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**PROCEEDINGS OF THE
FOURTH NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES**

**April 22-23, 1986
Holiday Inn Crowne Plaza Hotel
Rockville, Maryland**

Editors

**Thomas P. Cameron, D.V.M.
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Ingeborg C. Blackwood**

NIH Publication No. 88-2960

**PROCEEDINGS OF THE
FOURTH NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
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FOURTH NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

APRIL 22-23, 1986

HOLIDAY INN CROWNE PLAZA HOTEL
ROCKVILLE, MARYLAND

AGENDA

(20 minutes for presentation, 10 minutes for discussion)

TUESDAY MORNING, APRIL 22

9:00 a.m. WELCOME TO WORKSHOP

Thomas P. Cameron
Special Assistant for Environmental Cancer
Division of Cancer Etiology, NCI
Program Chairman

INTRODUCTION

Susan Sieber
Deputy Director, Division of Cancer Etiology, NCI

SESSION I - EXPERIMENTAL STUDIES

9:15 a.m. Chairman: Edward Bresnick, Eppley Institute
for Research in Cancer and Allied Diseases

9:20 a.m. Biochemical, Pharmacological and Tumorigenic Studies on
a Composite of Drinking Water Carcinogens and Mutagens
Utilizing Aquatic Animals

Presenter/Contractor: Robin M. Overstreet, Gulf Coast
Research Laboratory

Project Officer: Thomas P. Cameron, NCI
Co-Project Officer: John Couch, EPA

9:50 a.m. Registry of Tumors in Lower Animals

Presenter/Contractor: John Harshbarger, Smithsonian Institution
Project Officer: Susan Sieber, NCI
Co-Project Officer: John Couch, EPA

10:20 a.m. BREAK

10:40 a.m. Influence of Disulfiram or Ethanol on 1,2-Dichloroethane Carcinogenicity

Presenter/Project Officer: Kenneth L. Cheever, NIOSH
Co-Project Officer: Elizabeth Weisburger, NCI
Contractor: Midwest Research Institute

11:10 a.m. Fractionation and Analysis of Asphalt Fumes for Carcinogenicity Testing

Presenter/Project Officer: B. R. Belinkj, NIOSH
Co-Project Officer: Thomas P. Cameron, NCI
Contractor: Arthur D. Little, Inc.

11:40 a.m. LUNCH

TUESDAY AFTERNOON, APRIL 22

SESSION II - EPIDEMIOLOGY

1:00 p.m. Chairman: Carl M. Shy, University of North Carolina

1:05 p.m. Mortality of Workers in the Painting Trades

Presenter/Contractor: Genevieve Matanoski, Johns Hopkins University
Project Officer: Marie Sweeney Haring, NIOSH
Co-Project Officer: Thomas P. Cameron, NCI

1:35 p.m. Mortality of Chrome Leather Tanning Workers

Presenter/Project Officer: Frank B. Stern, NIOSH
Co-Project Officer: Robert Hoover, NCI
Contractors: Dynalelectronics, Inc. and Stokes Services

2:05 p.m. Mortality Among Industrial Workers Exposed to Formaldehyde

Presenter/Project Officer: Aaron Blair, NCI
Co-Project Officer: Leslie Stayner, NCI
Contractor: Westat, Inc.

2:35 p.m. BREAK

3:00 p.m. Mortality Analyses Using United Auto Workers' Cohorts

Presenter/Contractor: Robert Park, International Union,
United Auto, Aerospace and Agricultural
Implement Workers of America
Project Officer: Robert Roscoe, NIOSH
Co-Project Officer: Debra Silverman, NCI

- 3:30 p.m. Research Leads from Occupational Mortality Data
- Presenter: Carol Burnett, NIOSH
 Project Officer: John Sestito, NIOSH
 Co-Project Officers: Gilbert Beebe and Robert Spirtas, NCI
 Contractor: National Center for Health Statistics
- 4:00 p.m. Exposure Characterization Study of Workers Exposed to Hydrazines
- Presenter/Project Officer: Thomas F. Bloom
 Project Officer: John F. Fajen, NIOSH
 Co-Project Officer: Aaron Blair, NCI
 Contractor: Clayton Environment Consultants
- 4:30 p.m. Exposure Characterization of Workers Exposed to Organotin
- Presenter/Project Officer: Thomas F. Bloom, NIOSH
 Co-Project Officer: Aaron Blair, NCI
 Contractor: Clayton Environment Consultants

WEDNESDAY MORNING, APRIL 23

SESSION III - EXPERIMENTAL STUDIES

- 8:30 a.m. Chairman: William Farland, EPA
- 8:35 a.m. Metabolism of 4,4'-Methylene-bis(2-chloroaniline) in
 Explant Cultures of Human and Dog Bladder and Dog
 Liver Cell Cultures
- Presenter/Contractor: Gary Stoner, Medical College of Ohio
 Project Officer: Mark Mass, EPA
 Co-Project Officer: Thomas P. Cameron, NCI
- 9:05 a.m. Characterization and Validation of Carcinogen-Induced
 Transformation of Human Cells In Vitro
- Presenter/Contractor: George Milo, Ohio State University
 Project Officer: Stephen Nesnow, EPA
 Co-Project Officer: Thomas P. Cameron, NCI

9:35 a.m. Report on International Workshop on "The Use of
In Vitro Transformation of Established Cell Lines
for the Prediction of Carcinogenic Chemicals

Presenter/Project Officer: Stephen Nesnow, EPA
Co-Project Officer: Thomas P. Cameron, NCI

9:45 a.m. BREAK

SESSION IV - EPIDEMIOLOGY

10:10 a.m. Chairman: Joseph F. Fraumeni, Jr., NCI

10:15 a.m. Time Trends and Risk Factors for Mesothelioma

Presenter/Project Officer: Robert Spirtas, NCI
Co-Project Officer: John Sestito, NIOSH
Contractors: Univ of Southern California; N.Y. State
Health Dept.; Veterans Administration;
Med. Follow-Up Agency, National Academy
of Sciences

10:45 a.m. Body Burden of Toxic Substances in Support of the
Total Exposure Assessment Methodology (TEAM) Study

Presenter/Project Officer: Lance Wallace, EPA
Co-Project Officer: Michael Dellarco, EPA
Co-Project Officer: Thomas Mason, NCI
Contractor: Research Triangle Institute

11:15 a.m. Workshop Wrap-Up

Edward Bresnick	Carl M. Shy
William Farland	Joseph F. Fraumeni, Jr.
Frode Ulvedal	Roy M. Fleming

• 11:45 a.m. ADJOURNMENT

POSTER PROJECTS

Survey of Compounds Which Have Been Tested for Carcinogenic Activity (PHS-149)

Presenter/Contractor: Anthony Lee, Technical Resources, Inc.
Project Officer: Thomas P. Cameron, NCI
Co-Project Officer: William Farland, EPA

Development of a National Data Base on Body Burden of Toxic Chemicals, Including Carcinogens

Presenter/Project Officer: Philip Robinson, EPA
Co-Project Officer: Thomas P. Cameron, NCI
Contractor: Department of Energy/Science Applications
International, Inc.

Interaction Between Normal Human Diploid Cells and Chemical Carcinogens

Presenter/Contractor: Shiu Huang, Environmental Health
Research and Testing
Project Officer: Michael Waters, EPA
Co-Project Officer: Thomas P. Cameron, NCI

WELCOME TO WORKSHOP

**Dr. Thomas P. Cameron
Special Assistant for Environmental Cancer
Division of Cancer Etiology
National Cancer Institute**

WELCOME TO WORKSHOP

Dr. Thomas P. Cameron
National Cancer Institute

Good morning, ladies and gentlemen. I'm Tom Cameron of the National Cancer Institute. Speaking on behalf of the organizing committee for this activity, I wish all of you the most hearty welcome.

I would now like to introduce Dr. Susan Sieber, Deputy Director of the Division of Cancer Etiology, National Cancer Institute.

INTRODUCTORY REMARKS

**Dr. Susan M. Sieber
Deputy Director
Division of Cancer Etiology
National Cancer Institute**

INTRODUCTORY REMARKS

Dr. Susan M. Sieber
National Cancer Institute

On behalf of Dr. Adamson, who is traveling in China this week, I am pleased to welcome you to this Fourth NCI/EPA/NIOSH Workshop. An important function of the NCI is to collaborate and exchange information on environmental and occupational carcinogenesis with other research institutions and with Federal regulatory agencies. The NCI/EPA/NIOSH Collaborative Program on Environmental and Occupational Cancer Studies is a good example of this activity. The Program Workshops, which are held on a regular basis, play an important role in information dissemination and coordination, and also in the development of new collaborative efforts which are of mutual interest.

The collaborative program with NIOSH initially began in 1976 as a result of a House Committee report. NCI has continued this collaborative program with NIOSH even though there has been no Congressional directive to do so for several years. In Fiscal Year (FY) 1982, it became NCI policy to bring individual research projects before the Division of Cancer Etiology Board of Scientific Counselors for outside peer review. In addition, a Project Review Group was established which now includes a member of the Division of Cancer Etiology Board of Scientific Counselors. This group has been most helpful in identifying projects of high priority to both agencies. The funding level on the pass-through dollars for FY 1984 was about \$950,000 and in FY 1986, the pass-through amount is budgeted at \$500,000. This decrease in funding is a result of rigorous concept review by the Board as well as the budgetary decreases we are all experiencing.

Examples of projects currently being supported by this program include an Ethylene Oxide Mortality Study, a Mortality Study of Polyurethane Foam Workers Exposed to Toluene Diisothionate, a Mortality Study of Workers Exposed to Halowax, a study of Occupational Cancer in the North Carolina Dusty Trade Industry and a study of Cancer Risk in X-Ray Technologists. We anticipate that the NCI/NIOSH collaborative agreement will begin to place more emphasis on epidemiologic studies in which the interaction of smoking with occupational factors will be considered. To facilitate such studies, a Conference on Obtaining and Using Information on Smoking in Occupational Epidemiologic Studies is being supported by the collaborative agreements and will be held in December 1986.

The NCI/EPA Collaborative Program on Environmental Cancer was formally constituted in FY 1978. The program included collaboration with the EPA Office of Toxic Substances as well as the EPA Office of Research and Development. The importance of this collaboration was indicated in both the House and Senate reports of the FY 1978 budget. The House report acknowledged that as much as 80 to 90 percent of all cancer can be attributed to environmental and lifestyle factors. The Senate similarly noted that there should be "increased overall, as well as interagency, activity as a result of heightened interest in environmental causes of cancer" and especially urged collaboration with EPA and NIOSH.

There has been no Congressional directive since 1980 concerning this collaboration. Nevertheless NCI has maintained our program with EPA and for FY 1986 \$500,000 is budgeted for our collaborative activities. A similar amount is budgeted for direct funding of EPA projects by NCI. While the number of projects and total dollars have decreased over the years due to budget constraints for both agencies, the quality of the research projects has greatly improved. As noted earlier for the collaboration with NIOSH, each NCI/EPA project is concept reviewed by the Division of Cancer Etiology Board of Scientific Counselors and a member of the Board has been appointed to the NCI/EPA Project Review Group. Examples of projects being supported with the EPA pass-through funds include Development of a National Data Base on Body Burden of Toxic Chemicals Including Carcinogens and Compilation and Publication of a reference known as "PHS-149"--A Survey of Compounds which have been Tested for Carcinogenic Activity. The NCI is also funding three projects concerned with various aspects of aquatic toxicology. One such project uses fish as a bioassay system to study drinking water carcinogens and mutagens. Another project is aimed at studying the carcinogenicity of Black Rock Harbor sediments in molluscs and fish. And we continue to support the Registry of Tumors in Lower Animals, which of course includes fish and shellfish.

