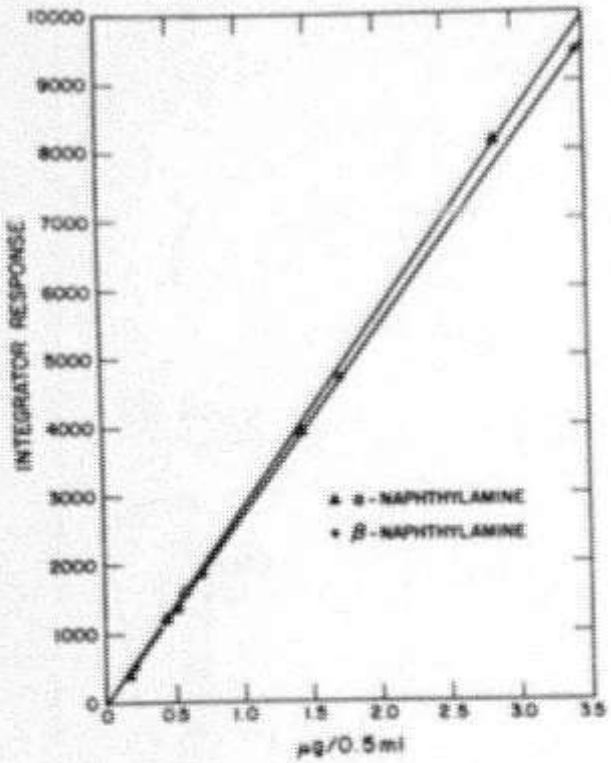

Fig. 19.
Calibration curves for α- and β-naphthylamine.

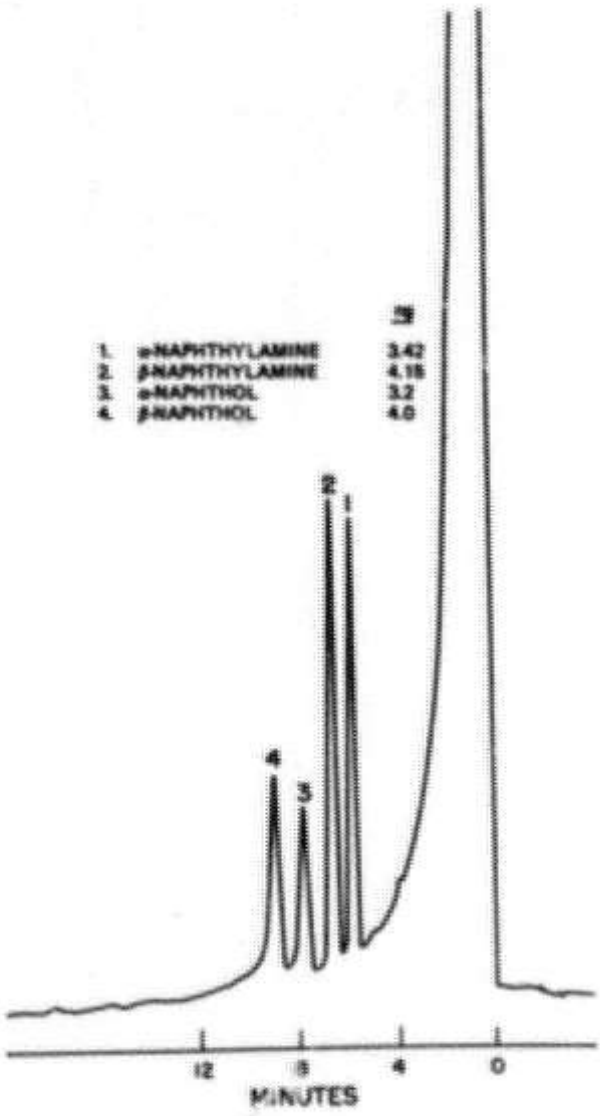

Fig. 20.
Chromatographic separation of naphthylamines and naphthols.

functioning properly. It appears that some loss of accuracy may be associated with the sampling and analysis of samples collected under varying conditions of temperature and humidity. Humidity, rather than temperature, appears to be tne major contributor to the error as shown in Table XVII where the data for the β-naphthylamine experiment are summarized. The mean β-naphthylamine concentration based upon all 36 observations was 81.6 µg/m³. A maximum error from the mean of 13% was observed in those samples collected at 26°C and 20% RH, which had a mean air concentration of 70.8 µg/m³.

