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January 1—June 30, 1975

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DEVELOPMENT OF SAMPLING AND ANALYTICAL METHODS FOR CARCINOGENS

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NIOSH-IA-74-35

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

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ABSTRACT

Activities pertaining to the development of sampling and analytical methods for chemical carcinogens are discussed. A compressed gas nebulizer has been designed to produce respirable, polydisperse aerosols of the aromatic amines on the Occupational Safety and Health Administration (OSHA) list of cancer-suspect agents. The aerosol passes through a sampling chamber which can be maintained at various environments from 25 to 40°C and 0 to 100% relative humidity. A two-stage sampling tube incorporating a filter followed by a sorbent is proposed for collecting aerosols and associated vapors from air. High-speed liquid chromatography (HSLC) and gas chromatography have been explored for analyzing these aromatic amines. HSLC appears promising for 4,4′-methylene-bis(2-chloroaniline)(MOCA), benzidine, and 3,3′-dichlorobenzidine; preliminary solvent systems and detection limits are reported. Isomers of phthalaldehyde have been tested for nonspecific field detection of selected aromatic amines on the list. Detection limits are given using these reagents and fluorescamine for amines on various surfaces. Results of glove permeability tests indicate that natural latex and polyvinyl chloride are good materials for protecting skin from contact with MOCA and other aromatic amines. A chamber is described for testing respirator cartridges for breakthrough of the carcinogens.

I. INTRODUCTION

The Los Alamos Scientific Laboratory (LASL) has been conducting research on the development of analytical methods, air-sampling techniques, and related procedures for each of the cancer-suspect agents designated by OSHA in the 14 regulations of 29 CFR 1910.93 c-p.[1] The research program is under

the sponsorship of a National Institute for Occupational Safety and Health (NIOSH) Interagency Agreement (IA-74-35). This report covers research activities for the period January 1 through June 30, 1975.

The research program has been divided into five phases.

1. Aerosol generation and air-sampling chamber systems (Sec. II).
2. Development and evaluation of nonspecific, nonquantitative sampling and analytical methods for carcinogens (Sec. III).
3. Development and evaluation of specific laboratory identification methods for the regulated carcinogens (Sec. IV).
4. Development and evaluation of quantitative air-sampling methods (Sec. V).
5. Evaluation of commercial respirator sorbents for protection against the carcinogens under study (Sec. VI).

These phases are proceeding with a revised schedule established by NIOSH.

A. Summary of Activities

All major construction has been completed for the laboratory. Most equipment has been purchased and is in satisfactory operation. The aerosol generation and sampling system has been completed. A seven-stage cascade impactor has been calibrated for use in sizing the aerosol produced by a compressed gas nebulizer. The sampling chamber has been designed for containment, sampling, and temperature and humidity stability.

New reagents for nonspecific field detection of selected aromatic amines included in this report are isomers of phthalaldehyde. The lower limit of detection for benzidine with o-phthalaldehyde is 3 ng/cm^2 on filter paper. Fluorescamine, using the surface leaching technique, detects 10 to 15 ng/cm^2 of 4,4′-methylene-bis(2-chloroaniline) (MOCA), benzidine, 3,3′-dichlorobenzidine, and β-naphthylamine on stainless steel and painted surfaces, respectively.

A two-stage air-sampling system which employs a filter followed by a sorbent is proposed for the first five compounds. Screening tests indicate that silica gel, Spherosil XOC-005, and Gas Chrom-P are satisfactory sorbents for collecting the vapors of MOCA from air for laboratory analysis. High-speed liquid chromatography appears the most promising analytical technique for the compounds tested. Employing a μBondapak C_{18} column and 60% dioxane in water as a mobile phase, $\leq$ 4 ng of MOCA per injection can be determined by an ultraviolet (UV) detector set at 254 nm. Using a similar system, $\leq$ 7 ng of benzidine or 3,3′-dichlorobenzidine per injection can be determined.

A respirator cartridge test chamber has been constructed, which will operate under conditions similar to those of the sampling chamber.

Gloves used for skin protection have undergone permeability tests. Natural latex and polyvinyl chloride appear to be the most practical glove materials for use with MOCA.

B. Laboratory Preparation

The laboratory facility is essentially complete. Many of the requirements in 29 CFR 1910.93 c-p[1] do not apply to a chemical laboratory; however, every effort is being made to exceed the applicable requirements. At the present time all minimum requirements have been met. The LASL Health Division approved the laboratory facility for work with cancer-suspect agents during the first quarter of this reporting period. The safety protocol developed for the LASL Cancer-Suspect Agent Laboratory has been completed and put into effect. Project personnel have been given medical clearance to work with the cancer-suspect agents and trained in standard and emergency procedures.

C. Equipment

All major instrumentation anticipated for analytical work on this project has been received except for the variable wavelength UV liquid chromatograph detector which is on order. During the reporting period an infrared spectrometer, Model SP-1100, (Pye-Unicam, Philips Electronic Instruments, Mt. Vernon, NY) and a microbalance, Model AD-2 (Perkin Elmer, Norwalk, CT) were purchased and received. To assist the carcinogen project, a UV-Vis-NIR spectrophotometer, Model DK-2A, (Beckman Instrument Co., Fullerton, CA), a spectrophotofluorometer, Model 5-81000 (Aminco Bowman, Silver Spring, MD), and other equipment were loaned to the program by LASL. An analytical gas chromatograph, Model 311, (Carle Instrument Co., Fullerton, CA) and a forward light-scattering photometer, Model JM-4000, (Sinclair-Phoenix, Virtis Co., Gardiner, NY) were purchased and received.

II. AEROSOL GENERATION AND AIR-SAMPLING CHAMBER SYSTEMS

The first five compounds to be studied (i.e., MOCA, benzidine, 3,3′-dichlorobenzidine, and α- and β-naphthylamine) are solids at room temperature. A system has been developed for producing test atmospheres of aerosols and their associated vapors (ppm-ppb concentrations). Stainless steel and glass were chosen as construction materials for all surfaces and tubing that contact the cancer-suspect agent. The entire aerosol generation and sampling system (Fig. 1) has been located in two interconnecting gloveboxes which serve as an isolation system (secondary confinement).

A. Description of Equipment

A compressed gas nebulizer (Fig. 2) appears to be a practical method for generating aerosols of high-boiling organic compounds. Vapor formation is a function of the temperature of the chamber and vapor pressure of the compound. The nebulizer operates at a temperature above the compound's melting point.

The generation and sampling systems operate as follows. Pure dry nitrogen at a flow rate of 1 to 2 liters/min is used to atomize the compound into a polydisperse respirable aerosol. The aerosol passes through a ^{85}Kr charge neutralizer, Model 3054, (Thermo-Systems, Inc., St. Paul, MN) connected directly to the nebulizer. The neutralized aerosol is diluted in a device which surrounds the particle stream with a sheath of humidified air. (The humidifier is shown in Fig. 3.) Clean dry air passes directly over water which is heated to a selected temperature to produce the required humidity. The airstream and aerosol pass through a heater (Fig. 4) where the proper temperature is established prior to

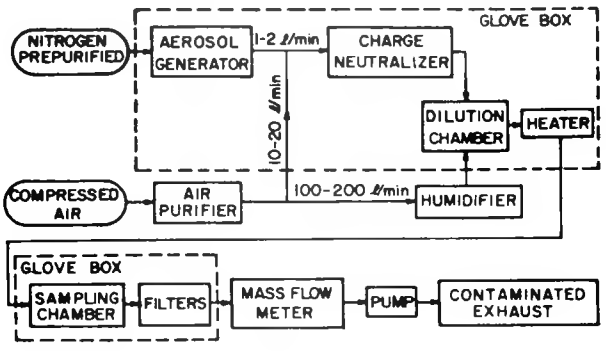


Fig. 1.
Flow diagram of the aerosol generation system.

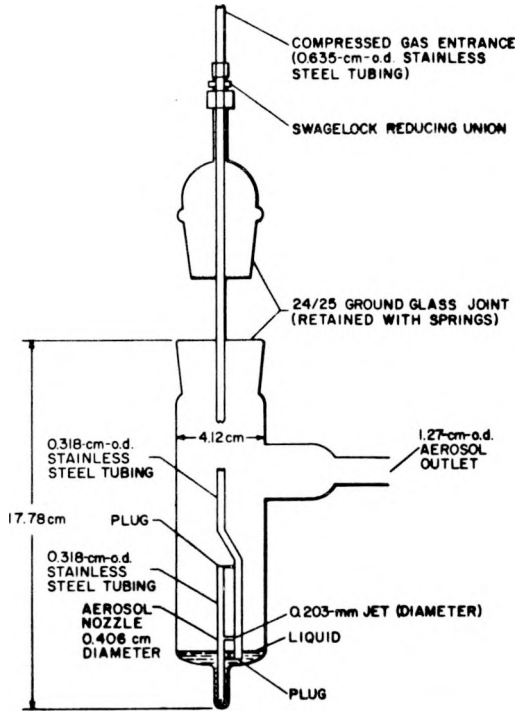


Fig. 2.
Compressed gas nebulizer.

entering the chamber. The sampling chamber was designed according to criteria[2] developed in other LASL aerosol studies (Fig. 5). Sampling ports are arranged to draw air from a laminar flow area. The effluent from the chamber is decontaminated by high efficiency particulate air (HEPA) and activated charcoal filters. The cleaned air is then pumped to the contaminated exhaust associated with the laboratory's air decontamination system.

Associated with the aerosol generator are monitors which measure the environmental conditions within the sampling zone (Fig. 5). Temperature and relative humidity are monitored by a temperature and relative humidity indicator, Model 400C, (General Eastern Corp., Watertown, MA) with dual linear 25-mV outputs. The aerosol concentration is

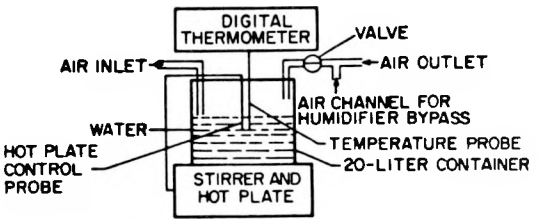


Fig. 3.
Humidifier.