NCI intends to continue its collaboration with NIOSH and EPA through this collaborative program and also by utilizing other mechanisms, although pass-through monies to each agency remain somewhat uncertain at the present time. It is hoped that in the future collaborative projects will take advantage of the expertise of each agency and, in particular, utilize the expertise NCI has in epidemiology.

In 1981 the NCI transferred the Carcinogenesis Testing Program to the National Institute of Environmental Health Sciences (NIEHS), an action which provides for direct management by the National Toxicology Program (NTP) of the NIH components of the program. Therefore, both NIOSH and EPA should, in future years, interact closely with the NTP, especially for studies relating to testing of chemicals, to assist in designing studies more relevant to risk assessment and for development and validation of testing methods.

I would like to conclude by thanking several individuals who have been involved in this program and in putting together the workshop this year. The NCI effort has been headed by Dr. Cameron and he has been ably assisted by Ms. Blackwood and Ms. Olbrich. Dr. Fleming, representing the NIOSH, has been associated with the collaborative program for the past two years. I would also like to thank Drs. Farland and Ulvedal, from EPA, for their efforts.

SESSION I—EXPERIMENTAL STUDIES

**Chairman: Dr. Edward Bresnick
The Eppley Institute for Research in Cancer
and Allied Diseases
University of Nebraska Medical Center**

Tuesday Morning, April 22, 1986

CHAIRMAN'S REMARKS/INTRODUCTION

**Dr. Edward Bresnick
The Eppley Institute for Research
in Cancer and Allied Diseases
University of Nebraska Medical Center**

CHAIRMAN'S REMARKS/INTRODUCTION

Dr. Edward Bresnick
University of Nebraska Medical Center

I would like to extend my welcome on behalf of the Board of Scientific Counselors for the Division of Cancer Etiology and also as a member of this project review team to which Tom alluded, the NCI/EPA Project Review Team.

I have just a few comments I'd like to make before getting the program under way. If you look at this morning's session, you will note that it really consists of three major areas, although there are four talks being presented.

The first major area deals with carcinogenesis or tumorigenesis using lower animal models, and the first talk is on aquatic organisms. For those of us involved in carcinogenesis, one of the crosses that we have to bear is the testing mechanism that we must use.

If you're using animal systems, it takes an inordinate length of time to get some sort of an answer out of the other end. And, again, to those of us in this business, it was quite surprising to find that fish, aquatic animals, and other lower animals, in fact, have a much, much shorter turnaround time for tumorigenesis experiments.

I think we'll see some of the utility of these models in the first talk.

Now, if you're going to use these lower animals as models for determining tumorigenesis of a variety of environmental types of agents, you have to have some sort of a basis. What is the noise level that you have to deal with? What's the baseline spectrum of the spontaneous cancers in these species? That really constitutes a little bit of the second talk, by John Harshbarger.

The second area represented in this morning's session deals with a problem that has been popping up with increasing frequency during the past few years--the problem of halogenated hydrocarbons. Because halogenated hydrocarbons find their way into our ground waters and, therefore, into us, they are becoming an increasing concern. In the third talk of today's session we're going to hear a little bit about effects of certain chemical agents on the carcinogenicity induced by halogenated hydrocarbons.

For the final talk, and perhaps it's fitting that it's the final talk of this morning's session, we return to one of the oldest problems that has been around in this area of carcinogenicity, and that's the business of coal tar pitch and asphalt. We'll hear a further progress report on the fractionation and analysis of asphalt fumes for carcinogenicity testing.

Now, with that as sort of an overview of what's going to be presented today, I'd like to turn our attention to the very first of the talks. Before doing this, Tom, I think I'll set some ground rules for this morning's session.

The important aspect of this session is supposed to be interaction, and that is discussion. The speakers have all been advised that they are supposed to have 20 minutes for presentation and then 10 minutes for discussion. So when it comes to around 18 minutes, I'm going to stand up. That will sort of warn you that you should be getting close to finishing up. If you've got 30 more slides to go, you know you're in a lot of trouble. I think we'll see if we can preserve the 10-minute discussion time to get the most out of what should be the real meat of this conference, the discussion.

Now with those introductory notes, I'd like to introduce Robin M. Overstreet from Gulf Coast Research Laboratory. Dr. Overstreet is going to be talking about biochemical, pharmacological, and tumorigenic studies on a composite of drinking water carcinogens and mutagens utilizing aquatic animals.

**BIOCHEMICAL, PHARMACOLOGICAL, AND TUMORIGENIC STUDIES
ON A COMPOSITE OF DRINKING WATER CARCINOGENS
AND MUTAGENS UTILIZING AQUATIC ANIMALS**

**Presented by Dr. Robin M. Overstreet
Gulf Coast Research Laboratory
Ocean Springs, Mississippi**

BIOCHEMICAL, PHARMACOLOGICAL AND TUMORIGENIC STUDIES ON A COMPOSITE OF DRINKING WATER CARCINOGENS AND MUTAGENS UTILIZING AQUATIC ANIMALS

Robin M. Overstreet, William E. Hawkins, and William W. Walker
Gulf Coast Research Laboratory, Ocean Springs, Mississippi 39564

The use of small fish species offers several advantages over rodent models for testing the carcinogenic effects of waterborne pollutants. In addition to being much cheaper to obtain, test, and maintain and to being available in identically treated large sample sizes, they are sensitive to a range of drinking water biorefractories and allow much easier contact with those chemicals than rodents which have to drink large amounts of contaminated water or be subjected to the toxicants by unnatural means. This study demonstrates that different fish respond to carcinogenic compounds differently, that a wide variety of neoplasms can develop in fish, that a combination of halomethanes can induce neoplasia in fish, and that halomethanes and other drinking water contaminants can influence the effect of the genotoxic compound methylazoxymethanol acetate (MAM-Ac) in our models.

Materials and Methods

All fish exposed to toxicants were laboratory reared. These included the Japanese medaka (Oryzias latipes), king cobra guppy (Poecilia reticulata), sheepshead minnow (Cyprinodon variegatus), Gulf killifish (Fundulus grandis), inland silverside (Menidia beryllina), rivulus (Rivulus marmoratus), and fathead minnow (Pimephales promelas). With the exception of the guppy, all are egg bearers. Fish either 6 days old or 6 to 10 days old were exposed at 27°C 1) to a single 2-hour exposure of MAM-Ac in a carcinogen glovebox, 2) to one or weekly 6- to 12-hour static pulse exposures of other chemicals in the glovebox or a hood, or 3) to chronic exposure of such chemicals in a closed flow-through system (Walker et al., 1985). Those chemicals included a mixture of six compounds referred to as trihalomethanes (THM) for convenience (chloroform, bromodichloromethane, chlorodibromomethane, bromoform, trichloroethylene, and carbon tetrachloride) and the single compounds vinylidene chloride (1,1-dichloroethylene), trichloroethylene, and bis(2-chloroethyl)ether (1,1'-oxybis[2-chloroethane]). After a specific period of exposure, the fish were transferred to 38-liter aquaria maintained in a 27°C waterbath for grow out for various periods after which they were either fixed in Lillie's fixative and stained with hematoxylin and eosin or prepared for electron microscopical evaluation.

Results and Discussion

Based on exposure to MAM-Ac, nearly half of the exposed individuals of guppy, medaka, sheepshead minnow, and inland silverside developed hepatic neoplasms. At 50 ppm MAM-Ac, the latent period was about 1 month for the guppy, medaka, and silverside but 2 months for the sheepshead minnow. The other three tested fish also developed neoplasms to a lesser degree, with the fathead minnow being the least sensitive. Only

11% of the fathead minnows exposed to 50 or 100 ppm developed neoplasms, and those cases required 7 months at 100 ppm and 14 months at 50 ppm (Hawkins, Overstreet, Walker, and Manning, 1985). Because of the more sensitive nature of the guppy and medaka in conjunction with the ease of breeding and maintaining experimental groups and the demonstration of an active mixed function oxidase system and other liver enzyme systems (Heard et al., in manuscript), we used those two species for exposure to the water biorefractories.

Fish have been questioned as useful carcinogenicity models because experimentally induced neoplasms have been restricted to the liver (Stewart, 1977; Dawe and Couch, 1984). This study, however, demonstrates a variety of neoplasms in tissues other than the liver (Table 2). Moreover, any neoplasm is rare in control fish; we have observed only two spontaneous neoplasms, limited to hepatic tissues, in several thousand controls. The neoplasms all occurred in specimens more than a year old, exceeding by several months our longest anticipated sampling period. Exposed individuals of the medaka, guppy, and sheepshead minnow all developed neoplasms of mesenchymal and nervous tissues. The sheepshead minnow developed a neoplasm in the choroid gland of the eye, the guppy developed neoplasms in the exocrine pancreas, and the medaka developed other neoplasms in the kidney, retina, skeletal muscle, smooth muscle, and connective tissue (Hawkins, Overstreet, Fournie, and Walker, 1985; unpublished data).

In addition to some small fish species being good model systems for carcinogenicity testing, some tumors in the fish make good models for human or rodent neoplasms. Ultrastructural analysis of MAM-Ac-induced hepatic neoplasms in both guppy and medaka resemble those in laboratory rodents (Hawkins et al., in press). After high-dose exposures, cytotoxic changes occurring by 3 weeks included loss of lipid and glycogen stores from hepatocytes, loss of relationships among parenchymal, biliary, and sinusoidal elements, and occurrence of hepatocytic megalocytosis. These megalocytic cells contained large fields of rough endoplasmic reticulum and had bizarre-shaped mitochondria. Later, some hepatocytes, but probably not the megalocytic ones, regained nearly normal nuclear morphology and glycogen content. Neoplastic changes involved both parenchymal and cholangiolar cells, with hepatocellular neoplasms tending to be more common in the guppy and cholangiolar ones more common in medaka. Neoplastic hepatocytes, smaller than normal cells, were basophilic, occurring in both multiple foci and nodules compressing normal tissue. We classify at least cystic lesions, cholangiomas, and cholangiocarcinomas as cholangiolar lesions.

In medaka exposed to 30 ppm or more MAM-Ac, 57% developed intraocular neoplasms (Hawkins et al., 1986). Early lesions of this model system consisted of complexes of neoplastic retinal epithelium and tubes of sensory retinal cells. These complexes differentiated into three patterns of medulloepitheliomas. The first consisted of solid masses of unpigmented cells, frequently showing photoreceptor differentiation and ductular formation. The second consisted of adenomatous patterns formed of heavily pigmented cuboidal to columnar cells resembling retinal epithelium. The most advanced lesions were

malignant teratoid medulloepitheliomas, consisting of heterogeneous, highly mitotic, invasive cells and containing heteroplastic elements including striated muscle, undifferentiated mesenchymal tissues, and hyaline cartilage.

Whereas the medaka is the first fish to serve as an experimental model for eye tumors, the guppy is the first to indicate a chemical etiology for pancreatic neoplasia in fish (Fournie et al., in manuscript). Seven percent of the fish exposed to 10 ppm MAM-Ac or less developed pancreatic tumors. Early lesions consisted of adenomas, usually hyperplastic well-differentiated acinar cells filled with zymogen granules. Apparently these cells developed into acinar cell carcinomas, ranging from well-differentiated to highly anaplastic lesions. The most aggressive lesion was a giant cell carcinoma. Dedifferentiation of acinar cells in the guppy differs from that suggested as the mechanism in rodents because rather than flattening and becoming cuboidal after losing their zymogen granules, the guppy cells become spindle-shaped, hypertrophy, and then lose their zymogen content. On the other hand, the guppy model supports the contention of Longnecker (1983) that many human pancreatic carcinomas may be of acinar origin.