2. Precision. The precision of the sampling/analytical method was determined by collecting and analyzing groups of 10 samples. Each group of samples was maintained in a zone of homogeneous aerosol or aerosol/vapor concentration at 30°C and 80% RH. The concentration in air and/or the sampling volume were adjusted to provide the desired amount of each compound. Critical orifices limited sampling flow rates at 0.8 liters/min. Samples were stored in the dark at -15°C until analyzed. Analyses were performed by HPLC or GC. No detectable quantities of benzidine or 3,3'-dichlorobenzidine were found in the silica sections of the samplers. The naphthylamines were found on the filters and the 100-mg silica sections.

The three groups of samples collected from 0.35 to 3.0 µg of benzidine, 1.1 to 7.0 µg of 3,3'-dichlorobenzidine, 0.35 to 2.7 µg of α-naphthylamine, and 0.26 to 2.3 µg of β-naphthylamine. The data are summarized in Table XVIII. Bartlett's Chi-Square test[14] showed no significant inhomogeneity of variance for either the

TABLE XVI

EFFECTS OF TEMPERATURE AND HUMIDITY ON THE SAMPLING/ANALYTICAL METHODS

Compound	Variable (Range)	Sample Vol (liters)	Challenge Conc (μg/m³)	F*	P*	Sp† (%)
Benzidine	Temp (26-35°C)	18	317	11.5	0.05<P<0.10	6.06
	RH (20-80%)			0.215	0.25<P	
	Temp x RH			2.42	0.10<P<0.25	
3,3'-Dichlorobenzidine	Temp (27-37°C)	16	128	7.70	0.10<P<0.25	3.90
	RH (20-80%)			0.684	0.25<P	
	Temp x RH			1.68	0.10<P<0.25	
α-Naphthylamine	Temp (26-36°C)	16	94	0.305	0.25<P	3.46
	RH (20-80%)			4.92	0.10<P<0.25	
	Temp x RH			2.97	0.05<P<0.10	
β-Naphthylamine	Temp (26-36°C)	16	82	0.402	0.25<P	4.00
	RH (20-80%)			3.75	0.10<P<0.25	
	Temp x RH			31.0	P <0.001	

*Based upon two-way ANOVA H_o: no significant effect, that is, no significant difference in mean sample concentration; upper one-sided test.

†Pooled estimate of S_m; assumes no significant difference in sample variances as confirmed by Bartlett's Chi-Square test.

TABLE XVII

INDICATED AIR CONCENTRATION* OF β-NAPHTHYLAMINE CALCULATED FROM SAMPLES COLLECTED IN VARIOUS ENVIRONMENTS

	Relative Humidity	
Temp (°C)	20%	80%
26	70.8 ± 4.64%	93.6 ± 4.28%
31	77.1 ± 5.62%	79.0 ± 2.50%
36	79.4 ± 1.54%	89.9 ± 3.98%

*Mean air concentration (μg/m³) from six replicate samples in each group ± relative standard deviation.

TABLE XVIII

PRECISION OF SAMPLING/ANALYTICAL METHODS

Compound	Amount (μg)	Sample Vol (liters)	No. of Samples	Air Conc ± σ_{rel}[b]	S_p(%)[c]
Benzidine	3.0	48	10	63.2 ± 1.43	4.19
	1.38	49	10	28.2 ±1.20	
	0.35	16	09[a]	21.1 ± 1.10	
3,3'-Dichlorobenzidine	7.0	51	10	13.4 ± 0.56	5.05
	3.4	51	08[a]	66.5 ± 2.53	
	1.1	51	10	20.2 ± 1.37	
α-Naphthylamine	2.7	73	10	36.8 ± 0.91	[d]
	1.3	32	10	39.9 ± 2.17	
	0.35	08	09[a]	45.9 ± 3.57	
β-Naphthylamine	2.3	73	10	31.2 ± 0.61	[d]
	1.0	32	10	32.4 ± 2.39	
	0.26	08	09[a]	30.8 ± 2.72	

[a]One or more samples were broken prior to analysis.