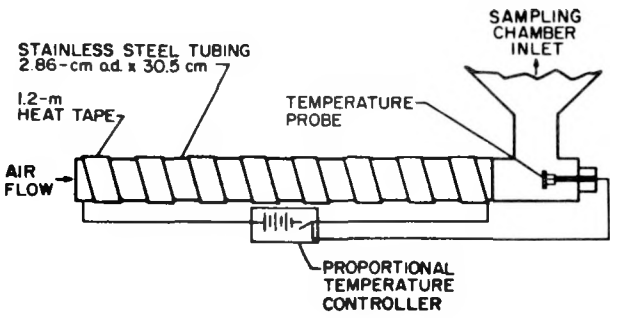


Fig. 4.
Air heater.

measured by a forward light-scattering photometer (FLSP) with an adjustable linear 0 to 25-mV output. Flow through the chamber is measured with a mass flow meter, Model AHL-10, (Teledyne-Hastings-Raydist, Hampton, VA), which produces a linear 0 to 5-V output. The analog signals from these devices are recorded by dual channel potentiometric strip-chart recorders with variable 1-mV to 100-V inputs and 10-to-1 attenuators, Model 400, (Linear Instrument Corp., Irvine, CA).

B. Evaluation of Aerosol Generation Equipment and Conditions

1. **Particle Size.** The particle size distribution of the aerosol produced by the compressed gas nebulizer is determined with the aid of a seven-stage, stainless-steel cascade impactor. The device is the same as a Lovelace Cascade Impactor[3] except that the number of jets per stage has been changed from one to four to increase the airflow and collection area. The modified stage configuration is shown in Fig. 6.

The effective cutoff aerodynamic diameter (ECAD, which correspondsto 50% collection efficiency) for each impactor stage was determined using the apparatus shown in Fig. 7. Distilled water suspensions of monodisperse latex spheres (0.1% to 0.01% V/V) were atomized in a commercial nebulizer (Retec Development Laboratory, Portland, OR). The resulting suspensions, produced at 3 to 4 liters/min, were diluted with approximately 30 liters of dry air and forced through the charge neutralizer into a cylindrical metal sampling chamber of 6 liters capacity. Adjustment of 3-way ball valves directed the aerosol into the FLSP where the photomultiplier gain was arbitrarily set at 100%. The valves were

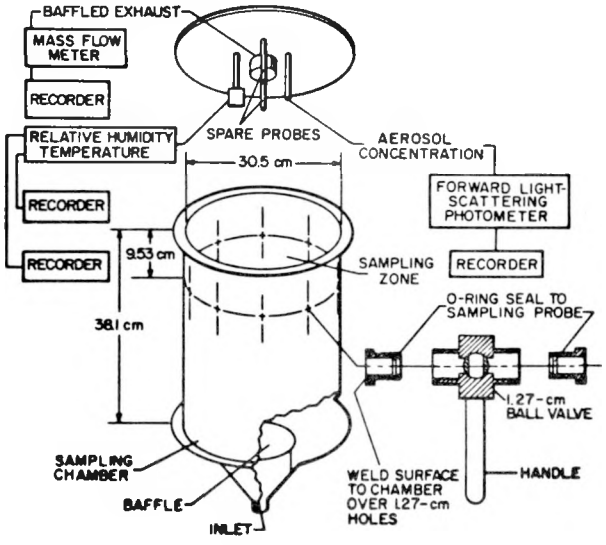


Fig. 5.
Air-sampling chamber.

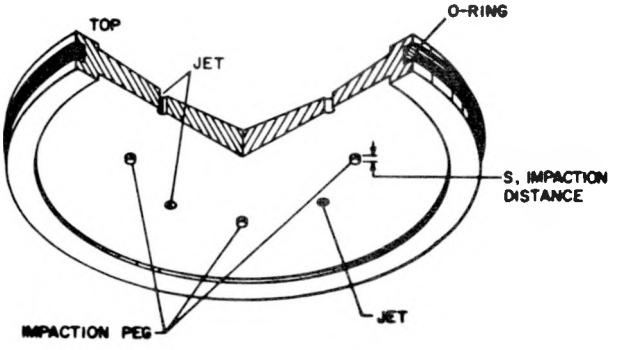


Fig. 6.
Single stage of modified cascade impactor.

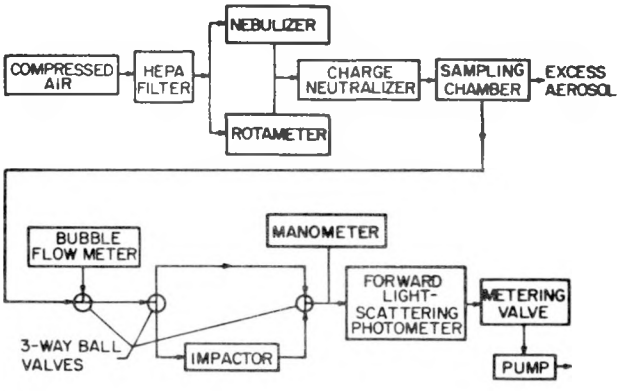


Fig. 7.
Flow diagram of cascade impactor calibration system.

switched to route the particles through stages 2, 3, or 4 of the impactor before entering the FLSP. The aerosol concentration reaching the FLSP was a direct measure of the collection efficiency of the stage being tested at a given flow rate. Polyvinyl chloride membrane filters of 25 mm diam (0.8 μm pore size) were placed on the collection plate to aid in the retention of particles impinged at the plate's surface. Repetition of this procedure at several flow rates (measured with a 1 liter bubble flow meter) defined the collection characteristics of each stage. Figure 8 shows the curves of collection efficiency vs $P^{1/2}$ where P is the dimensionless impaction parameter.[4]

$$P = \frac{\rho_p d^2 U_0 C}{9\eta W}$$

where

ρ_p = particle density (g/cm^3),

d = particle diameter (cm),

U_0 = velocity of particle in the impaction jet (cm/s),

C = Cunningham (slip) correction factor,

η = viscosity of air, poise, and

W = diameter of impaction jet, (cm).

Table I shows the $(P_{50})^{1/2}$values ($P^{1/2}$ at 50% collection efficiency) which were determined experimentally for stages 2, 3, and 4. The average of these values, 0.535, was selected to describe the collection characteristics of stages 1, 5, 6, and 7 on the basis of the following considerations. First, experimental data for the three calibrated stages are in close agreement, revealing a maximum deviation from the mean of 5.6% for the $(P_{50})^{1/2}$ values. Second, the geometries of all seven stages are identical and their S/W ratios (jet-to-exit distance/jet diam) ratios fall within a narrow range. Finally, the flow characteristics of all stages should be quite similar, as indicated by the Reynolds numbers (Re) which extend from 214 (stage 1) to 2723 (stage 7).

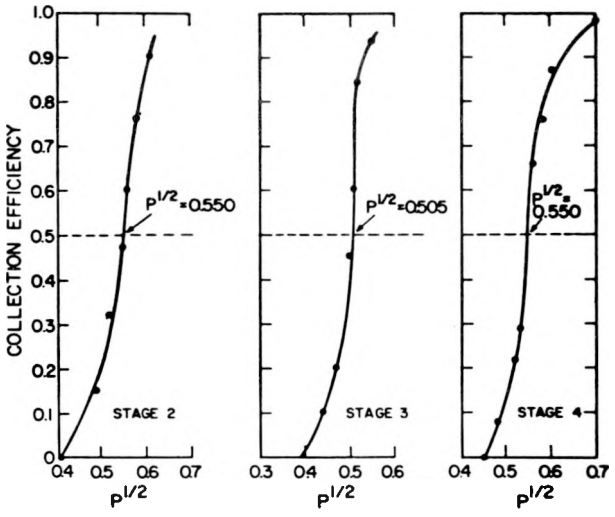


Fig. 8.
Single stage efficiencies of cascade impactor.

Table II shows the collection characteristics of all seven impactor stages. The ECADs are based on a total volumetric flow rate of 1.43 liters/min (0.36 liters/min per jet) which corresponds to critical flow through stage 7. Flow corrections were made for stages 5, 6, and 7 because of their significant pressure drops. Particles smaller than 0.13 μm are collected on a membrane filter at the impactor outlet.

2. Temperature. Temperature stability of the system between 25 and 40°C was evaluated in the following manner. Thermocouples were placed at four points in the sampling zone through sample ports (Fig. 5) and the proportional temperature controller was set at the level required to produce the desired chamber temperature. Airflow was set at 120 liters/min and the system was allowed to equilibrate for at least 1 h. Then, using a digital multipoint thermometer, Model BAT-8C (Bailey Instruments Co., Saddle Brook, NJ), having a resolution of 0.1°C, the temperatures at the four sampling points were recorded each minute over a 10-min period. The stability ranged from 0.0 to 0.1°C at 25°C to 0.1 to 0.3°C at 40°C. Additional temperature readings taken at 15-min intervals over a 2-h period gave identical results.

3. Humidity. Humidity control was evaluated with the temperature-compensated humidity indicator previously described. The sensor (sulfonated polystyrene) was calibrated at relative humidities of 0, 33, and 96.5%, using a desiccant and saturated solutions of $MgCl_2$ and K_2SO_4 respectively.[5] It was mounted in the chamber as shown in Fig. 5 with an airflow through the chamber of 120 liters/min. The humidifier heater was turned on and the relative humidity was monitored with the recorder until equilibrium was established.