Preliminary chronic exposures of the THM-mixture to medaka in the closed flow-through system show that a combination of chemicals can induce hepatic neoplasia in a few fish by 6 months, whether exposed to a concentration of the mixture approximating an assumed maximum acceptable level for drinking water, a higher level occurring in some drinking water supplies, or even higher levels that could be achieved during accidents but that do not cause acute pathological effects in model fish (Table 3). Additional lesions, some associated with neoplastic development, occurred in other specimens. Flow-through tests, each initially involving about 1200 guppies or medaka, are presently underway to confirm and better assess these responses.

Fish used for flow-through bioassay tests of various chemicals were grown out after their 28-day exposures. Even though sample sizes of processed fish ranged from about 3 to 17, two medaka exposed to 20 ppm vinylidene chloride developed what appear to be hepatic neoplasms by 18 and 36 weeks and by 24 weeks at 40 ppm. Two guppies demonstrated similar lesions by 36 weeks at 10 and 20 ppm. After 45 weeks, both species exposed to 10 and 20 ppm trichloroethylene had neoplasms. One medaka developed a hepatic neoplasm by 36 weeks after exposure to 80 ppm bis-chloroethyl ether.

To examine for enhancement effects, we conducted preliminary studies by exposing a relatively small number of both guppies and medaka to a single 10 ppm MAM-Ac induction exposure followed by a 28-day exposure of relatively high concentrations of the THM mixture in the flow-through system (Table 4). The medaka test resulted in no neoplasms at 12 weeks, none in the positive flow-through controls by 24 weeks, and possibly a dose-related prevalence of tumors in the THM-exposed fish at 24 weeks. After 36 weeks, tumor prevalence in the THM-exposed fish remained higher relative to the aquarium control and increased from the 24-week level at the lower THM exposure

concentrations. The low tumor prevalence in the higher THM concentration was probably a result of excessive mortality of unexamined fish within this group. In the guppy test, neoplasms showed up at 12 weeks, and the positive control group had a 50% prevalence at 24 weeks. Consequently because of those findings and because of high dose-related mortalities which occurred in the flow-through system, we used for the next series of tests an induction dose of 2 ppm MAM-Ac, lower THM concentrations, and larger sample sizes (Table 5). Results for the medaka test had a similar pattern to those in the first series, even though prevalence of neoplastic livers was less in the lower MAM-Ac dose. No neoplasms occurred by 8 weeks, but then few neoplasms occurred in any of the MAM-Ac-exposed medaka at 24 or 29 weeks. On the other hand, some guppies developed tumors by 8 weeks. An unexpected and unexplained finding at 8 weeks was the higher prevalence of hepatic neoplasms in the flow-through positive guppy control group compared to a similar control group maintained outside the system. By 24 weeks, no difference was evident between those two groups and by 36 weeks all MAM-Ac-exposed fish had a similar prevalence. The much higher levels of prevalence in the guppy test than in the medaka test could have resulted from the longer exposure (28 vs. 12 days), the saltwater exposure (12 vs. 0 ppt), or the species differences. A similar test with a small number of the sheepshead minnow produced neoplasms only in the positive controls at 24 weeks when the test was terminated. Additional studies on large numbers of guppies and medaka with high and low THM concentrations need to be conducted with 28-day exposures and samples taken at least at 8 and 24 weeks.

When 2 ppm MAM-Ac was exposed to relatively large groups of medaka after a 12-day exposure to the THM mixture, there occurred initially a lower prevalence of hepatic neoplasms in fish exposed to higher concentrations of the THM mixture (Table 6). As with medaka, but not guppy, presented MAM-Ac before the THM mixture, there occurred a lower prevalence of neoplasms in the positive control group maintained within the closed system compared to those kept outside at 24 weeks. By 36 weeks the prevalence increased for all three groups in the system that had been exposed to the MAM-Ac. Additional tests are necessary to confirm the apparent effect of THM concentration on tumor latency period in both medaka and guppy. A similar test with a small number of the sheepshead minnow at 24 weeks had 0 of 17, 1 of 15 and 7 of 9 fish with liver tumors for out-of-system control without MAM-Ac, out-of-system positive control with 2 ppm MAM-Ac, and flow-through positive control, respectively. The fish exposed to the THM had died by 24 weeks, but 2 of 13 fish exposed to 2 ppm THM had possible neoplastic lesions at 6 weeks even though none of the controls showed any signs of such lesions.

Conclusions

Our studies confirm that some small aquarium fish provide good carcinogenicity models because they are sensitive to induction of a wide variety of neoplasms, the neoplasms have a relatively short latency period, the fish have a low number of spontaneous neoplasms, they are relatively cheap to rear and maintain, large sample sizes can be maintained and examined, and most susceptible tissues can be screened for

neoplasms in one or a few microscopic slides. Unless pooling of livers or other organs and serial, time-sequence procedures are acceptable, many biochemical tests are more reliably conducted with larger fish or with other animals. Our studies also show that significant differences occur in propensity to develop tumors among species. The freshwater egg-laying Japanese medaka, live bearer king cobra guppy, and marine egg-layer sheepshead minnow all proved to be adequate models for a variety of carcinogenicity tests. Those fishes also developed a wide range of neoplasms, with about 15 different neoplasms developing in the medaka exposed to a single pulse dose of MAM-Ac. Both medaka and guppy provide especially good models for hepatic neoplasia, the medaka is a good model for retinal neoplasms, and the guppy is a good model for pancreatic acinar cell carcinoma. Based on preliminary data, THM's in drinking water, difficult to test using rodent models, induce hepatic neoplasms in a relatively small number of both guppies and medaka after chronic exposure to both low and high concentrations of test compounds in a closed flow-through system. Also based on a few cases, other drinking water biorefractories including vinylidene chloride, bis-chloroethyl ether, and trichloroethylene also appear capable of inducing tumors in our models. Moreover, enhancement of tumors, reduction of end-point determination, and an increase in latency period can, in some cases, be achieved by MAM-Ac given to models before or after they are exposed to drinking water biorefractories. Procedures for both our exposure systems and our fish models have been perfected, but a more definitive evaluation of the effect on our models by drinking water biorefractories will require additional tests that are either presently underway or planned.

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Table 1. Neoplastic lesions induced by MAM-Ac in livers of small fish species.

Species	Exposure dose	No. of livers examined	No. of neoplasms and prevalence	Earliest neoplasm noted in weeks	Numbers and percentage of neoplastic cell types		
					Cholangiolar	Hepatocellular	Mixed
Medaka	control	178	0 (0%)	-	-	-	-
	10 ppm	95	28 (29%)	8	7/28 (25%)	13/28 (46%)	8/28 (29%)
	30 ppm	33	21 (64%)	8	6/21 (29%)	9/21 (43%)	6/21 (29%)
	50 ppm	174	91 (52%)	4	24/91 (26%)	50/91 (54%)	17/91 (19%)
	80 ppm	9	6 (67%)	8	2/6 (33%)	1/6 (17%)	3/6 (50%)
	100 ppm	32	12 (37%)	4	6/12 (50%)	2/12 (17%)	4/12 (33%)
Guppy	control	56	0 (0%)	-	-	-	-
	10 ppm	106	70 (66%)	8	5/70 (7%)	52/70 (74%)	13/70 (19%)
	50 ppm	39	29 (74%)	4	9/29 (31%)	16/29 (55%)	4/29 (14%)
	100 ppm	73	47 (64%)	3	8/47 (17%)	25/47 (53%)	14/47 (30%)
Sheepshead minnow	control	48	0 (0%)	-	-	-	-
	10 ppm	33	22 (66%)	12	0/22 (0%)	19/22 (86%)	3/22 (14%)
	50 ppm	28	14 (50%)	8	1/14 (7%)	11/14 (79%)	2/14 (14%)
	100 ppm	7	0 (0%)	-	-	-	-

Table 1. (Continued)

Species	Exposure dose	No. of livers examined	No. of neoplasms and prevalence	Earliest neoplasm noted in weeks	Numbers and percentage of neoplastic cell types		
					Cholangiolar	Hepatocellular	Mixed
Inland silverside	control	37	0 (0%)	-	-	-	-
	50 ppm	28	14 (50%)	4	-	13/14 (93%)	1/14 (7%)
	100 ppm	20	12 (60%)	8	-	11/12 (92%)	1/12 (8%)
Gulf killifish	control	41	0 (0%)	-	-	-	-
	10 ppm	15	0 (0%)	-	-	-	-
	50 ppm	17	8 (47%)	12	-	8/8 (100%)	-
	100 ppm	21	0 (0%)	-	-	-	-
Rivulus	control	6	0 (0%)	-	-	-	-
	50 ppm	25	3 (12%)	8	1/3 (33%)	2/3 (67%)	-
	control	115	0 (0%)	-	-	-	-
Fathead minnow	10 ppm	65	0 (0%)	-	-	-	-
	50 ppm	63	7 (11%)	56	1/7 (14%)	5/7 (71%)	1/7 (14%)
	100 ppm	56	6 (11%)	28	1/6 (17%)	4/6 (67%)	1/6 (17%)
	control	115	0 (0%)	-	-	-	-

Table 2. Occurrence of MAM-Ac-induced neoplastic lesions and lesions associated with neoplasia in seven small fish species.

Species	Organ, tissue or cell of origin	Lesion
Japanese medaka	Liver	Hepatocellular and ductal neoplasms
	Eye	Medulloepitheliomas
	Skeletal muscle	Rhabdomyosarcomas
	Smooth muscle	Leiomyosarcomas
	Fibroblasts	Fibromas; fibrosarcomas
	Mesenchymal tissues	Undifferentiated sarcomas
	Kidney	Nephroblastomas
Guppy	Nervous tissue	Neuroblastomas
	Liver	Hepatocellular and ductal neoplasms
	Mesenchymal tissue	Undifferentiated sarcomas
	Exocrine pancreas	Acinar cell carcinomas
Sheepshead minnow	Kidney	Nephroblastomas
	Liver	Hepatocellular and ductal neoplasms
	Eye (choroid gland)	Hemangioma
	Mesenchymal tissues	Fibroma; undifferentiated sarcoma
Inland silverside	Nervous tissue	Neuroblastomas
	Liver	Hepatocellular neoplasms
Gulf killifish	Liver	Hepatocellular neoplasms
Rivulus	Liver	Hepatocellular neoplasms
Fathead minnow	Liver	Hepatocellular and ductal neoplasms

Table 3. Neoplastic lesions in the livers of the Japanese medaka that had been exposed for 2 months¹ to a mixture of halomethanes (THM) in a closed flow-through system (FT26, 36) before transfer to grow out facilities.

Exposure group	12 weeks ²	24 weeks
aquarium control	0/11 ³	0/25
flow-through control	0/32	0/26
0.2 ppm THM	1/11	1/25
1.6 ppm THM	0/22	4/32
4.6 ppm THM ⁴	0/10	1/21
12.5 ppm THM	1/20	0/12
flow-through control	0/49	0/27
0.2 ppm THM	1/89	0/53
1.7 ppm THM	0/74	0/45
15.9 ppm THM	1/117	4/38

¹These data represent two tests which were conducted before methodology for maintaining fish in tests extending longer than 28 days had been worked out. Consequently, considerable mortality occurred in all groups in the flow-through system.

²The time periods indicated were the regular sampling periods, but they include some moribund fish at periods of 1-18 and 19-30 weeks.

³Number of fish with neoplastic lesions related to number of fish examined.

⁴This group was not part of the normal experiment. The exposure was conducted following an earlier test to determine whether a biological filter had an effect on the THM concentration in the system. Fish were 3-6 months old when introduced for a 27-day exposure.

Table 4. Neoplastic lesions in livers of the Japanese medaka and guppy exposed to 10 mg/l MAM-Ac for 2 hours followed by a 28-day exposure of a halomethane (THM) mixture in a closed flow-through system (FT10, 12).