[b]In μg/m³.

[c]Pooled estimate of S_{rel}; assumes no significant difference in sample variances, as confirmed by Bartlett's Chi-Square test.

[d]Bartlett's Chi-Square test showed differences in variances, therefore, S_p values were not calculated.

benzidine ($0.10<P<0.20$) or 3,3'-dichlorobenzidine ($0.30<P<0.40$) samples. Values of the pooled estimate of S_{rel} were 4.19 and 5.05% for benzidine and 3,3'-dichlorobenzidine, respectively. When coupled with a personal-pump error of 5%, the precision of the methods as applied to field sampling should be 6.52 and 7.11% for benzidine and 3,3'-dichlorobenzidine, respectively. Bartlett's Chi-Square test did show significant difference in variances for the α- ($0.01<P<0.025$) and β-naphthylamine ($0.0005<P<0.005$) samples; thus values of S_p were not calculated. Values of S_{rel} increased from 2.61 to 7.77% for α-naphthylamine indicating that field sampling precision levels should be between 5.64 and 9.25%. The corresponding S_{rel} values found for β-naphthylamines were between 1.96 and 8.82% with expected field precision of between 5.37 and 10.1%.

V. EVALUATION OF COMMERCIAL RESPIRATOR CANISTERS FOR PROTECTION AGAINST 4,4'-METHYLENEBIS(2-CHLOROANILINE) AND THE NAPHTHYLAMINES

In order to evaluate the protection that could be afforded by commercially available air-purifying respirators, several of the all-purpose canisters used with these devices were tested to determine their effectiveness in removing 4,4'-methylenebis(2-chloroaniline) and the naphthylamines from an airstream. The test chamber used was designed to accommodate four respirator canisters and is shown in Fig. 21. Because there is a possibility that both aerosols and vapors of the compounds may be found in certain environments, the canisters that were evaluated were of the two-stage type. The first stage

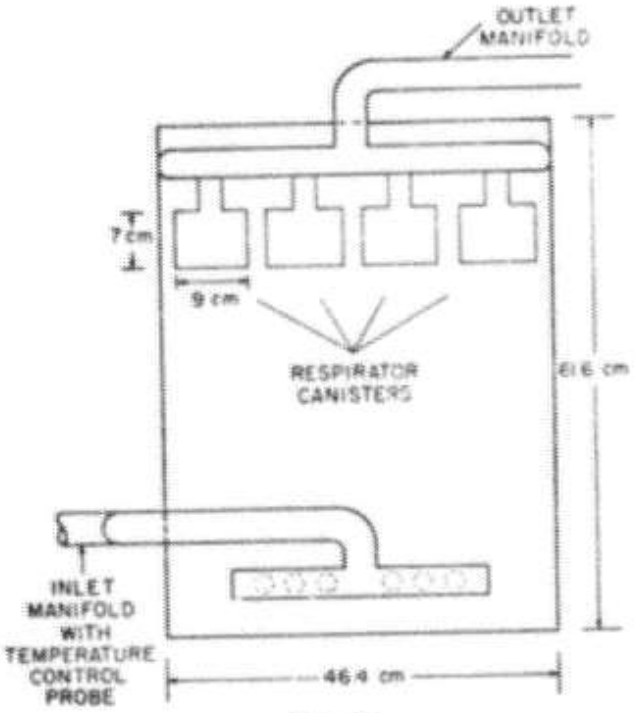

Fig. 21.
Respirator-canister test chamber.

consists of a high-efficiency particulate air (HEPA) filter followed by an activated charcoal stage. The canisters were pretested[15] for leaks in the HEPA filter by challenging them with a sodium chloride aerosol (MMAD = 0.6 μm, σ_g = 2, concentration = 15 mg/m³) at 32 liters/min, and monitoring the downstream concentration with a flame photometer. All canisters showed collection efficiencies greater than 99.95%.