These tests showed that 30-100 ±2% relative humidity could be maintained at chamber temperatures between 25 and 35°C. At chamber temperatures >35°C the humidifier output was great enough to cause condensation in the lines leading to the chamber. Thus, relative humidities between 50 and 75% will be the maximum attainable

TABLE I

EXPERIMENTAL IMPACTOR STAGE CALIBRATION (585 TORR)

Stage	S[a] (cm)	W[b] (cm)	S/W	$(P_{50})^{\frac{1}{2}}$[c]	Calibration Aerosol[d] Material	Density (gm/cm^3)	Diameter (μm)
2	0.163	0.104	1.56	0.550	Polyvinyltoluene	1.025	2.02
3	0.097	0.069	1.41	0.505	Polystyrene	1.056	1.011
4	0.064	0.043	1.48	0.550	Polystyrene	1.056	0.79

[a] Distance from exit of jet to collection plate.

[b] Diameter of jet.

[c] Obtained by interpolation from Fig. 8 at 50% collection efficiency points.

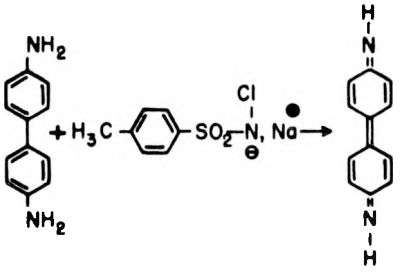
[d] Obtained from Dow Chemical Company, Midland, MI, monodisperse particles with geometric standard deviations of 1.0135 (2.02 μm), 1.0054 (1.011 μm), and 1.0044 (0.79 μm).

at temperatures above 35°C. Relative humidity of 0% is established by by-passing the humidifier.

III. DEVELOPMENT AND EVALUATION OF NONSPECIFIC, NONQUANTITATIVE SAMPLING AND ANALYTICAL METHODS FOR CARCINOGENS

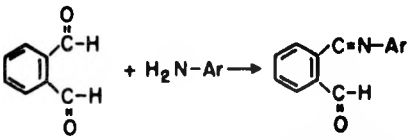
The development of nonspecific, nonquantitative field detection methods (field spot tests) has proceeded well because of previous experience with analogues and homologues of the carcinogens.[6] In addition to the spot test reagents previously discussed, the following new reagent reactions have been studied.

1. Chloramine-T with primary aromatic amines.



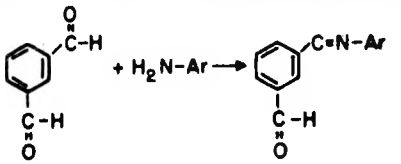
HIGHLY COLORED QUINOIDAL STRUCTURE

2. o-Phthalaldehyde with primary aromatic amines.



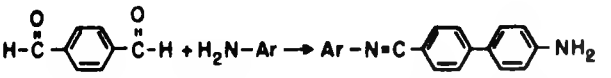
OR *bis* (SCHIFF BASE)

3. m-Phthalaldehyde with primary amines.



OR *bis* (SCHIFF BASE)

4. p-Phthalaldehyde with primary amines.



OR *bis* (SCHIFF BASE)

These reactions were not completely general in their applicability and each was individually evaluated.

A. Experimental Reagents and Procedure

Reagents and chemicals described in Ref. 6 were also used in this study. Additional reagents are:

- o-Phthalaldehyde, J. T. Baker, Baker grade (1 mg/cm^3 in glacial acetic acid).
- m-Phthalaldehyde, Aldrich, 27% (1 mg/cm^3 in glacial acetic acid).

TABLE II

CASCADE IMPACTOR COLLECTION CHARACTERISTICS (585 TORR)

Stage	S[a] (cm)	W[b] (cm)	S/W	Re[c]	$(P_{50})^{\frac{1}{2}}$	ECAD (μm)
1	0.272	0.178	1.53	214	0.535[d]	5.89
2	0.163	0.104	1.56	367	0.550	2.60
3	0.097	0.069	1.41	553	0.505	1.25
4	0.064	0.043	1.48	890	0.550[d]	0.62
5	0.048	0.032	1.51	1285	0.535[d]	0.36
6	0.038	0.028	1.36	2531	0.535[d]	0.21
7	0.038	0.028	1.36	2723	0.535[d]	0.13

[a] Distance from exit to jet to collection plate.

[b] Diameter of jet.

[c] Reynolds number = $\frac{WU_0\rho_a}{\eta}$ where ρ_a = density of air (g/cm^3).

[d] Based upon average of stages 2, 3, and 4.

- p-Phthalaldehyde, Matheson, Coleman, and Bell, mp-115-116°C. (1 mg/cm^3 in glacial acetic acid).
- Fluorescamine (fluram), Aldrich Chemical Co. (0.35 mg/cm^3 in tetrahydrofuran).

The solvent initially proposed for fluorescamine was 1,4 dioxane; however, because it may be termed a cancer-suspect agent,[7] we chose a less potentially hazardous solvent, tetrahydrofuran, for field use.

Procedures used here were the same as those described in the previous progress report.[6] The leaching technique limited the area to be tested and made the survey of vertical surfaces difficult. The use of swipe techniques appeared to be more applicable and could increase the sensitivity by increasing the area examined.

B. Results and Discussion

o-Phthalaldehyde has been reported[9] capable of forming highly fluorescent derivatives with benzidine. To determine its general applicability to other primary aromatic amines, a number of compounds have been studied. Based upon considerations of steric hindrance and resonance structure, m- and p-phthalaldehyde were expected to be more sensitive for some amines than o-phthalaldehyde. Contrary to this, the o-isomer gave generally lower limits of detection for the homologues and analogues than did either m- or p-phthalaldehyde (Table III). A high degree of selectivity and specificity was shown for benzidine by o-phthalaldehyde. The lower limit of detection of benzidine with o-phthalaldehyde was 3 ng/cm^2 which is an order of magnitude lower than for other amines. (Subsequent studies not reported here have shown a similar high degree of o-phthalaldehyde specificity for β-naphthylamine.) The use of o-phthalaldehyde shows promise in field tests as a monitoring agent for benzidine and β-naphthylamine contamination.

The values given in Table IV show detection limits for MOCA, benzidine, and 3,3′-dichlorobenzidine by both fluorescamine and the colorimetric visualization reagents. As seen, the use of fluorescamine as a detection reagent for MOCA and benzidine affords an order of magnitude greater sensitivity than may

TABLE III

LIMIT OF DETECTION VALUES FOR AROMATIC AMINES[a] VISUALIZED WITH ISOMERIC PHTHALALDEHYDES

Aromatic Amines	Isomeric Phthalaldehyde (ng/cm^2)		
	ortho	meta	para
Aniline	30	b	b
o-Chloroaniline	b	---	---
m-Chloroaniline	30	---	---
p-Chloroaniline	30	b	b
o-Toluidine	800	b	b
m-Toluidine	30	---	---
p-Toluidine	30	---	---
2-Chloro-4-methylaniline	30	b	b
3-Chloro-4-methylaniline	30	b	800
2-Aminobiphenyl	30	---	---
Methylenedianiline	30	800	800
Methylenedianiline dihydrochloride	30	---	---
N-Methylaniline	b	b	b
N,N-Dimethylaniline	b	---	---
MOCA	800	800	800
Benzidine	3	80	80
3,3′-Dichlorobenzidine	800	150	75

[a] Cancer-suspect agents and their homologues and analogues on filter paper (Whatman 42).

[b] Not detected at the level of 800 ng/cm^2.

be obtained with any of the colorimetric visualization reagents studied. Field sampling by "swipe" testing the surface under investigation may improve the detection limits shown in Table IV.

C. Field Detection of Carcinogens on Contaminated Surfaces

Test methods and techniques reported in Ref. 6 are continuing. Although field detection methods are not directly applicable to the NIOSH format, its outline serves as a guide for the detailed description of the field test.

These methods have been (internally) tested: two field industrial hygienists and three LASL laboratory technicians have performed the tests as written without supervision. The tests were made on surfaces contaminated with various amines, MOCA, and "blank" controls. No one performing the tests was familiar with them or was involved in this program. Each person was able to detect the type of contamination under consideration.

Table V gives the limit of detection values using fluorescamine and Table VI gives those for Ehrlich's reagent. The values obtained using these reagents and the leaching techniques were all less than the 0.2 $\mu g/cm^2$ on filter paper, stainless steel, and painted surfaces as requested by NIOSH.

The more common practice of wiping an area (swipe) with a suitable material to determine surface contamination will continue. A prototype kit for field use is being designed and the detailed assembly and construction will be completed during the next reporting period. Evaluation of contaminated test surfaces will continue.

TABLE IV

LIMIT OF DETECTION VALUES[a] FOR CANCER-SUSPECT AGENTS ON FILTER PAPER[b]

Visualization Reagents	Cancer-Suspect Agent		
	MOCA	Benzidine	3,3'-Dichlorobenzidine
Fluorescamine (1,4-dioxane solvent)	3	3	3
Fluorescamine (tetrahydrofuran solvent)	3	3	30
p-Dimethylaminobenzaldehyde	160	30	30
p-Dimethylaminocinnamaldehyde	30	30	30
Chloranil	800	800	800
Chloramine-T	c	30	30

[a] In ng/cm^2.

[b] Whatman 42.

[c] Not detected at the level of 800 ng/cm^2.

IV. DEVELOPMENT AND EVALUATION OF SPECIFIC LABORATORY IDENTIFICATION METHODS FOR THE REGULATED CARCINOGENS

We are in the process of surveying various methods for carcinogenic compound identification and selecting specific techniques for feasibility testing. The method selected need not be limited to sample size or carcinogen concentration.

Consideration has been given to various instrumental and chemical methods potentially applicable to the laboratory qualitative identification of the 14 specified carcinogens. Time-consuming chemical isolations and reactions appear to have only limited application. Instrumental methods are generally preferred because they are rapid, provide some specificity, and are very sensitive.