Fish	Exposure group	Time after MAM-Ac exposure		
		12 weeks ¹	24 weeks	36 weeks
medaka ²	aquarium control	0/8 (0%) ³	0/10 (0%)	0/4 (0%)
	flow-through control 10 ppm MAM/0 ppm THM	0/11 (0%)	0/11 (0%)	2/9 (22%)
	10 ppm MAM/15.4 ppm THM	0/6 (0%)	3/9 (33%)	3/5 (60%)
	10 ppm MAM/24.1 ppm THM	0/7 (0%)	3/5 (60%)	1/3 (33%)
guppy ⁴	aquarium control	0/2 (0%)	0/4 (0%)	
	flow-through control 10 ppm MAM/0 ppm THM	1/17 (6%)	4/8 (50%)	
	10 ppm MAM/15.5 ppm THM	2/12 (17%)	2/10 (20%)	
	10 ppm MAM/31.4 ppm THM	0/3 (0%)	1/2 (50%)	

¹The time periods indicated above were the regular sampling periods, but they include some moribund fish at periods 11-18, 19-30, and 31-36 weeks for medaka and 6-18 and 19-24 weeks for guppy.

²There occurred a dose-dependent mortality of fish while in the flow-through system. Mortality values for 0, 15.4, and 24.1 ppm THM by 28 days were 0, 25, and 45%, respectively.

³Numbers relate to fish with neoplastic livers, total fish examined, and calculated percentage.

⁴Similar mortality occurred as in superscript², with values of 0, 5, and 48%.

Table 5. Neoplastic and related lesions in the Japanese medaka, guppy, and sheepshead minnow exposed to 2 mg/l MAM-Ac followed by an exposure¹ of a halomethane (THM) mixture in a closed flow-through system (FT31, 32, 35).

Fish	Exposure group	Time after MAM exposure ²			
		8 weeks	24 weeks	29 weeks	36 weeks
medaka	aquarium control 2 ppm MAM/0 ppm THM	0/28 (0%)	7/92 (8%)	0/33 (0%)	
	flow-through control 2 ppm MAM/0 ppm THM	0/30 (0%)	0/68 (0%)	1/22 (5%)	
	2 ppm MAM/1.8 ppm THM	0/29 (0%)	2/90 (2%)	0/26 (0%)	
	2 ppm MAM/21.8 ppm THM	0/45 (0%)	1/45 (2%)	1/13 (8%)	
guppy ³	control in grow out 0 ppm MAM/0 ppm THM	0/19 (0%)	0/19 (0%)		0/9 (0%)
	aquarium control 2 ppm MAM/0 ppm THM	1/16 (6%)	8/18 (44%)		11/13 (85%)
	flow through control 2 ppm MAM/0 ppm THM	5/13 (38%)	7/18 (39%)		8/9 (89%)
	2 ppm MAM/6.0 ppm THM	4/16 (25%)	12/18 (67%)		11/13 (84%)
	2 ppm MAM/23.8 ppm THM	2/16 (13%)	12/19 (63%)		15/16 (94%)
sheepshead minnow ⁴	aquarium control 2 ppm MAM/0 ppm THM	0/11 (0%)	0/12 (0%)		
	flow-through control 2 ppm MAM/0 ppm THM	0/13 (0%)	2/14 (14%)		
	2 ppm MAM/1.7 ppm THM	0/11 (0%)	0/14 (0%)		
	2 ppm MAM/13.2 ppm THM	0/10 (0%)	0/13 (0%)		

¹The medaka THM exposure lasted 12 days and was conducted in fresh water, the guppy exposure lasted 28 days in 12 ppt seawater, and the sheepshead minnow exposure lasted 10 days in 12 ppt seawater.

²Some moribund fish were examined before and after the regular sample dates indicated.

³There was 100% survival of all fish in the flow-through system during the 28-day exposure.

⁴There was considerable mortality of fish at 10 days and exposures were terminated.

Table 6. Neoplastic and related lesions in the liver of the Japanese medaka exposed for 12 days to a mixture of halomethanes (THM) in a closed flow-through system followed by a 2-hour static exposure of 2 mg/l MAM-Ac (FT33).

Exposure group	Time after initial THM exposure ¹		
	6 weeks	24 weeks	36 weeks
aquarium control 0 ppm THM/ 0 ppm MAM	0/31 (0%)	0/47 (0%)	0/26 (0%)
aquarium control 0 ppm THM/ 2 ppm MAM	1/28 (4%)	33/57 (58%)	18/32 (56%)
flow-through control 0 ppm THM/ 2 ppm MAM	1/36 (3%)	16/56 (29%)	11/25 (44%)
1.6 ppm THM/ 2 ppm MAM	0/22 (0%)	22/80 (27%)	3/5 (60%)
24.3 ppm THM/ 2 ppm MAM	0/23 (0%)	1/43 (2%)	6/19 (32%)

¹Some moribund fish were examined before and after the regular sample dates indicated.

²There occurred a dose-dependent mortality of fish in the flow-through system, with cumulative values for the 12 days of 0, 3, and 29%.

QUESTIONS AND DISCUSSION

DR. BRESNICK: This paper is now open for discussion. I will start off by asking the first question. Robin, in your experience with either the medaka or the guppy, what is the incidence of malignant tumors, actually invasive tumors, versus benign tumors over the spectrum of carcinogens that you have used?

DR. OVERSTREET: Most of our malignant tumors have developed after a one-year period of time. We really don't understand that aspect well yet because most of our studies are still short-term. For fish in general, most neoplasms do not metastasize; most of ours, however, are locally invasive.

I might also mention that the prevalence of spontaneous tumors in our controls was zero. Out of the many thousands of fish that we've looked at we have only seen two tumors in control fish. Both of those showed up after one year.

Back to malignancy, if you characterize a malignant tumor as being invasive, we have seen malignant tumors in fish exposed to both high and low doses of both direct- and indirect-acting compounds. With the drinking water carcinogens in the chronic tests, most tumors do not appear as invasive as tumors induced by the stronger carcinogens at this time. When we look at samples at nine months, we really don't know what we're going to find. We do have some invasive tumors in our fish exposed to drinking water carcinogens.

DR. BRESNICK: Yes, please?

DR. MARZULLI: F. Marzulli, National Academy of Sciences. Have you looked at pH as a factor?

DR. OVERSTREET: We're using well water that has a pH of around 8.5. We were initially rather worried about the effect of pH both on breeding and on the toxic effect of exposed compounds on fish.

By lowering the pH, you generally expect to see a more toxic effect. We have not looked histologically at any fish exposed at a lower pH. All of the exposures described here were conducted at the relative high pH level, a level that we presume either did not affect or reduced the toxicity of the compounds. In some of our early pilot studies with phenol, we found that when we lowered the pH, it really made a considerable difference in the toxic effect. However, we have not evaluated the relationship between toxicity and carcinogenicity. That is a topic that interests us, but our primary interest now is to evaluate the carcinogenicity of halomethanes.

DR. BRESNICK: Dr. Farland?

DR. FARLAND: Bill Farland, Environmental Protection Agency. Dr. Overstreet, could you just talk a little bit about the rationale for selecting the MAM acetate as a positive control and what other types of chemicals you might have used as a positive control?

DR. OVERSTREET: Well, I didn't mention it, but we also tried MNNG. We needed a direct-acting carcinogen, and DMN would probably have to be exposed for a long period. In our exposures of both MAM and MNNG, we utilized single two-hour exposures. The primary reason we used both compounds was because other people had used them with positive results. Our results turned out better than others, including those of some Japanese workers who also used MAM with medaka. Consequently, we continued to use MAM. As we developed our system, procedures, and biochemical studies, the choice still appeared appropriate. There are a number of factors that could be involved with our success with MAM and other carcinogens. One of them is that we rear our fish at 27 degrees. In the contrasting case of trout, trout are reared at 12 degrees, and that may be a primary reason why many tumors in trout require much longer to develop and why most trout samples are taken at 18 months.

DR. BRESNICK: Just a continuation of that question and then Dr. Kraybill. What's the stability of MAM acetate in the aquarium waters?

DR. OVERSTREET: It's supposed to break down at a reasonably rapid rate, but I think there are still questions about the active components and their stability. We exposed MAM for two hours in a beaker, and then we disposed of the solution, so our MAM never entered into our evaporatory pond or even into our flow-through system. Thus, we're not in a position to evaluate the stability of the products.

Our MAM work has all been done on a dose level. In our studies with the halomethanes, we've monitored actual water concentrations by electron-capture gas chromatography. We monitored the systems three or four times a week. Now we look at the water twice weekly and find we are able to maintain a constant concentration of our halomethanes over a three-month period of time, usually with less than 10 percent variation throughout that entire period.

DR. BRESNICK: I wonder if it might be useful after the two hours to take the spent water and test it for mutagenic activity and continue to do that just as sort of a confirmation that you're getting rid of the alkylating agent. I know it's reasonably unstable, but I think it still worries me a little bit.

DR. OVERSTREET: Yes, it would be very interesting to conduct that study with or without bacteria in the water.

DR. BRESNICK: Herman?

DR. KRAYBILL: Herman Kraybill, ex-NCI. I think you partly answered the question I'm going to ask you. I've always been concerned about your concentrations. And you said something about your monitoring, that you did this several times a week. Is that correct?

DR. OVERSTREET: Yes, what we do is extract all six compounds simultaneously from the water and analyze them under 2 GC conditions, which separates each compound from the others. The concentration of each individual halomethane is then quantitated.

DR. KRAYBILL: Yes, well let's say we were trying to get a concentration of 200 parts per billion, the lowest level. Through your analytical chemistry, would you see any excursions away from that value or fluctuation?

DR. OVERSTREET: Do you mean in our system or in the methodology?

DR. KRAYBILL: In the actual concentrations from your analysis, how close did you come to that 200 parts per billion?

DR. OVERSTREET: Quite close. More importantly, however, our measured concentrations proved to be stable throughout the entire three-month exposure period, as evidenced by coefficients of variation generally ranging between 10 and 15 percent.

DR. BRESNICK: Thank you very much, Robin.

REGISTRY OF TUMORS IN LOWER ANIMALS

**Presented by Dr. John Harshbarger and Dr. Esther Peters
National Museum of Natural History
Smithsonian Institution**

REGISTRY OF TUMORS IN LOWER ANIMALS

**Dr. John Harshbarger
Dr. Esther Peters
Smithsonian Institution**

DR. HARSHBARGER: The purpose of the Smithsonian Institution's registry of tumors in lower animals is to facilitate the study of neoplasms and related disorders in cold-blooded vertebrates and invertebrate animals by serving as a diagnostic center, a specimen depository and information resource, and as a research collaborator.

Specimens have been received from nearly 50 countries for diagnosis and accessioning. Specimens are also acquired from outside experimental and survey projects in which the registry provides histotechnology and histopathology. In these collaborative studies, specimens are diagnosed first, and only those with neoplasms or related lesions are actually accessioned into the collection.

Registered specimens represent the lower 99 percent of the animal kingdom. We only exclude birds and mammals. Over half of the lesions, are neoplasms. We try to exclude repetitious specimens with non-neoplastic lesions but that's not always easy. For example, one contributor from Florida finds many fish in Biscayne Bay with grossly disoriented scales. He is convinced this condition is an alarming indicator of pollution, that it is getting worse, and that my accessioning of numerous examples into the registry would give it the visibility it deserves. While he may be right on all counts, it is outside the mission of the registry as now defined.

However, the registry is interested in token reference cases of non-neoplastic diseases that may be confused with neoplasms, such as proliferative kidney disease, which is a parasitic disease in fish, and token reference cases of non-neoplastic diseases commonly encountered in specimens being studied along with their neoplasms. For example, we recently received a clam with a hemic sarcoma that also had rickettsia infecting its digestive diverticulum, ciliates infesting its gill lamellae, and greporines infecting its gill tissues.