Four canisters of each type (MSA GMR-S: Mine Safety Appliances, Pittsburgh, Pennsylvania; Welsh-Protex 7500-83: Norton Safety Products Division, Cranston, Rhode Island; Scott 282-OAP-R: Scott Co., South Haven, Michigan) were placed in the test chamber and challenged with 4,4'-methylenebis(2-chloroaniline) aerosol concentrations of 100-160 μg/m³ for 7 h at 25°C and 50% RH. Air from the chamber was drawn through each canister at the rate of ~32 liters/min to determine the possibility of carcinogen breakthrough. Two-stage sampling tubes[4] were used to collect air samples downstream from each canister. Downstream samples were collected from time 0 to 4 h after the challenge started and also from the fourth through the seventh hour of the run. All downstream samples showed no evidence of 4,4'-methylenebis(2-chloroaniline) being present, that is, <0.15 μg/sample. Thus, the maximum concentration of the compound that could have penetrated the canisters would have been much less than 1 μg/m³.

Similar respirator canister tests were performed with the naphthylamines. However, it was decided that a 30°C and 80% RH air environment would be a more rigid test for the canisters. The canisters challenged with aerosols and vapors of the naphthylamines were: MSA GMR-S, Welsh Protex 7500-83, Scott 282-OAP-R, and Scott 282-OVR. The canisters were challenged for 8 h with naphthylamines concentrations of 100-300 μg/m³. Consecutive 4-h samples taken downstream from each canister indicated no detectable amounts (<0.01 μg/m³) of the amines. These results indicate that the canisters efficiently removed and retained the aerosols and vapors of the carcinogens under the conditions studied.

VI. CONCLUSIONS

The personal sampler and analytical procedures described here should provide a means for the industrial hygienist to monitor worker exposure to benzidine, 3,3'-dichlorobenzidine, and the naphthylamines. The methodology was developed with the idea that it should be practical and applicable under various conditions. The sampling tubes proposed are small and light and should not interfere with a worker's functions in the performance of his duties. The collection media of the sampler are able to collect both aerosols and vapors of the compounds from an airstream. The analytical procedures developed employ chromatographic techniques to provide the needed sensitivity as well as selectivity by resolving the analyte from possible contaminants that may be present in the same environment. All materials and instrumentation required to implement the methodology described are commercially available.

Although both the sampling and analytical procedures that were developed were designed to be applied in the occupational environment, no field samples were obtained to determine the reliability of the methods under various field conditions. The methods may, therefore, be applicable under many field conditions but may have to be modified in certain situations, for example, in the case of a contaminant (not evaluated in this study) that interferes with the analysis. Sufficient information is included in this report to allow the industrial

hygienist and the analytical chemist to make necessary modifications.

Commercially available all purpose respirator canisters (HEPA plus charcoal) were found to provide no detectable breakthrough when the canisters were challenged for 7 h with 4,4'-methylenebis(2-chloroaniline) aerosol concentrations of 100-160 $\mu g/m^3$. Canisters challenged for 8 h with naphthylamine environments containing aerosol and vapor (primarily the latter) concentrations of 100-300 $\mu g/m^3$ also indicated no breakthrough of the compounds. It can be expected, therefore, that the canisters tested should also provide protection against benzidine, 3,3'-dichlorobenzidine, and the salts of these amines.

The results presented in this report can be summarized to include the following highlights for the industrial hygienist.

Benzidine: A two-stage (filter and silica gel) sampler is used to collect the analyte. The range of the analytical method is 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 a HPLC. On the basis of canisters tested against 4,4'-methylenebis(2-chloroaniline) aerosols, the MSA GMR-S, Welsh-Protex 7500-83, and Scott 282-OAP-R can also be expected to provide protection against benzidine.

3,3'-Dichlorobenzidine: A two-stage (filter and silica gel) sampler is used to collect the analyte. The range of the analytical method is 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 a HPLC. Protection against aerosols of dichlorobenzidine can be expected from those canisters recommended for benzidine.

α- and β-Naphthylamine: A three-stage (filter and two beds of silica gel) sampler is used to collect the analyte(s). The range of the analytical method is 4-70 $\mu g/m^3$ for a 50-liter air sample using 0.5 ml of desorbing solution and a 1-μl injection into a gas chromatograph. Protection against aerosols and vapors of the naphthylamines can be expected from the above recommended canisters as well as the Scott 282-OVR.

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