Only a limited number of instrumental techniques are available for evaluation. Gas chromatography and high-speed liquid chromatography are suitable for quantitative and semiqualitative work when the sample origin and history are well known (these techniques are discussed in Sec. V.B). Spectrophotometric methods normally require laboratory processing of the sample and are not as suitable for routine identification. The mass spectrometer (MS) coupled with a gas chromatograph (GC) offers the most promising method to identify mixtures of carcinogens with their associated contaminants in practically any type (above trace concentrations) of samples. No more than two of the listed carcinogens are expected to be present in any given sample: it follows that GC/MS is a practical analytical approach. Any good mass spectrometer with a high temperature injection port should be suitable for laboratory identification of the 14 listed cancer-suspect agents. A laboratory required to perform identification of any of the cancer-suspect agents by GC/MS must develop its own techniques and methods to carry out the analysis. A preliminary evaluation of this instrumental technique for MOCA and some additional aromatic amines was conducted.

The equipment used at LASL was a Model 1800 GC (Varian, Los Angeles, CA) with thermal conductivity (TC) detector interfaced with a heated splitter to a Time-of-Flight Mass Spectrometer (TOFMS), Model MA-2 (Bendix Corp., Rochester, NY) equipped with a high-speed differential pumping system. Companion studies were performed using a Model 21-104 mass spectrometer (Du Pont, Wilmington, DE) equipped with both molecular leak and heated direct-probe sample introduction systems.

TABLE V

LIMIT OF DETECTION VALUES USING
FLUORESCAMINE VISUALIZATION REAGENT

Contaminated Surface	Limit of Detection (ng/cm^2)		
	Filter Paper[a]	Stainless Steel[b]	Paint[c]
Technique	Direct[d]	Leaching[e]	Leaching[e]
Cancer-Suspect Agent			
MOCA	5	10	15
Benzidine	5	10	15
3,3′-Dichlorobenzidine	5	10	15
α-Naphthylamine	30	30	30
β-Naphthylamine	5	10	15

[a] Whatman 42.

[b] Type 316, roughness 0.5 - 3.5 μin.

[c] Machine gray, Glidden Company, Cleveland, OH.

[d] Direct application of reagent to surface being studied.

[e] Sample "leached" from surface by the following sequences of steps:
1. Place filter paper over spot.
2. Apply five drops of methanol to filter paper center while maintaining intimate surface/paper contact.
3. Allow methanol to evaporate; apply visualization reagent by drawing dropper from one edge of paper, across, onto, and to the opposite edge.
4. Examine for yellow fluorescent product while irradiating with 366 nm ultraviolet light.

Two μl of a 1% solution of carcinogen analogues in diethyl ether were injected into a heated (110°C) septum. Helium was used as a carrier gas at a flow rate of 50 cm^3/min through a 5.3-mm-i.d. x 1.0-m-long stainless steel column packed with 10% (W/W) Supelco-SP 2100 on Supelcoport (100/120 mesh). The detection limit of the GC/TC detector for the amines tested was 0.1 μg/injection under isothermal conditions at 150°C. The TOFMS total-ion monitor is ~100 times more sensitive for aromatic amines than the GC's TC detector. Still, identifiable mass spectra are difficult to obtain at low concentrations. The approximate detection limits for isomeric o-, m-, and p-chloroaniline was 20 ng, whereas for 4,4′-methylenedianiline the value was ~1 ng.

Based on the May and July 1975 audit by NIOSH project officers, effort in the identification portion of the program was reduced. Redirection of this phase is to include the application of GC/MS to the identification of major contaminants associated with the "technical" grade materials. Contaminants in industrial process bulk samples may also require identification in order to evaluate specificity of the air sampling and analytical methods.

V. DEVELOPMENT AND EVALUATION OF QUANTITATIVE AIR-SAMPLING METHODS

Under the terms of the LASL/NIOSH agreement, quantitative methods are to be developed for a minimum of 6 of the 14 carcinogens. The first five carcinogenic compounds under study are solids at room temperature with low vapor pressures; an effective air-sampling system must be capable of collecting both the aerosol and the vapor. Experience at this laboratory[10] indicates that a two-stage collection system (filtration and adsorption) is practical and consistent with the criteria developed by NIOSH/LASL. To improve on previously designed systems, the two stages are in a single unit. Selection of the sorbent requires maximization of sorption from air and controlled desorption in the laboratory with minimum interference in subsequent analytical procedures. The desorption technique must be compatible with the analytical procedure selected. Analytical techniques that appear most applicable for routine use in the determination of the listed carcinogens[1] are gas chromatography and high-speed liquid chromatography.

TABLE VI

CANCER-SUSPECT AGENT LIMIT OF DETECTION VALUES USING
p-DIMETHYLAMINOBENZALDEHYDE VISUALIZATION REAGENT

Contaminated Surface	Limit of Detection (ng/cm^2)		
	Filter Paper[a]	Stainless Steel[b]	Paint[c]
Technique	Direct[d]	Leaching[e]	Leaching[e]
Cancer-Suspect Agent			
MOCA	175	150	150
Benzidine	30	30	150
3,3′-Dichlorobenzidine	30	30	150
α-Naphthylamine	800	150	150
β-Naphthylamine	30	30	150

[a] Whatman 42.

[b] Type 316, roughness 0.5 - 3.5 μin.

[c] Machine gray, Glidden Company, Cleveland, OH.

[d] Direct application of reagent to surface being studied.

[e] Sample "leached" from surface by the following sequence of steps:
1. Place filter paper over spot.
2. Apply five drops of methanol to filter paper center while maintaining intimate surface/paper contact.
3. Allow methanol to evaporate; apply visualization reagent by drawing dropper from one edge of paper, across, onto, and to the opposite edge.
4. Examine.

A. Air Sampling

1. Air-Sampling Tube. The basic design of the proposed air-sampling tube is shown in Fig. 9. The first stage is an 8-mm filter retained in the tube with Teflon rings. These 8-mm-i.d. Teflon rings are the same as those described by Wood.[11] The back-up sorbent section (6.4-mm o.d.) was held in position with stainless-steel screens, 200 mesh, and plastic cap-tube seals. The sampling system is capable of being inserted through the two O-rings in the sampling valve shown in Fig. 8. This arrangement allows the entire sampler to be exposed and maintained under proper environmental conditions, yet ensures installation and removal without contamination of the secondary confinement (glovebox) of the sampling chamber.

2. Sorbent Desorption Tests. Microgram quantities of MOCA were eluted with solvents from portions of commercially available sorbents. Aliquots of the eluates were analyzed by gas chromatography with parameters given in Table VII. Desorption efficiencies were calculated, and three potentially useful sorbents were selected.

The first test compared desorption efficiencies of the two most commonly used sorbents, i.e., silica gel, 30/60-mesh, GC grade (Applied Science Laboratories, Inc., State College, PA) and activated charcoal 30/60-mesh GC grade (Applied Science). Using the desorption calibration technique described by White et al,[12] ~100-mg portions of these sorbents were measured in calibrated tubes and placed in 1-cm^3 microreaction vessels. Ten μl of dichloromethane containing 28.5 μg of MOCA were added to the sorbents with a 10-μl Hamilton syringe. The vessels were sealed with Teflon-lined rubber septa. After a minimum of 24 h, 500 μl of solvent (n-hexane, dichloromethane, or methanol) were added to the duplicate experimental sorbents, blanks, and MOCA controls. The vessels were resealed and swirled occasionally over a 30-min period. They were left to stand a minimum of 2 h at room temperature, after which duplicate aliquots were injected into the GC. The results are shown in Table VIII. No response was observed for aliquots of the charcoal sorbent eluates; thus, recoveries are reported as less than the minimum detectable level for the injection volume. As expected, silica gel recoveries increased with solvent polarity to 100% sorbent desorption of MOCA using methanol.

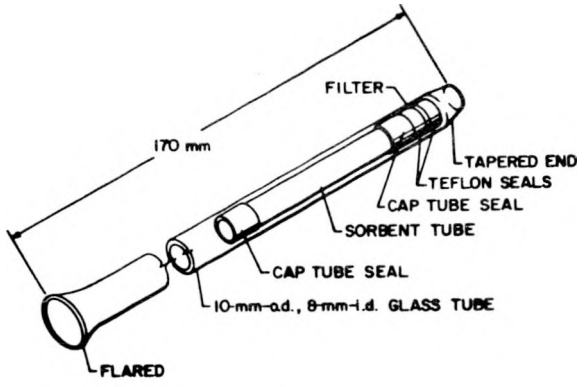


Fig. 9.
Two-stage air-sampling tube.

The suitability of various solvents to desorb MOCA from silica gel was further evaluated. Triplicate samples and controls were treated as before and desorbed with 500 μl of ethyl acetate, isopropanol, and methanol. The results indicated 100% recovery of MOCA with all three solvents.

Recoveries of lower levels of MOCA from various sorbents were determined in the final study. The porous polymers, Chromosorb 101, 60/80 mesh, and Tenax GC, 60/80 mesh were obtained from Applied Science as were Deactigel 45/60 mesh, (chemically deactivated silica gel) and Gas Chrom-P, 60/80 mesh (acid-washed diatomaceous earth). Gas Chrom-P is the acid- and base-washed form of Gas Chrom-S used by Yasuda[13] for the collection of MOCA vapors in air. Spherosil XOC-005, 100/200 μm (porous silica beads) and Amberlite XAD-8, 15/50 mesh (macroporous resin) were obtained from Supelco (Bellefonte, PA). These two sorbents were selected because they have the smallest surface areas and largest pore sizes of their respective classes. Triplicate samples were tested by the same procedure discussed above using isopropanol as the eluting solvent. As can be seen in Table IX, the most promising sorbents from this group are silica gel, Spherosil XOC-005, and Gas Chrom-P. Essentially complete recoveries were found for these three sorbents at the level of 2.85 μg of MOCA per 100 mg of sorbent. Recoveries from the other sorbents were lower or not observed either at the 2.85 μg or 14.5 μg levels.

Note that these desorption trials were intended as screening techniques with an estimated accuracy within 10 to 20%; they should not be considered as refined quantitative methods. Sorbents which are selected on the basis of these and subsequent screening tests will be further evaluated at lower detection limits by the analytical techniques currently being developed.