During the previous 12 months, specimens were accessioned at the traditional rate of one per day. However, cases actually processed and diagnosed were much higher due to the collaborative studies. Neoplasms represented 60 percent of the total number of accessions. Bony fish had the most tumors, followed by amphibians, reptiles, bi-valved mollusks, corals, sharks, and crabs.

The coral cases, contributed by Dr. Esther Peters, are highly significant, not only because they are the most primitive animal yet found with neoplasms, but also because one reason the Smithsonian Institution originally accepted the registry in 1966 was curiosity about the real nature of tumor-like growths on corals in the U.S. National Collection, which it houses.

Dr. Peters has developed a technique for decalcifying and preparing tissue sections of the coral lesions so that diagnosis could finally be made. She interprets the lesions as neoplasms originating from skeleton-forming cells, the

calicoblasts. Her manuscript describing these lesions will be published in the May issue of the JNCI (76:895-912.1986), including cover photographs. Since Dr. Peters is presently working at the Smithsonian, I invited her to present a synopsis of her coral tumor studies.

DR. PETERS: Thank you, Dr. Harshbarger. I will first discuss a specimen of Acropora palmata, the elkhorn coral, from the Key Largo National Marine Sanctuary, with raised whitened lumps protruding over the branches. These lumps can appear anywhere on the surfaces of the branches on certain colonies of the coral.

The normal branch surface of the coral is composed of numerous polyps, which resemble small sea anemones, except that they produce a calcified skeleton. The corals are connected by tissue covering the surface called the coenosarc, and a skeleton produced by the calicoblasts, as Dr. Harshbarger mentioned, binds all the polyps together. In this particular group of corals, the polyps are connected beneath the surface of the skeleton by a series of tissue-lined channels known as the gastrovascular canals.

The tumor appears as raised and lifted tissue above the skeleton, with a bubbly effect around the edge. It is a calcified material covered by a thin layer of tissue with porous canals throughout the mass. This mass of skeleton grows over and covers the normal polyp structures.

In the tumor area of the coral, there are no longer any normal polyp structures, and there's a proliferation of the gastrovascular canals and their associated tissues. Comparing the number of cells per area, there is a significant increase in the number of cells produced per area of tissue in the tumorous region compared to the non-tumorous region.

In the tumor, we found a decrease in the number of mucous secretory cells on the outer epidermis. We also found a total lack of zooxanthellae, or one-celled algae that live within the coral tissues and provide nutrients to the coral, and in this particular species, give its brown color. And that is why the tumors appear quite white.

Also, the calicoblastic epithelial cells in this region are more cuboidal to columnar, rather than squamous, as you usually find in the normal areas of the coral. These cells resemble the juvenile, more metabolically active cells found at the growing tips of the branches in the coral.

Now, because the coral loses its mucous secretory cells, the tumors may become ulcerated. The mucous is used to remove particulates falling on the coral surface; when it can no longer do this, the tumors become ulcerated and are invaded by filamentous algae and sediments and the tissue is destroyed. These tumors are life-threatening to the coral because they are quite porous and may cause the branches to break apart more easily during storms. And, of course, because the tumors overgrow the normal polyp structures, the coral is no longer able to reproduce sexually.

DR. HARSHBARGER: Thanks a lot, Esther. Another major function of the registry, in addition to the specimen collection, is the gathering, abstracting, computerizing, and disseminating of information. Relevant literature in this field increases at the rate of 20 to 30 papers per month for a current total of 4,800 papers.

We computerize both the reprint database and the specimen database by the same 60 descriptors, so that they are complementary. The database recently served to identify tissue-specific fish tumor literature and specimens for the various groups that are currently preparing a fish tumor pathology atlas. Registry cases are providing the bulk of the material for the atlas.

Since the 1983 series of Cable News Network TV pieces exposing the apparent relationship of fish liver tumors to pollution, followed by a congressional hearing, the registry has been bombarded daily with requests for information. There has been no letup in media coverage, as you can see by the article in the April 21, 1986, issue of Forbes Magazine. Channel 7 will have a 3-1/2 minute piece in May. Channel 9 is working on two pieces. CNN had an excellent six-part documentary in December.

I would now like to discuss some recent cases to help you appreciate the nature of the lesions and to give you an idea of the registry's collaborations and interactions. Dr. Cameron had already mentioned the NCI contract with the EPA Narragansett Laboratory in Rhode Island where they are exposing clams and fish to Bridgeport, Connecticut, harbor sediment (black rock). Several weeks ago, Dr. George Gardner from that laboratory brought two large boxes of microslides of the resulting lesions to the registry for evaluation.

One of the slides shows an adenoma in the gut of a soft clam that had been siphoning suspended harbor sediment for several months. The epithelium of the gut mucosa is thickened, forming glandular structures. Cilia are absent from the luminal surface. Mitoses are plentiful, so it is rapidly growing, but not yet invasive. Similarly treated clams are being fed to winter flounder to see if the carcinogens are transferred up the food chain.

We are collaborating with the Fish and Wildlife Service on both experimental and survey studies. Dr. Paul Merle in Columbus, Missouri, has exposed rainbow trout to dioxin for two months, followed by a one-month holding period. The highest dose was 20 nanograms per liter. At the highest dose for the longest period, little clusters of tubules formed in the livers of all the fish on the test.

This may be the beginning of cholangiocarcinoma, but at this point I am calling it ductal hyperplasia. Unfortunately, the two-month exposure was not long enough to reveal how far these lesions could have progressed, but the fact that something came up suggests it would be worth redoing for a longer period.

Surveys in the Black River and the Cuyahoga River in Ohio, the Fox River in Wisconsin, and in waterways, have shown a large prevalence of liver cancer in brown bullhead. Examples include a highly invasive cholangiocarcinoma that has grown through the liver capsule and is invading the gut; a hepatocellular carcinoma compressing normal tissue, with a different staining pattern from normal liver tissue, and which is more eosinophilic; and a cholangiocarcinoma that does not reach the surface, which illustrates that you cannot always go by surface

lesions. Sometimes there are huge tumors down inside the liver, and so you really have to do histology to find them.

You look at the edge of tumors to determine if they are invasive. At the edge of one example you can clearly see fingers of tumor tissue infiltrating the normal liver tissues. Tumors like this start small and look innocent, but there is a continuous progression. At what point you say this is malignant rather than benign is hard to tell.

A spectacular cholangiocarcinoma in a white sucker from the Fox River takes practically the entire liver. There is just a little rim of normal liver around the edge; the rest is all cancer.

One winter flounder with liver cancer came eight miles out of Boston harbor in the ocean. Multiple tumor nodules on the liver represent at least four different lesions. One is a solid hepatocellular carcinoma, consisting of solid fields of irregular hepatocytes that do not form the normal two-cell wide cords separated by sinusoids into a regular pattern. Another is a non-neoplastic lesion that is cystic with a fibrous matrix. The cysts appear to be lined with endothelial cells, so they are probably capillaries; I'm calling it toxic cirrhosis.

Vacuolar cell lesions consist of cords of vacuolate hepatocytes. They range from an individual vacuolated cord to large areas where all liver cords have vacuolated cells. Vacuolar cell lesions so far are unique to winter flounder. They are not neoplasms, but cholangiocarcinomas often originate within them.

Philosophizing a bit, an analysis of registry specimens and reprint databases suggest that fish liver cancer is the specific result of carcinogen exposure. The reasons are sixfold.

Epidemiologically, fish with liver cancers are clustered about concentrations of chemicals in their habitat; sediment extracts from these areas of chemical concentrations produce similar cancer in fish in the laboratory. Experimentally, approximately three dozen known carcinogens have produced liver cancer in approximately three dozen fish species in the laboratory. Physiologically, fish have the same liver enzymes as mammals. Historically, liver cancer was not seen in wild fish prior to World War II, when relatively few synthetic organics were in use; now we have a score of fish liver tumor epizootics. Biologically, we can find no evidence of viruses in any of these liver tumors.

On these bases, a task force recently recommended that fish liver cancer be considered evidence of carcinogen injury. This brings us one step closer to the acceptance of fish as indicators of environmental carcinogens and as bioassay models for testing individual chemicals and chemical mixtures for carcinogenicity.

QUESTIONS AND DISCUSSION

DR. BRESNICK: I have two questions relative to the coral. Can you grow coral in culture in the laboratory? And if you can, can you reproduce this cancer process in the laboratory?

DR. PETERS: Yes. Certain species of coral can be grown quite well in the laboratory when they're relatively adaptable to laboratory conditions. Unfortunately, the species that I showed here does not do well in the laboratory. It seems to require the constant replenishment of fresh ocean waters over its surface. And so I'm not sure that we'll ever be able to grow and try to reproduce this in the laboratory.

DR. BRESNICK: Do you have any idea how long a process this is, before you get this full-blown creature that you've shown us?

DR. PETERS: No. We're not sure. The slides that I showed you were on older specimens. I visited a site near that one where we had originally found the tumors a couple of years ago and I found two younger corals that might have been offspring from those adults, based on where they were situated on the reef. And so right now, it appears that there are some genetic factors that may contribute to the development of these tumors on this particular species of coral. And Dr. Daniel Cheney, who originally had described similar tumors in Pacific acroporids, also thought that there were genetic factors because he found them with a very patchy distribution.

DR. ZEEMAN: Maurice Zeeman, Food and Drug Administration. Did I correctly understand that the location of this particular tumor in coral was fairly discrete, and in a fairly small area?

DR. PETERS: You mean in ...

DR. ZEEMAN: In that reef, there was only a small area where you noticed that problem?

DR. PETERS: Yes, fairly discrete. However, colonies containing these tumors have been found in several different areas in the Key Largo Sanctuary, also in the Netherlands Antilles. And as I mentioned, in related Pacific acroporids in several different areas.

DR. ZEEMAN: A second question I have relates to the zooxanthellae loss in that tissue there. I was wondering why that happens and what particular significance it has for the survival of the coral.

DR. PETERS: That's very interesting. We're not sure why that happens except that the cells making up the tumor appear to be juvenile cells similar to those found in the growing branch tips.

You also do not have the zooxanthellae in the growing branch tips of the coral. We don't know whether that's because they secrete some compound that prevents the zooxanthellae from invading those tips or whether the zooxanthellae just haven't multiplied enough to invade those cells.

But the tumor is growing much faster than the surrounding coral, certainly. And, let's see, what was the second part to your question?

DR. ZEEMAN: The survival.

DR. PETERS. Oh. The survival of the coral. Well, the tumors seem to survive quite well. Another investigator down in the Netherlands Antilles looked at the growth of branch tips related to whether they had tumors. And within 25 centimeters of the branch tip, the branch tip stopped growing. This indicated there was translocation of nutrients to that tumor.

DR. NEWELL: Gordon Newell, Electric Power Research Institute. Dr. Harshbarger, with the many suppliers of specimens and your extensive background, have you seen tissues or organs where similar tumors predominate? In other words, are there environmental sources that might cause certain types of tumors which could relate back to specific pollutants?

DR. HARSHBARGER: Well, some generalizations would be that bottom fish seem to be more sensitive or have more tumors than surface-feeding fish.

DR. BRESNICK: How about within the fish, within the bottom fish, liver or ...

DR. HARSHBARGER: Tumors occur in every organ or tissue system. Some are caused by viruses, some are genetic, and some are cryptogenic.

However, we think all the liver tumors, pancreas tumors, and most of the skin tumors are caused by chemicals for the six reasons I gave. If you find a liver tumor in a wild fish you can almost be sure that there is a source of carcinogens in that area. As brought out by Dr. Overstreet, fish liver cancer almost never occurs spontaneously in laboratory studies.

DR. BRESNICK: John, in that regard, is there a species of fish that actually does have a high spontaneous incidence of any type of tumor that is similar to those seen in mice, for example?

DR. HARSHBARGER: Not in the liver but some hybrids develop melanomas.