3. Sorbent Breakthrough Tests. Selection of a sorbent for any one of the cancer-suspect agents depends primarily on its ability to collect and retain the compound under a dynamic airflow situation. Preliminary studies are in progress to evaluate the sorbents that appeared most promising in the

TABLE VII

GAS CHROMATOGRAPHIC PARAMETERS FOR SORBENT DESORPTION TESTS

Parameter	Description
Instrument	Carle model 311 analytical gas chromatograph
Detector	Flame ionization (FID)
Column	1.83-m stainless steel 0.32 cm o.d. x 0.22 cm i.d., packed with 5% (W/W) OV-101 on 80/100 mesh, Supelcoport (Supelco, Inc., Bellefonte, PA)
Temperatures	Column Oven: 250°C isothermal
	Inlet and detector: 270°C
Flow rates of gases	Carrier (Helium): 40 cm^3/min (80 cm^3/min total)
	Hydrogen: 35 cm^3/min
	Air: 400 cm^3/min
Recorder	1 mV full scale
Retention time	MOCA: 7 min
Quantitation method	Peak height
Injection volume	1 to 5 μl

TABLE VIII

RECOVERIES OF MOCA FROM SILICA GEL AND ACTIVATED CHARCOAL

Sorbent	Weight (mg)	Standard Deviation (mg)	Recovery[a] (wt%) n-Hexane	Dichloro-methane	Methanol
Activated charcoal	102.32	2.33	<24.6	<24.6	<42.1
Silica gel	97.96	0.76	<22.8	33.0	118

[a]28.5 µg MOCA added, 500 µl solvent, detection limit varied with injection volume.

desorption study. A flame ionization detector is used to monitor the presence of the carcinogen vapor downstream from a sorbent tube maintained at temperatures between 100° and 200°C. Nitrogen is used as a carrier gas to reduce the possibility of chemical changes that could occur at these temperatures in air. Retention characteristics and breakthroughs at room temperature may be estimated from these data.[14] This preliminary evaluation allows for rapid screening of selected sorbents prior to chamber sampling and quantitative analysis.

B. Development of Quantitative Analytical Methods

Preliminary work on calibration and standardization of the gas chromatograph (GC), Model 3920, (Perkin-Elmer, Norwalk, CT), and the high-speed liquid chromatograph (HSLC), Model ALC 202/401, (Waters Associates, Milford, MA), was conducted using homologues and analogues of the first five cancer-suspect agents. The model compounds were aniline, N-methylaniline, o-toluidine, o-, m-, and p-chloraniline, o-tolidine, and methylenedianiline. Although development of specific conditions was not practical until the carcinogens could be used in the laboratory, analytical skills and instrument performance characteristics were developed. Experimental work with the carcinogens began as soon as the laboratory was approved for use in April 1975.

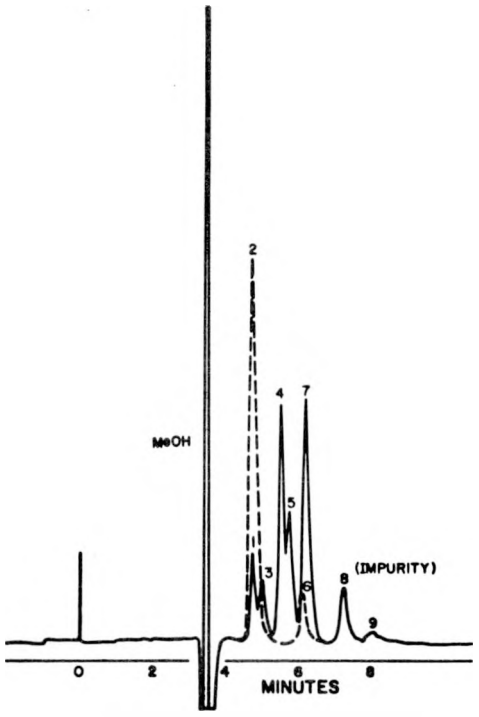
1. High-Speed Liquid Chromatography of Analogues and Homologues. Various packing materials (C_{18} Corasil, µBondapak C_{18} and -NH_2, and Partisil) and mobile phases were evaluated for the separation of the model compounds by HSLC. The µBondapak columns indicated the most promise in terms of good resolution and short retention times. Rather long retention times (>30 min) were unexpectedly encountered with C_{18} Corasil; in the case of Partisil, some of the amines were irreversibly adsorbed. Figure 10 shows an HSLC separation of some of these amines.

TABLE IX

RECOVERIES OF MOCA FROM SORBENTS

Sorbent	Weight (mg)	Standard Deviation (mg)	Recovery[a] (wt%) 14.5 µg MOCA	2.85 µg MOCA
Chromosorb 101	96.06	0.51	<28.0	---
Spherosil XOC-005	89.44	0.51	109	105
Silica gel	97.96	0.76	129	96.6
Deactigel	91.01	0.65	<30.5	---
Gas Chrom-P	99.56	1.47	---	96.5
Amberlite XAD-8	96.05	2.43	67.8	28.4
Tenax GC	48.15	0.28	---	<10.6

[a]Eluted with 500 µl isopropanol, detection limit varies with injection volume.

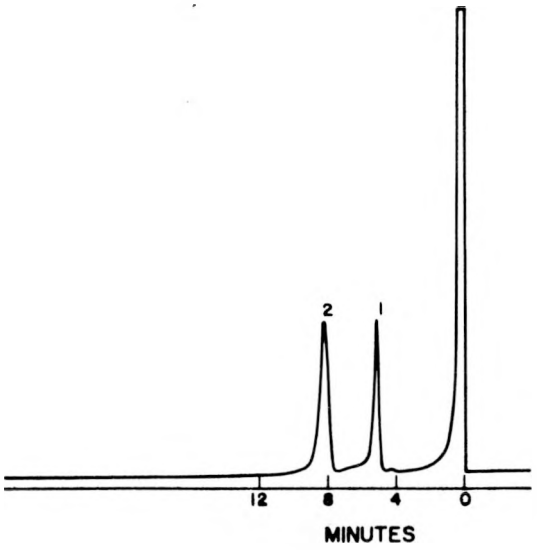


Column	μBondapak C_{18} (0.63 x 30 cm)
Flow	0.8 mℓ/min
Detector	UV: 254 nm (0.08 AUFS)
Mobile phase	60% dioxane/water
Injection	1 μℓ (100 ng of dianiline and 66 ng of all other amines)
Sample	1. Aniline 2. Methylenedianiline 3. o-Tolidine 4. p-Chloroaniline 5. m-Chloroaniline 6. o-Chloroaniline 7. N-Methylaniline 8. o-Chlorotoluidine

Fig. 10.
HSLC of aromatic amines.

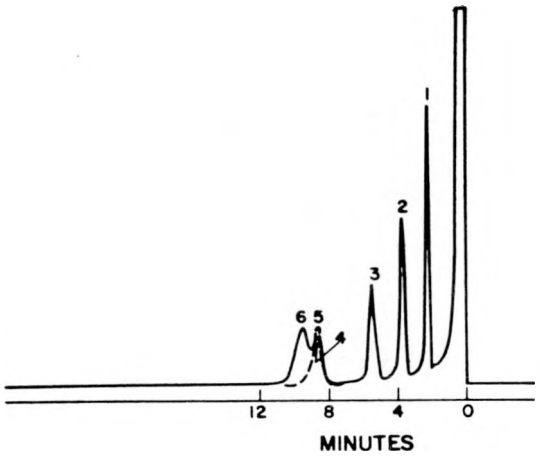
2. Gas Chromatography of Cancer-Suspect Agent Analogues and Homologues. Gas chromatography column selection studies were also carried out with the same model compounds used above. Chromosorb 103, 4% Carbowax 20M + 0.8% KOH/Carbopack B, 10% OV-25/Supelcon AW, 10% Apiezon-L+2% KOH/Supelcon AW, 3% SP-2100/Supelcoport, and Tenax GC were evaluated under various temperature conditions. The last three columns suggested good efficiencies and reasonable separation from the solvent peak. This was particularly the case for the MOCA analogue, methylenedianiline (Fig. 11a), and for other amines (Figs. 11b and 12).

3. High-Speed Liquid Chromatography of Carcinogens. Because of the structural similarities between the first three carcinogens, i.e., MOCA,



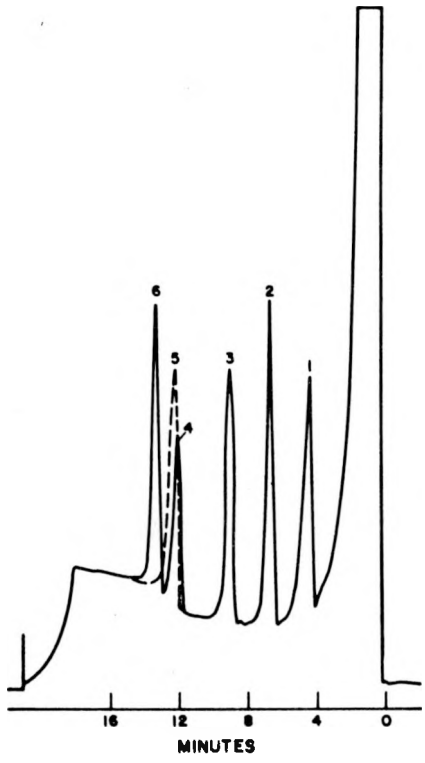
Column	0.9 m x 2 mm, glass, Tenax GC (60/80 mesh)
Temperature	Column: 285°C Injector: 300°C
Flow rate	35 mℓ/min (helium)
Detector	FID, at attenuation of 10 x 4
Injection	1 μℓ
Sample	1. Methylenedianiline (300 ng) 2. o-Tolidine (257 ng)

Fig. 11a.
GC of aromatic amines.



Column	0.9 m x 2 mm, glass, Tenax GC (60/80 mesh)
Temperature	Column: 175°C Injector: 200°C
Flow Rate	35 mℓ/min (helium)
Detector	FID, at attenuation of 10 x 8
Injection	1 μℓ
Sample	1. Aniline 2. N-Methylaniline 3. o-Chloroaniline 4. m-Chloroaniline 5. p-Chloroaniline 6. o-Chlorotoluidine

Fig. 11b.
GC of aromatic amines.



Column	1.8 m x 2 mm, glass, 10% Apiezon L + 2% KOH on Supelcon AW
Temperature	100°C initial 4 min; 4°/min to 140°C
Flow rate	30 mℓ/min (helium)
Detector	FID, at attenuation of 1 x 16
Injection	1 µℓ
Sample	1. Aniline 2. N-Methylaniline 3. o-Chloroaniline 4. m-Chloroaniline 5. p-Chloroaniline 6. o-Chlorotoluidine

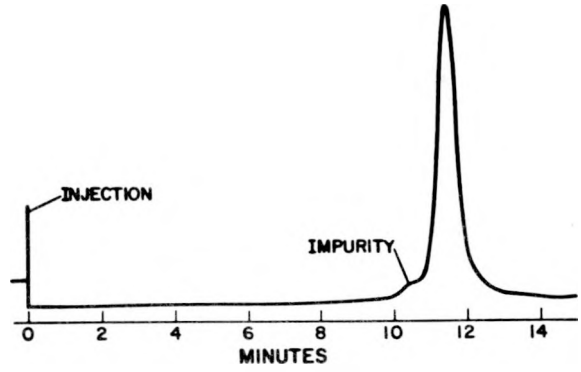
Fig. 12.
GC of aromatic amines.

benzidine, and 3,3′-dichlorobenzidine, these compounds were studied concurrently by HSLC. Numerous chromatographic conditions were explored to determine the optimum efficiency and resolution parameters required to develop a separation free of interferences from expected contaminants. Solutions of the amines (500-1500 ppm) were prepared in methanol and allowed to "age" at laboratory temperatures (20 to 28°C) while being exposed to unfiltered laboratory fluorescent lighting. Under these conditions, some degradation of the compounds in solution was expected. This was not a controlled kinetic study, nor should it be interpreted as such. These studies were performed to obtain HSLC conditions which would minimize interferences from sample impurities. Refrigerated reference solutions were assumed to be stable.

Based on the results obtained with the amine analogues discussed above, emphasis was placed on the separation of the first three carcinogens using the Waters µBondapak - NH_2 and C_{18} columns. Some work was also conducted with Waters µGPC (gel permeation) columns but the initial results did not offer promise either in terms of resolution or efficiency. Retention times for the first three carcinogens did not differ by more than 30 s, suggesting that it would be difficult to optimize the present µGPC columns for the separation of impurities closely related to the parent compound. Figure 13 shows a typical chromatogram of technical grade MOCA with dioxane as the mobile phase. (Another solvent may have to be substituted for dioxane for reasons previously cited.)

The µBondapak - NH_2 and C_{18} columns, on the other hand, displayed good efficiency and resolving characteristics. The µC_{18} column consists of a monomolecular layer of nonpolar octadecyltrichlorosilane chemically bonded to 10-µm porous silica particles. The µNH_2 is an intermediate polarity packing, consisting of a monomolecular layer of aminopropylsilane for normal phase or weak anion exchange chromatography. Both of these 0.63 x 30-cm columns are stable at pH 1-8 and have more than 3000 theoretical plates.

a. MOCA. Because of the high efficiencies obtained with the µBondapak columns, various mobile phases were investigated to ascertain that the impurities indicated by particular chromatographic conditions were truly indicative of all of the contaminants present. For example, with a µNH_2 column, only three impurities were easily



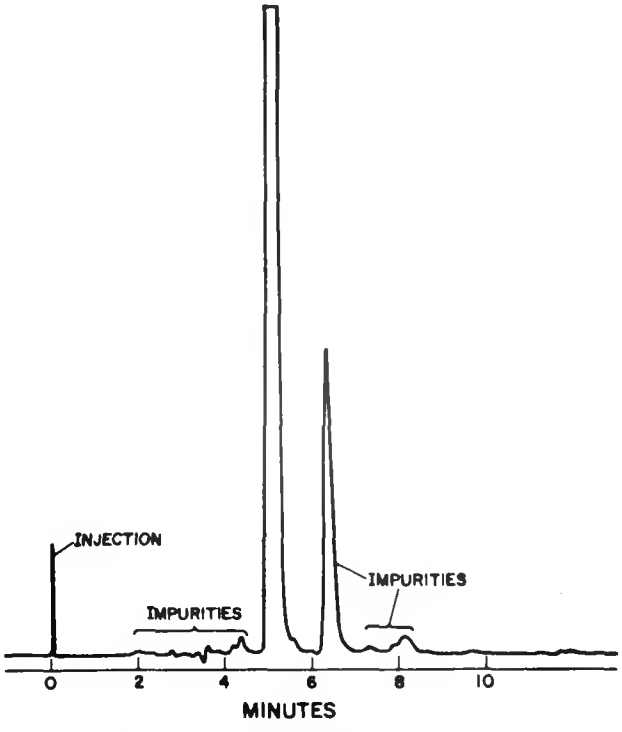
Column	µGPC: 500 Å (1 x 30 cm) + 200 Å (2 x 30 cm)
Flow	2 mℓ/min
Detector	UV: 254 nm (0.32 AUFS)
Mobile phase	Dioxane
Injection	1500 ng (1 µℓ)

Fig. 13.
HSLC of aged MOCA.

discernible in aged MOCA solutions with a 30% acetonitrile/chloroform mobile phase, but a 45% dioxane/hexane system indicated at least seven.

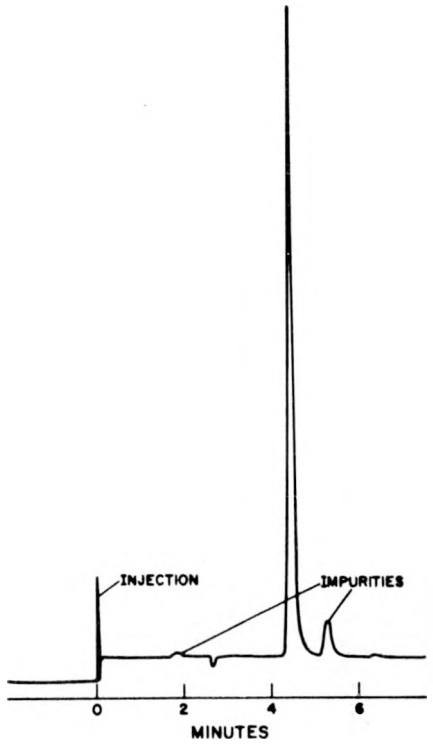
Figures 14 and 15 (C_{18} column) contrast an aged MOCA solution with one stored in the refrigerator and away from light. It is obvious from the intensity and number of peaks that some degradation has taken place in the solution kept under ambient room conditions. Operating conditions detailed in Fig. 16 are being contemplated for the preparation of a MOCA analytical standard and will also be investigated for their sensitivity merits in the development of a trace analytical method. Extrapolated data suggests that a sensitivity of ≤ 4 ng/injection should be attainable using the UV detector at 254 nm.

b. Benzidine. HSLC systems investigated for MOCA were also explored with benzidine; as with MOCA, the mobile phase had a marked effect on the resolution of the carcinogen from its impurities and also had an effect on the sensitivity. Figures 17 and 18 are representative chromatograms for benzidine. The use of μBondapak C_{18} with a 70/30 acetonitrile/water mobile phase and UV (254 nm) detector, shown in Fig. 18, appears to offer the best possibility for the purification and analysis of benzidine. Extrapolated results indicate a sensitivity of ≤ 7 ng/injection to be attainable.



Column	μBondapak C_{18} (0.63 x 30 cm)
Flow	1.8 mℓ/min
Detector	UV: 254 nm (0.32 AUFS)
Mobile phase	80% acetonitrile/water
Injection	3708 ng (3 μℓ)

Fig. 14.
HSLC of aged MOCA.

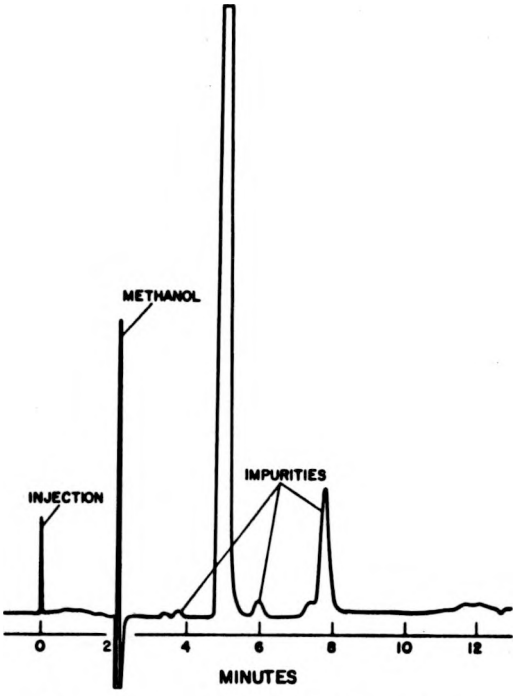


Column	μBondapak C_{18} (0.63 x 30 cm)
Flow	2 mℓ/min
Detector	UV: 254 nm (0.32 AUFS)
Mobile phase	80% acetonitrile/water
Injection	1236 ng (1 μℓ)

Fig. 15.
HSLC of refrigerated MOCA.

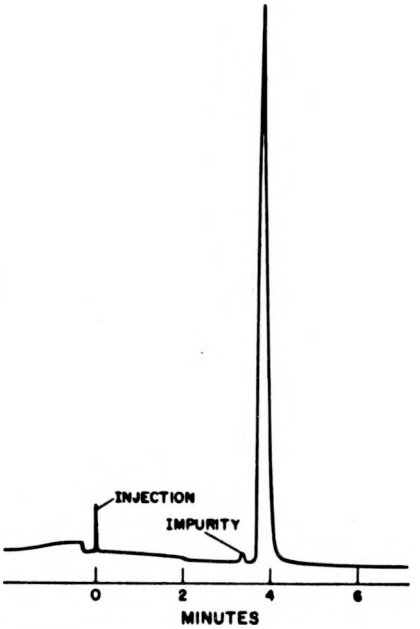
c. 3,3′ -Dichlorobenzidine. Investigations of HSLC chromatographic conditions for 3,3′ -dichlorobenzidine were more limited than for the first two carcinogens.