DR. BRESNICK: Let me ask one more. We have one or two more minutes.

Can you transplant any of these tumors into other fish? I'm looking for establishing models in the fish that are similar to those established in the eukaryotes, or in mice, rather.

DR. HARSHBARGER: I don't know of any fish tumor xenografts or heterografts, but Schlumberger made tumor autografts to the anterior chamber of the eye.

**THE INFLUENCE OF DISULFIRAM OR ETHANOL
ON 1,2-DICHLOROETHANE CARCINOGENICITY,
METABOLISM, AND DNA BINDING**

**Presented by Kenneth L. Cheever
Division of Biomedical and Behavioral Science
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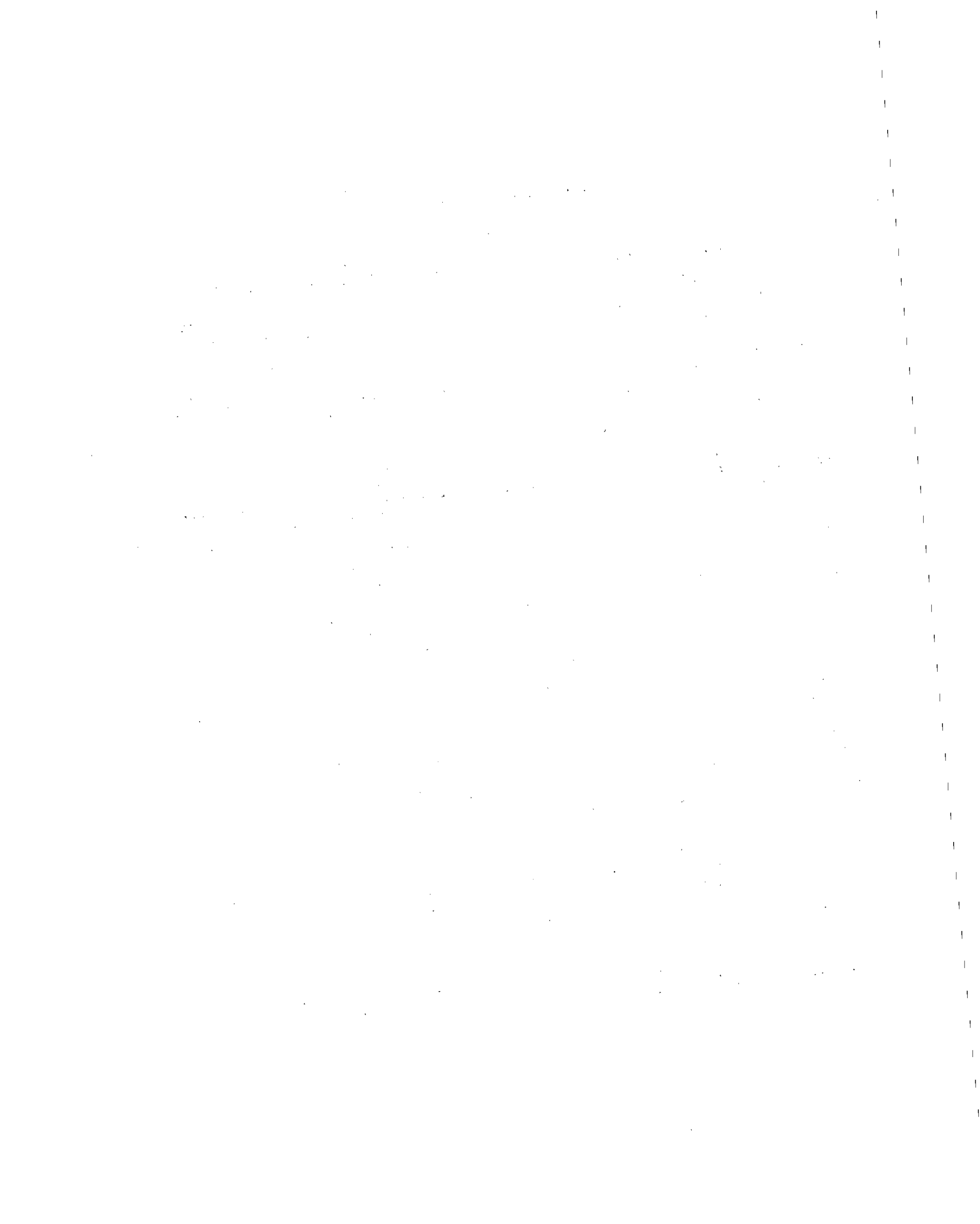
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Work carried out at the Midwest Research Institute, Kansas City, MO.

A 24-month inhalation study of 1,2-dichloroethane (EDC), with concomitant disulfiram or ethanol exposure, was conducted. Sprague-Dawley rats of both sexes received either 50 ppm of EDC, 0.05% disulfiram in the diet, 5% ethanol in the drinking water, 50 ppm of EDC and 0.05% disulfiram in the diet, 50 ppm of EDC and 5% ethanol in the drinking water, or control air. The combined treatment of EDC and disulfiram resulted in a high incidence of intrahepatic bile duct cholangiomas in both male and female rats (18% for males, 34% for females), a lesion not observed in the control animals. This combined treatment also resulted in increased incidence of neoplastic nodules (hepatocellular adenomas) in male rats (12% for EDC and disulfiram vs 0% for controls), interstitial cell tumors in the testes (22% for EDC and disulfiram vs 4% for controls), subcutis fibroma (20% for EDC and disulfiram vs 4% for controls), and mammary adenocarcinomas in female rats (25% for EDC and disulfiram vs 8% for controls). Male rats treated with EDC and ethanol showed a slight increase in incidence of neoplastic nodules (8% for EDC and ethanol vs 0% for controls). At the end of the 24-month period of treatment animals from each group were further studied to determine the rate of elimination, metabolism, and DNA binding of EDC. Blood levels of EDC in male and female rats after exposure at 50 ppm EDC/7 hr were low with 0.22-0.28 ug EDC/mL blood, and 0.30-0.38 ug/mL after EDC and ethanol. Blood levels showed a five fold increase after EDC and disulfiram, with 1.08-1.54 ug EDC/mL. This treatment also affected the elimination of a single oral dose of radiolabeled EDC. In control rats 47 to 55% of 14-C elimination was urinary, and 28 to 30% as EDC was eliminated unchanged in the breath. Trace amounts were eliminated as CO₂ or in the feces. In disulfiram treated rats, 14-C in the breath was 41-55% and 35-36% in the urine. Rats given EDC and disulfiram, however, had 58% expired as EDC and only 25-28% in the urine. The urinary metabolite profile was qualitatively unchanged by longterm EDC, disulfiram or ethanol, and consisted of thiodiglycolic acid (54-71%) and its sulfoxide (19-33%), monochloroacetic acid (1-4%) and two unidentified metabolites (2-7%). Liver DNA binding of radiolabeled EDC was not significantly affected by these longterm treatments.

Results of this study indicate that the interaction of disulfiram with EDC resulted in increased carcinogenicity, an effect which appears to be related to, but not completely explained by, the modification of EDC elimination.

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INTRODUCTION 1,2-Dichloroethane (EDC, CAS Registry No. 107-06-2, 1977), the largest volume synthetic organic chemical manufactured in the United States, is used primarily in chemical synthesis, as a fuel additive, and as an industrial solvent (Drury and Hammons, 1979; NIOSH, 1978a; IARC, 1979; Gold, 1980; Davidson, et al., 1982). The annual United States production of EDC in the last decade has ranged from 3.6 to 4.3 million tons (USITC, 1976-1984), and NIOSH (1978a, b) has estimated that as many as 200,000 workers are continuously exposed on the job to EDC. EDC has been shown to readily absorbed by either the oral or inhalation exposure routes, and absorption by the skin may be significant (Reitz et al., 1980, 1982; Spreafico et al., 1980). Previous studies in the rat have produced contradictory results on the carcinogenic effect of EDC. In one study it was reported that the daily administration of 47 or 95 mg/kg of EDC for 78 weeks by gavage to Osborne-Mendel rats resulted in increased squamous-cell carcinomas and hemangiosarcomas in males and mammary adenocarcinomas and fibrosarcomas in female rats (Weisburger, 1977; NCI, 1978a). These effects were similar, albeit of lower toxicity, to those noted for the bromine analog, 1,2-Dibromoethane (EDB), administered by the same route (Weisburger, 1977; NCI, 1978b). However, in an inhalation study conducted by Maltoni et al. (1980) using Sprague-Dawley rats, no significant changes in the incidence of tumors were found after exposure to EDC at concentrations as high as 250-150 ppm for 78 weeks.

The metabolism of EDC is thought to be complex. Metabolites resulting from in vivo or in vitro metabolism of EDC are reported to include 2-chloroethanol, chloroacetic acid, thiodiglycolic acid, and cysteine and

glutathione conjugates (Yllner et al., 1971; Anders et al., 1980; Guengerich et al., 1980; Reitz et al., 1980, 1982). One reactive intermediate, 2-chloroacetaldehyde, has been shown to cause mutagenic effects in bacterial TA 100 system (McCann et al., 1975), and the formation of the more potent bromoacetaldehyde, resulting from metabolism of EDB in the rat, has been reported (Hill et al., 1978). However, the weak mutagenic effects of EDC in the TA 1535 bacterial system were increased by addition of rat liver cytosol fraction (Guengerich et al., 1980; Reitz et al., 1980; Rannung, 1980a, b). Guengerich and his coworkers suggested that other possible reactive products of EDC metabolism, such as episulfonium ion, may be formed by glutathione-dependent reactions. Certain chemicals, thought to alter the metabolism of halogenated hydrocarbons, have been shown to cause significant synergistic carcinogenic effects in rats when given in combination with those halogenated compounds. Sprague-Dawley rats, when exposed to 20 ppm of EDB and 0.05% disulfiram (DS) in the diet, showed increased mortality and carcinogenicity in comparison with rats exposed to EDB alone (Plotnick, 1978; Plotnick et al., 1980a, b; Wong et al., 1982). A similar synergistic effect was reported between ethanol (ET) and vinyl chloride (Radike et al., 1977). One possible explanation for this increased toxicity is that inhibition of the microsomal aldehyde dehydrogenase system, either directly with DS or competitively with ET, would allow buildup of the reactive haloacetaldehyde. The reported similarities in the metabolism of EDB and vinyl chloride (e.g. formation of the mutagenic haloacetaldehyde intermediate) suggested that the ingestion of DS or ET would have a similar effect on EDC metabolism and the induction of tumors

in the rat. Thus, a study was initiated to evaluate the carcinogenicity, metabolism, and DNA binding of EDC in rats exposed at the current U.S. occupational standard of 50 ppm, with and without either DS in the diet or ET in the drinking water.

METHODS

Chemicals and Solutions The test compound, 1,2-dichloroethane (>99%) was purchased from Aldrich Chemical Company, Milwaukee, WI². Uniformly labeled ¹⁴C-1,2-dichloroethane (EDC), having a radiochemical purity of greater than 98% was synthesized by New England Nuclear (Boston, MA). The radiochemical purity of this radioisotope was verified by high-performance liquid chromatography (hplc) with radioactivity detection prior to use in the metabolism and binding experiments. Commercially available reference compounds corresponding to possible metabolites were obtained at the highest purity. Chloroacetic acid (98%) was purchased from the Fisher Scientific Company, Fairlawn, NJ. Thiodiglycolic acid (97%) and Disulfiram (98%) were purchased from Sigma Chemical Company, St. Louis, MO, and ethanol (95%) was purchased from Aaper Alcohol and Chemical Company (Shelbyville, KY). Thiodiglycolic acid sulfoxide was prepared by the reaction of thiodiglycolic acid with 5% hydrogen peroxide for 2 hr at room temperature in accordance with the method of Rietz (1982). Purity and stability studies were conducted on EDC, DS in diet, and ET in water prior to the initiation of the study, and reanalysis of EDC and DS was conducted periodically throughout the study.