The data, nevertheless, indicate that HSLC should provide an efficient separation and a sensitivity in the same range as for benzidine, i.e., ≤ 7 ng/injection. Figures 19 and 20 show chromatograms of 3,3′-dichlorobenzidine obtained with μBondapak-C_{18} and -NH_2 columns. The chromatographic conditions shown in Fig. 20 will be investigated further because they promise good efficiency and sensitivity. Also under consideration is the coupling of two amino columns to determine whether impurities other than those currently resolved may be present in 3,3′ -dichlorobenzidine. Degradation similar to MOCA in solution had been expected for 3,3′ -dichlorobenzidine; this, however, was not observed.



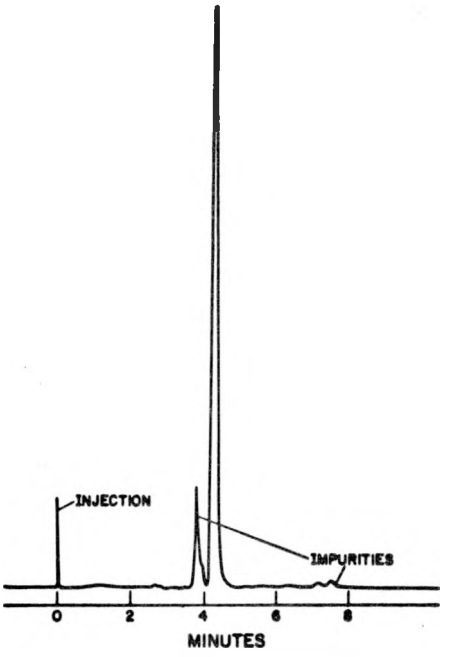
Column	μBondapak C_{18} (0.63 x 30 cm)
Flow	1.5 mℓ/min
Detector	UV: 254 nm (0.32 AUFS)
Mobile phase	60% dioxane/water
Injection	3708 ng (3 μℓ)

Fig. 16.
HSLC of aged MOCA.



Column	μBondapak C_{18} (0.63 x 30 cm)
Flow	1 mℓ/min
Detector	UV: 254 nm (0.32 AUFS)
Mobile phase	70% methanol/water
Injection	460 ng (1 μℓ)

Fig. 17.
HSLC of benzidine.

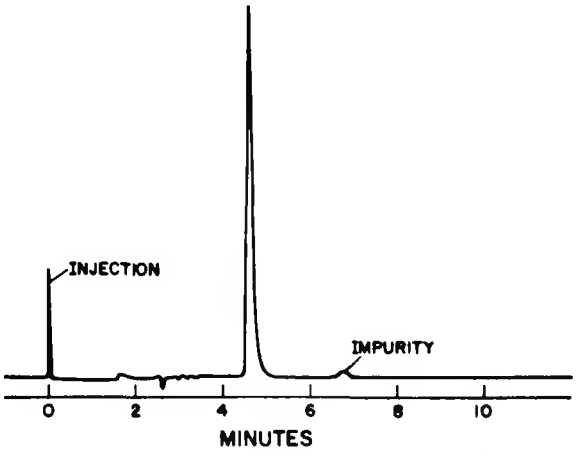


Column	μBondapak C_{18} (0.63 x 30 cm)
Flow	1 mℓ/min
Detector	UV: 254 nm (0.32 AUFS)
Mobile phase	70% acetonitrile/water
Injection	1000 ng (1 μℓ)

Fig. 18.
HSLC of benzidine.

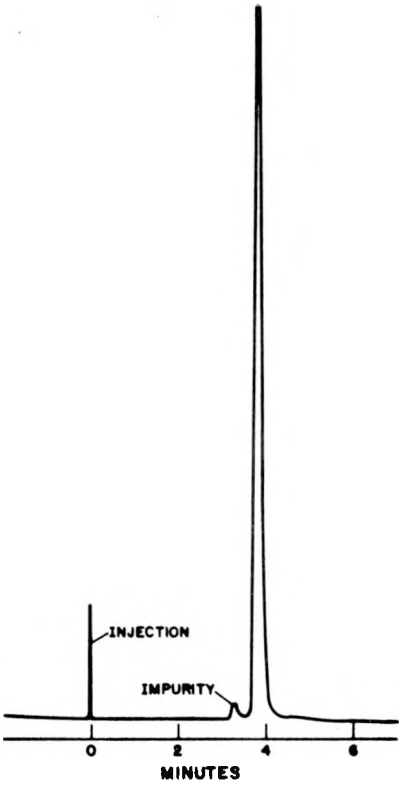
A chromatographic separation of the first four cancer-suspect agents to be studied is shown in Fig. 21. Retention times for the carcinogens when run singly, indicated full resolution between the four amines. When a solution of the four was made in methanol, however, an extra peak resulted (peak 4). Various binary combinations of these amines were made, and it was discovered that only a solution of benzidine with α-naphthylamine produced this particular unknown product.

4. Gas Chromatography. The electron capture detector (ECD) was delivered and installed. No investigations have been conducted with either the ECD or nitrogen detectors. All efforts until now have centered on evaluating various columns for the determination of MOCA with the flame ionization detector. Glass columns which have shown promise are 10% Apiezon-L+2% KOH/Supelcon AW, Tenax GC, and 3% SP-2100/Supelcoport. On these columns MOCA has displayed a minimum of tailing; retention times can be varied from 4 to 12 min, depending upon the conditions selected. Calibration curves with technical MOCA have been obtained down to 2 ng/injection, but reproducibility of these curves from day to day has proved erratic, particularly at those minimum attenuation settings which are required for trace analysis. This irreproducibility appears to



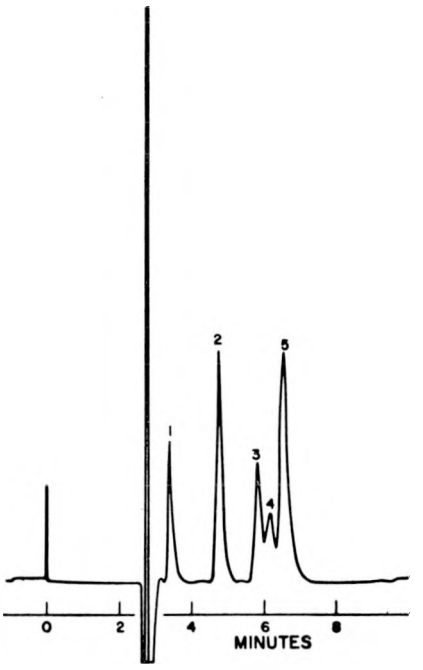
Column	μBondapak C_{18} (0.63 x 30 cm)
Flow	1 mℓ/min
Detector	UV: 254 nm (0.32 AUFS)
Mobile phase	80% acetonitrile/water
Injection	1000 ng (2 μℓ)

Fig. 19.
HSLC of 3,3′-dichlorobenzidine.



Column	μBondapak-NH_2 (0.63 x 30 cm)
Flow	1 mℓ/min
Detector	UV: 254 nm (0.32 AUFS)
Mobile phase	Acetonitrile
Injection	2000 ng (1 μℓ)

Fig. 20.
HSLC of 3,3′-dichlorobenzidine.



Column	μBondapak C_{18} (0.63 x 30 cm)
Flow	1.0 mℓ/min
Detector	UV: 254 nm (0.08 AUFS)
Mobile phase	60% dioxane/water
Injection	1 μℓ (100-200 ng of amine)
Sample	1. Benzidine
	2. α-Naphthylamine
	3. 3,3′-Dichlorobenzidine
	4. Unknown
	5. MOCA

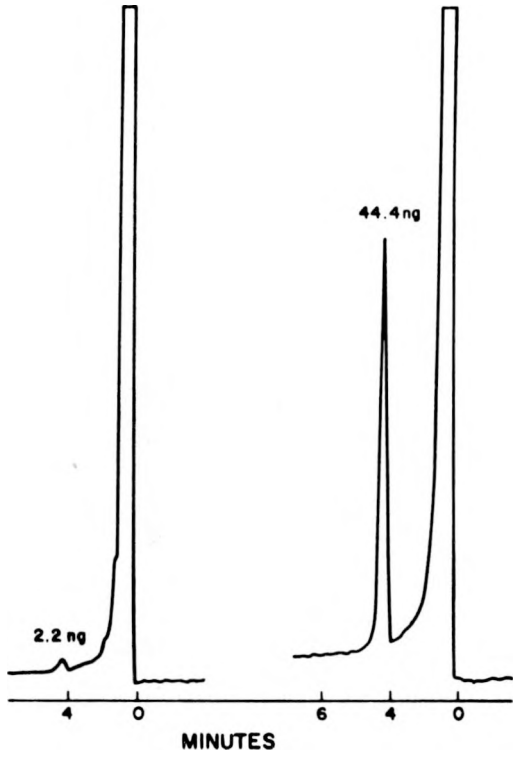
Fig. 21.
HSLC of carcinogenic amines.

be a result of electrical noise which distorts the output peak shape and thus affects quantitation. Electronic filters have not eliminated the problem. The Perkin-Elmer representative is attempting to diagnose the source of this aberration.

Typical gas chromatograms of MOCA analysis on Tenax GC are shown in Fig. 22 along with the calibration curve (Fig. 23). Retention time for this carcinogen under the conditions cited is ~4 min. Relative to current procedures,[13] greater selectivity and sensitivity may be obtained using the nitrogen detector. Work will continue in this area to develop a GC procedure to cross-check the HSLC method.