Animals Sprague-Dawley [Cr1:CD (SD)BR outbred], cesarean-derived rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA and maintained on powdered Purina 5002 Rodent Chow (Ralston Purina Company, St. Louis, MO) and distilled water ad libitum. The animals used for the chronic study, weighing 51 to 75 g, were observed for 12 days and were 5.5-6 weeks old at initiation of the study. For preliminary studies on EDC blood levels, metabolism, and excretion, 4- to 5-month old male rats weighing 502 to 522 g and female rats weighing 267 to 322 g from the same supplier were used.

Experimental design After the 12 day quarantine period, six groups consisting of 50 male and 50 female rats were established. The animals were exposed to either 1,2-dichloroethane (50 ppm) or filtered air 7 hours/day, 5 days/ week for a 24-month exposure period. During this period, certain groups of rats were given either DS in the diet or ET in the drinking water according to the following experimental design:

GROUP	EDC	DIET	WATER
1	50 ppm EDC	0.05% Disulfiram	Distilled Water
2	50 ppm EDC	Standard Diet	5% Ethanol
3	50 ppm EDC	Standard Diet	Distilled Water
4	Filtered Air	0.05% Disulfiram	Distilled Water
5	Filtered Air	Standard Diet	5% Ethanol
6	Filtered Air	Standard Diet	Distilled Water

Rats were individually caged and each of the exposure groups was housed in a separate Hazleton Model 1000 stainless steel exposure chamber (Hazleton Systems, Inc., Aberdeen, MD) throughout the course of the study. Water or 5% ET was supplied to these chambers using an automatic watering system

obtained from Edstrom Industries (Waterford, WI) except during the period when water consumption was measured. Powdered rat food was removed during daily EDC exposure, and both 24-hr food and water consumption was determined periodically during the 24-month period. Environmental conditions in the chambers was maintained at $74 \pm 4^{\circ}\text{F}$ with a relative humidity of $50 \pm 15\%$ throughout the course of the study and a 12 hr light-dark cycle was provided using fluorescent light. At the end of this 24-month exposure period, certain rats from each of these groups were tested to determine either blood levels of EDC, EDC metabolism or DNA binding.

Dosage and treatments Radiolabeled EDC for use in the metabolism and DNA binding studies was administered by gavage at a dose of 150 mg/kg bw. Solutions were prepared by mixing appropriate amounts of the ^{14}C -EDC with EDC in corn oil to give a concentration of 75 mg/mL. For the metabolism studies, each rat received 115 to 140 uCi/kg bw, and for DNA binding studies each rat received 241 to 260 uCi/kg bw.

Generation and analysis of test atmospheres The 50 ppm EDC test atmospheres were generated by bubbling compressed air through undiluted EDC at room temperature to form a concentrated vapor. This vapor was diluted to the desired concentration with filtered compressed air, mixed, and transported to the appropriate chamber through Teflon lines. The chambers which received only filtered air were housed in a separate room. The atmospheric concentration of EDC was monitored for each chamber throughout the daily exposure period by gas chromatography (gc) using an automated sampling

system in conjunction with a Model 2400 gas chromatograph (Varian Associates, Palo Alto, CA), fitted with a 1.8-m-long X 2-mm-i.d. nickel column packed with 10% Carbowax 20M-TPA on 80/100-mesh Chromosorb W AW (Supelco, Inc., Bellefonte, PA). The oven temperature was maintained at 70°C and a nitrogen carrier gas flow rate was 30 cm³/min. Peaks were detected by flame ionization and areas were determined using a Varian CDS 111 integrator. The system was standardized externally by manual injection of EDC standards and quality control samples, and chamber concentrations were adjusted as necessary.

Preparation and analysis of disulfiram feed mix DS diet was prepared weekly by mixing 15 g of DS with 30 kg of powdered food using a PK twin-shell blender, analyzed for DS content, and stored at -32°C until use. For analysis 10 g samples of mixed feed were extracted with 100 mL acetonitrile by shaking for 30 min. The extracts were centrifuged, a 10-mL aliquot was mixed with 3 mL of an internal standard solution containing 0.75 mg/mL of butyrophenone, and each extract was adjusted to 25 mL with methanol:water (75:25). These solutions were filtered through a 0.45 µm pore size Acrodisc^R (Gelman Sciences, Ann Arbor, MI). Duplicate samples were analyzed using a modular Waters Associates (Milford, MA) liquid chromatographic system equipped with a Waters Z-Module radial compression column packed with 10 µm particle size Bondapack C18 and a Waters Model 440 UV detector set at 280 nm. The mobile phase was methanol:water (75:25) and the flow rate was maintained at 3 mL/min. Data was accumulated and processed using a Nelson 4400 Data System (Nelson Analytical, Inc., Cupertino, CA).

Preparation and analysis of ethanol in drinking water Drinking water containing ET was prepared by mixing an appropriate amount of 95% ET with distilled water to give a final concentration of 5%. ET concentrations were determined using a Varian Model 920 gas chromatograph (Palo Alto, CA) equipped with a 3.0-m-long X 2-mm-i.d. nickel column packed with 100/120-mesh Chromosorb 105 (Supelco, Inc.). The oven temperature was maintained at 170°C and a helium carrier gas flow rate was 30 cm³/min. Peaks were detected using a thermal conductivity detector with a filament current set at 150 mA in accordance with the method of Hillbrom (1971).

Histological examination Rats which died during the course of the study and those killed at the end of the 24-month EDC exposure period were given a complete gross and histopathologic examination in accordance with the NCI Guidelines for Carcinogen Bioassay in Small Rodents (1976). All tumors as well as specified organs and tissues were fixed in 10% neutral buffered formalin. The organs and tissues were trimmed, processed through paraffin wax, sectioned at 5-6 microns, stained with hematoxylin and eosin, and examined by light microscopy.

EDC blood levels Blood levels of EDC were determined for 24-month exposure animals from the Control, EDC, EDC/DS, and EDC/ET groups following termination of the 7-hr exposure period. Blood samples were taken from five male and five female rats by the orbital sinus 15 min and 2 hr after cessation of EDC generation during week 103 of exposure. Immediately after collection 1-mL blood samples were injected into 10-mL septum vials containing 3 mL of an internal standard solution (0.86 ug

trichlorethylene/mL blood) with 10% citric acid, and stored at 0°C until analysis. The samples were mixed and allowed to equilibrate at 40°C prior to analysis of 0.1 cm³ headspace aliquots. The headspace analysis was performed using a Varian Model 3700 gas chromatograph equipped with a 6.0-ft-long X 4-mm-i.d. glass column packed with 20% SP-2100/0.1% Carbowax on 100/120-mesh Supelcoport (Supelco, Inc.). The oven temperature was maintained at 65°C and a nitrogen carrier gas flow rate was 70 cm³/min and peaks were detected using a electron capture detector in accordance with the method of Zuccato et al., (1980).

Metabolism and elimination of EDC The metabolism and elimination of radiolabeled EDC was determined for 24-month exposure animals from the Control, DS, ET, EDC, EDC/DS, and EDC/ET groups following termination of 24-months of exposure. Doses of 150 mg/kg bw of ¹⁴C-EDC were administered to three male and three female rats by gavage one week after cessation of 103 weeks of EDC generation. The appropriate animals continued to receive DS or ET as designated during this period. Immediately following the administration of the dosing solution, the rats were placed into separate Roth-type metabolism cages for the collection of exhaled compounds, urine, and feces. Airflow through the cages was maintained at 200 mL/min and unmetabolized EDC was sampled sequentially at 1, 3, 6, 12, and 24 hr by absorption on activated charcoal. Radiolabeled CO₂, trapped by bubbling through 500 mL of 5 M ethanolamine in 2-methoxyethanol, was determined at 3, 6, 12, and 24 hr. Urine was collected at 6, 12, and 24 hr and feces was

taken at 24 hr. Urine and feces samples were stored at -20°C prior to analysis. Charcoal samples (1 g) were desorbed in 15 mL of Bio-solvTM (Beckman Instruments, Fullerton, CA), a toluene based scintillation fluid. The elimination of ^{14}C -EDC was determined using liquid scintillation spectroscopy by counting appropriate aliquots of the charcoal desorbate or aliquots of urine or ethanolamine in 10 mL of PCS scintillation cocktail (Amersham Corporation, Arlington Heights, IL). Feces was burned in a Tri-Carb Model B306 sample oxidizer (Packard Instrument Co.). The $^{14}\text{CO}_2$ from the combustion was absorbed in Carbo-Sorb^R and analyzed in Permafluor^{RV} (Packard Instrument Co.).

Determination of urinary metabolite profiles Conditions for hplc chromatography of possible EDC metabolites were determined using a Waters model 440 absorbance detector set at 214 nm (Waters Associates). The hplc analysis was performed using a Waters chromatograph equipped with a Model 6000A solvent pump and using a 30-cm-long X 4-mm-i.d. stainless steel column packed Bio-Rad Aminex HPLX-87H ion exchange resin. Rat urine samples were analyzed by hplc for identification and quantitation of EDC urinary metabolites. Each urine sample was thawed and filtered using a 0.45- μm pore size Acrodisc^R (Gelman Sciences, Ann Arbor, MI), a 100- μL aliquot was injected into the hplc, and metabolites were chromatographed at 0.5 mL/min using a 0.005 N sulfuric acid mobile phase in accordance with the method of Rietz et al. (1982). The column eluent was mixed 1:3 with Scintiverse LC^R scintillation fluid (Fisher Chemical Co.), and radioactive

components were detected and quantified using a Radiomatic Flo-One Model HP radioactivity monitor.

Liver DNA binding The hepatic binding of radiolabeled EDC was determined for 24-month exposure animals following termination of 24-months of exposure. The appropriate animals continued to receive DS or ET as designated during this period. Doses of 150 mg/kg bw of ^{14}C -EDC were administered to three male and three female rats from each group by gavage 10 to 14 days after cessation of 103 weeks of EDC generation. The animals were anesthetized with ether 6 hr after dosing and killed by esanguination. The livers were removed, rinsed with 0.1 M tris/0.01 M EDTA, weighed, and immediately frozen in liquid nitrogen and stored at -70°C until DNA isolation.

Isolation and purification of DNA Livers were homogenized in two volumes of 0.1 M tris/0.01 M EDTA, pH 7.7, using a Polytron Model PCU-1 homogenizer equipped with a PC-10 probe (Kinematica GmbH, Luzern, Switzerland) and a 0.5-mL aliquot was combusted for determination of total liver radioactivity. The homogenate was then adjusted to a concentration of 1.5% by addition of an appropriate amount of 15% sodium dodecyl sulfate, and incubated at 37°C with 100 ug/mL of pronase B (Cal Biochem, La Jolla, CA) in accordance with the DNA isolation procedure of Joyce and Daniel (1982). After the incubation period, the homogenate was extracted with an equal volume of Kirby's phenol (phenol:m-cresol:8-hydroxyquinoline:water, 500:70:0.5:55, by weight) at room temperature by shaking followed by

centrifugation for 30 min at 4°C at 8500 X g. The upper layer was extracted by shaking with an equal volume of chloroform:isoamyl alcohol (24:1, v/v), centrifuged for 30 min at 4°C at 8500 X g, and nucleic acids precipitated by the addition of 1.5 volumes of cold absolute ET. The solution was allowed to stand at -20°C overnight, and the nucleic acids collected by spooling on a glass rod. The nucleic acids were resuspended in 0.1 M tris/0.01 M EDTA, pH 7.7, at approximately 2 mg/mL, and incubated for 1 hr at 37°C with 100ug/mL of RNase A-amylase (Sigma Chemical company). The incubate was then extracted with an equal volume of Kirby's phenol (phenol:m-cresol:8-hydroxyquinoline:water, 500:70:0.5:55) and again centrifuged. The final extract was made 1% with respect to a saturated sodium chloride solution, and DNA was precipitated by the addition of 1.5 volumes of cold absolute ET. The solution was allowed to stand at -20°C overnight, and the DNA collected. The DNA was dried for 1 hr under a stream of nitrogen, weighed, and stored desiccated at 4°C.