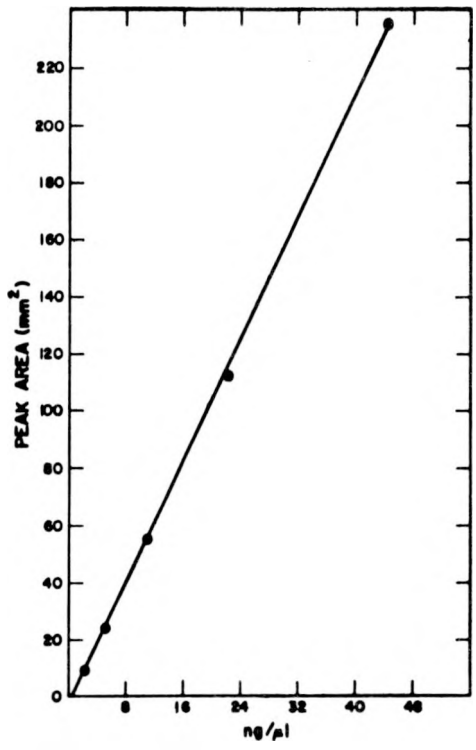
VI. EVALUATION OF COMMERCIAL RESPIRATOR SORBENTS FOR PROTECTION AGAINST CARCINOGENS UNDER STUDY

To expedite the testing of commercial respirator cartridge sorbents, a special chamber has been constructed (Fig. 24). The cartridge test chamber functions parallel to the air-sampling chamber and can



Column	0.9 m x 2 mm, glass, Tenax GC (60/80 mesh)
Temperature	Injector: 320°C Column: 320°C
Carrier	45 mℓ/min (helium) 190 mℓ/min (H_2)
Detector	FID, at attenuation of 1 x 4
Sample	1 µℓ MOCA/propanol

Fig. 22.
GC of MOCA.



Column	0.9 m x 2 mm, glass, Tenax GC (60/80 mesh)
Temperature	Injector: 320°C Column: 320°C
Carrier	45 mℓ/min (helium) 190 mℓ/min (H_2)
Detector	FID, at attenuation of 1 x 4
Sample	1 µℓ MOCA/propanol

Fig. 23.
MOCA calibration curve.

be operated continuously to determine the protection characteristics of as many as four respirator cartridges simultaneously. Sampling upstream and downstream from the cartridge at regular intervals allows for evaluating the breakthrough and estimating the cartridge's capacity.

VII. GLOVE PERMEATION STUDY

Some solvents and chemicals used in this program may require protective membrane (glove) permeation evaluation. Each carcinogen requires a glove permeability test to meet the industrial hygiene requirements of the Industrial Hygiene Group (H-5).

A. Background

Regulations[1] relating to laboratory procedures similar to the work performed in the LASL Cancer-Suspect Agent Laboratory do not require a stringent skin protection program. The requirement for skin protection during transfer from or opening of a closed system (Ref. 1, Para. C.4) is that impervious gloves are to be worn. No data is available as to the impermeability of glove materials, nor is a particular material recommended; therefore, it is desirable to determine the degree of skin protection provided by commercially available gloves and to select the most satisfactory ones.

Eutsler, Campbell, and Stein[15] developed a cell (Fig. 25) to estimate the permeability of materials with respect to various solvents as well as chemicals dissolved in a solvent. The test materials served as a semipermeable membrane between the solvent or solute/solvent and physiological saline. In brief, the procedure was to cut a portion of the glove material from the palm area and clip it between the two halves of the permeation cell. One side of the cell was filled with physiological saline; the other side with the solution of compound being tested. The cells were allowed to stand at room temperature; aliquots were taken from the saline solution at regular intervals and analyzed for the chemical under study. The authors found that only polyethylene

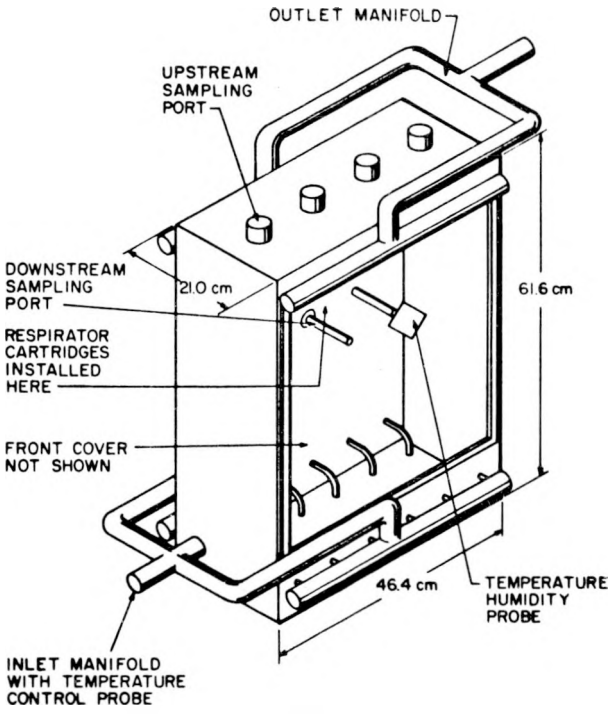


Fig. 24.
Respirator cannister test chamber.

was satisfactory for skin protection against dimethylsulfoxide and dissolved metals. Using this same technique, Yasuda, Douglas, and Hermes[16] found that a combination of polyethylene and latex afforded maximum protection against dimethylformamide. Due to the requirements of the analytical procedure, water was used instead of physiological saline in the Yasuda et al. study.

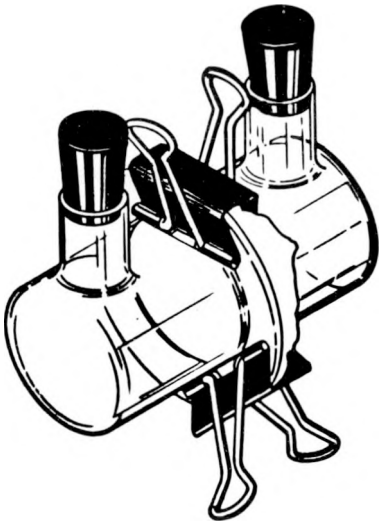


Fig. 25.
Permeation cell.

B. Method

A study similar to the one described above, an evaluation of various protective gloves, was conducted for solutions containing aromatic amines. The study used a methanolic solution of 500 $\mu g/cm^3$ of the various amines routinely used in the Cancer-Suspect Agent Laboratory. These aromatic amines were measured in the saline solution at regular intervals. A 1-cm^3 aliquot of the saline solution was removed, diluted to 6.5 cm^3 with methanol and two drops of Ehrlich's reagent added to develop the color. The absorbance of the Ehrlich's reagent product was measured at 435 nm on a Spectronic 20. The saline solution in the permeation cell was brought back to its original volume for further permeation testing. Extrapolation of timed aliquot analysis of the saline solution to the detection limit or breakthrough value* is shown in Table X.

The data shown in Table X indicate the breakthrough time of low concentrations of the aromatic amines in methanol solutions to saline. Neat aniline (technical grade) as the challenge solution resulted in a breakthrough time of <30 min. A 10 000-$\mu g/cm^3$ solution of MOCA in methanol broke through every glove material shown on Table X in <2 h. Permeation studies are continuing on other compounds and, in particular, other carcinogens.

C. Conclusions

Natural latex gloves appear to provide ample skin protection for short periods of time at relatively high concentration of the chemical (aromatic amines) in methanol. Neither the Hypalon/Neoprene glove system nor butyl rubber gave permeation breakthrough times as long as natural latex for the amines studied. Polyvinyl chloride gloves provide the highest degree of protection for MOCA, methylenedianiline, and p-toluidine.

Gloves made of the materials tested here only afford short-term (<30 min) skin protection from neat liquid amines.

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*The detection limit (breakthrough value) is defined as that value (ppm) obtained from a Beer's Law plot wherein $A = (0.0000 + 2\,\sigma_{A,0})$. $\sigma_{A,0}$ is the standard deviation of the absorbance at zero concentration and was determined individually for each compound studied.

TABLE X

GLOVE MATERIAL PERMEATION BY AROMATIC AMINES

Estimated breakthrough concentration ($\mu g/cm^3$)[b]		Aniline[a] 1.1	p-Chloro-aniline[a] 1.7	p-Toluidine[a] 1.6	Methylene-dianiline[a] 1.8	MOCA 3.5
Material	Thickness (mil)	Breakthrough[c] (h)				
Natural latex	8	49 ± 2	47 ± 1	45 ± 1	45 ± 1	35 ± 2
Hypalon/Neoprene[d]	30	26 ± 1	26 ± 1	26 ± 1	25 ± 1	---
Hypalon	30	21 ± 1	21 ± 1	21 ± 1	20 ± 1	---
Neoprene	30	15 ± 2	15 ± 2	14 ± 2	15 ± 1	---
Butyl	30	4 ± 1	4 ± 1	4 ± 1	4 ± 1	---
Polyethylene	2	4 ± 1	4 ± 1	4 ± 1	4 ± 1	---
Nitrile	11	1 ± 0.5	1 ± 0.5	1 ± 0.5	1 ± 0.5	---
Polyvinyl chloride	9	21 ± 1	21 ± 1	55 ± 4	80 ± 5	51 ± 1

[a]Challenge concentration was 500 µg of aromatic amine per cm^3 of methanolic solution.

[b]Lower detection limit or breakthrough concentration is defined as that value (ppm) obtained from Beer's Law plot wherein $A = (0.0000 + 2\sigma_{A,0})$. $\sigma_{A,0}$ is the standard deviation of the absorbance at zero concentration; it was determined individually for each compound studied.

[c]Breakthrough given in hours with estimated standard deviation.

[d]Neoprene in contact with physiological saline solution and Hypalon in contact with the methanolic solution of the amine.

Each chemical and its solvent should be evaluated independently and under conditions comparable to occupational situations and the solute/solvent concentration used in the process of interest.

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