DNA analysis Hepatic DNA analysis was performed using resuspended DNA in 0.1 M tris, pH 7.7, with standards prepared from calf thymus DNA (Sigma Chemical Company). Appropriate dilutions of a 5 mg/mL standard solution and hepatic DNA samples were diluted 1 to 10 with 1 N NH₄OH, and 10-μL aliquots were dried at 60°C and dissolved in diaminobenzoic acid dihydrochloride solution. Samples were then incubated for 30 min at 60°C and diluted with 2 mL of 0.6 N perchloric before analysis by the fluorometric method of Kissane and Robins (1958) as modified by Setaro and Morley (1976). An

Aminco-Bowman model SPF 500C Spectrofluorometer (SLM/Aminco Instruments, Inc., Urbana, IL) set at an excitation wavelength of 420 nm and an emission wavelength of 520 nm was used for the analysis. An 0.5-mL aliquot of each hepatic DNA sample was digested by incubation at 80°C for 30 min with 0.2 mL of 35% perchloric acid. Samples were diluted with 10 mL of PCS cocktail and the amount of ^{14}C bound to the hepatic DNA was determined by scintillation spectroscopy.

Scintillation spectroscopy Duplicate samples prepared for counting by scintillation spectroscopy were cooled for a minimum of 24 hr prior to analysis using a refrigerated Tri-Carb model 3255 liquid scintillation counter (Packard Instruments). The counting efficiency was determined using the automatic external standard method and background was subtracted. Quench standards were prepared for use under the same conditions used throughout the experiment, and sample counts per minute (cpm) were converted to disintegrations per minute (dpm). Dpm per g or mL of sample, based on amounts counted, was calculated, and the ug equivalents/g or mL was determined by dividing this value by the specific activity of the ^{14}C -labeled EDC.

Statistical analysis The significance of differences between control and test values for absolute and relative organ weights, body weight, and food and water consumption was statistically determined using Dunnett's Multiple Comparison Test. Data on tumor incidence were analyzed by Fisher's Exact Probability Test. Differences between control and test values for blood levels, metabolism, and DNA binding was determined using Student's t test.

Statistical significance was assumed at the $p < 0.05$ level.

RESULTS

Clinical signs of toxicity There were no noteworthy differences between control and treatment groups for clinical signs of toxicity throughout the study.

Test chemical concentrations Chamber concentrations of EDC, determined by gc, during the 24-month exposure period were 50.35 ± 0.96 ppm (mean $\pm$ SD). Dietary DS, determined by hplc was $0.0493 \pm 0.0001\%$ (mean $\pm$ SD) for the diet lots used, and ET in the drinking water was $5.07 \pm 0.03\%$ (mean $\pm$ SD), as determined by gc.

Body Weights The average body weight of male and female rats exposed to 0.05% DS in the diet or to a combination of 50 ppm EDC and DS was significantly lower than corresponding controls throughout the study. This depression of weight gain in these two treatment groups was apparently due to DS intake alone since there was no significant difference between the mean body weights of the DS and EDC/DS groups. Body weights of rats in the other exposure groups did not show statistical differences ($p > 0.05$) when compared with their respective controls. The 24-month body weights for each group of animals is shown in Figures 1 and 2.

Food and water consumption A summary of the average food and water consumption for rats during the 24-month exposure period is shown in Tables

1 and 2. Based on these values, calculation of the average daily intake of DS ranged from 10.0 to 10.6 mg/day for male rats and from 7.0 to 7.1 mg/day for female rats. For ET, the average daily intake ranged from 2.41 to 2.59 g/day for male rats and from 2.25 to 2.40 g/day for female rats. Summaries of the food and water consumption is shown in Tables 1 and 2.

Mortality There was no significant difference in the survival of rats in the various treatment groups when compared with the control group during the 24-month exposure. In male rats 52% of the control group, 70% of the DS group, 52% of the ET group, 52% of the EDC group, 44% of the EDC/DS group, and 52% of the EDC/ET group survived to the end of the study. In female rats 54% of the control group, 66% of the DS group, 54% of the ET group, 64% of the EDC group, 44% of the EDC/DS group, and 44% of the EDC/ET group survived to the end of the study. The slightly higher survival at 24 months of animals in the DS group may be related to the significantly lower body weights of those animals. The 24-month mortality for these animals is shown in Figures 3 and 4.

Necropsy observations and organ weights Certain tissue lesions and masses were noted at necropsy with increased frequency in treated male and female groups compared to their control groups. Male rats in certain groups were found to have increased liver masses (32% for the EDC/DS group vs. 8% for controls), kidney lesions (30% for the EDC/ET group vs. 8% for controls) or testicular lesions (24% for the EDC group vs. 10% for controls). In female rats the most noteworthy observations was an increased incidence of liver masses (46% for the EDC/ET group vs. 6% for controls) at necropsy.

The absolute and relative organ weights are shown in Tables 3 and 4.

Histopathology The tumor incidence for rats exposed to EDC/DS was increased when compared with other control or exposure groups (Table 5,6). This increase was due, primarily, to the high incidence of cholangiomas of the intrahepatic bile duct in the livers of both male and female rats in the EDC/DS group (Table 7,8). These lesions consisted of a proliferation of bile ducts lined by a flattened epithelium (Figure 9). The cholangiomas did not appear to be locally invasive, and there were no metastases. However, these histopathological observations in the liver were reflected by an increased relative liver weight. The incidence of mammary neoplasms was increased for female rats exposed to EDC/DS; the combined total of mammary adenomas, adenocarcinomas, and fibroadenomas was 71% compared with 42% in controls. For male EDC/DS rats, interstitial cell tumors of the testis were increased (22% for treated vs 4% for controls). Congestion of the mesenteric lymph node, an unusual finding, was prevalent in the DS and EDC/DS groups for both sexes, indicating a probable DS related effect. Female rats exposed to EDC showed an slightly increased incidence of basophilic focal cellular changes in the pancreas which was not apparent in male rats. Male and female rats in both the ET and EDC/ET exposure groups had neoplastic and non-neoplastic lesions similar to those seen in controls.

EDC blood levels Blood levels for EDC at 15 min or 2 hr postexposure were determined for both male and female rats. The control rats showed no EDC in blood and a sample chromatogram is shown in Figure 6. In rats exposed to

EDC alone, small amounts of the parent compound, 0.28 ug/mL for males and 0.25 ug/mL for females, were detected in the blood at 15 min. These blood levels were not significantly decreased after a 2-hr period. Similar concentrations were noted for animals exposed to EDC/ET. However, both male and female rats exposed to EDC/DS showed a five-fold increased level of the parent compound, 1.46 ug/mL for males and 1.54 ug/mL for females, at 15 min. These blood levels of EDC were decreased to 1.20 ug/mL for males and 1.08 ug/mL for females after 2 hr as shown in Table 9.

Metabolism and elimination of EDC Preliminary studies with 4-month old rats, dosed with ^{14}C -EDC at 150 mg/kg bw, showed that elimination of the radioactivity was essentially complete in 24 hr. The ^{14}C was excreted primarily in the urine with 49.7 to 51.5% of the administered dose eliminated by that route as shown in Tables 10 and 11. A significant portion of the dose, 35.5 to 39.6%, was eliminated unchanged in the breath, and small amounts of radioactivity were found either as expired CO_2 or in the feces. The pattern of elimination of radiolabeled EDC was similar for control animals from the chronic study. Rats exposed to either EDC or ET during the 24-month period showed an elimination pattern similar to their controls. However, differing patterns of elimination were apparent for rats exposed to DS, EDC/DS, or EDC/ET. In both DS and EDC/DS groups the amount of EDC eliminated unchanged in the breath, 40.9 to 55.2% for DS animals and 57.5 to 57.7% for EDC/DS animals, was increased. Radioactivity in the urine, 35.2 to 36.4% for DS animals and only 24.9 to 27.6% for EDC/DS animals, was significantly decreased. The EDC/ET group, however,

had 51.1 to 55.1% eliminated in the urine and only 17.6 to 17.7% in the breath.

Urinary metabolite profiles Urine collected from rats treated with ^{14}C -EDC was used to determine hplc urinary metabolite profiles. The urine samples from these animals showed 5 radioactive peaks and were qualitatively the same for each of the treatment groups. The major metabolite, accounting for 54.3 to 71.0% of the urinary radioactivity, was identified as Thiodiglycolic acid. The second most abundant metabolite, accounting for 18.7 to 32.7% of the activity was determined to be the sulfoxide of Thiodiglycolic acid. Trace amounts of chloroacetic acid, ranging from 0.7 to 4.3%, were found along with two unidentified metabolites which accounted for 1.5 to 3.7% and 4.3 to 7.3% of the total. A sample chromatogram is shown in Figure 7. The amounts of chloroacetic acid excreted by animals of the EDC/DS group appears to be higher than for other control or treatment groups, and was the only quantitative difference observed as shown in Tables 12 and 13.

Liver DNA binding Male and female rats from each group were treated with ^{14}C -EDC and their livers were removed at 6 hr to evaluate the DNA binding potential in 24-month exposure animals. Low levels of ^{14}C -EDC material, 36 to 44 um EDC/mole DNA, were detected in these animals. Although the binding was similar for each of the groups tested, female rats generally showed lower EDC binding than males as indicated in Table 14.

DISCUSSION

The results of this study indicate that rats exposed to 1,2-dichloroethane at 50 ppm, the current U.S. occupational standard, and given disulfiram in the diet showed increased numbers of tumors when compared with those exposed to filtered air and given a standard diet. This combined EDC/DS treatment resulted in a high incidence of an unusual lesion, intrahepatic bile duct cholangiomas in both male and female rats. In addition, male rats were shown to have a significantly higher incidence of interstitial cell tumors of the testes, fibroma of the subcutis, and hepatic neoplastic nodules. Female rats exposed to EDC/DS were shown to have a significantly higher incidence of mammary adenocarcinoma than the corresponding controls. Although significant, such enhancement of carcinogenicity by DS, an antioxidant, is in contrast with reported protective effects of DS on the tumor production of certain other compounds (Wattenberg, 1978; El-hawari et al., 1986). Previous NIOSH and NCI sponsored studies, however, have demonstrated a similar increase in the carcinogenic response of the bromine analog of EDC, 1,2-dibromoethane (EDB), administered in combination with DS (Plotnick, 1978; Stein et al., 1978; Plotnick et al., 1980a, b; Elliott and Ashby, 1980; Wong et al., 1982). Although the types of tumors noted in those EDB studies (i.e. hemangiosarcoma and liver carcinoma) differ from those found after exposure to EDC and DS in the present study, the results of this study show that the DS interaction observed for EDB is not limited to that particular halocarbon.

In the present study, no significant increase in tumor production was

observed in rats after long-term exposed to either EDC alone at 50 ppm or to EDC in combination with ET.

For EDC administered in combination with DS, a possible explanation of the observed carcinogenicity may be related to the presence of higher blood concentrations or changes in the disposition of the compound. Blood levels of rats were low following exposure to EDC of rats treated with ET. The animals exposed to EDC and DS, however, were shown to have significantly higher blood levels of unchanged EDC than those exposed to EDC alone. This difference appears to be due to a slower rate of biotransformation in DS-treated rats. The EDC/DS rats were found to eliminate a significantly larger portion of the administered radiolabel in the breath as unchanged EDC and less as urinary metabolites, a finding consistent with the known enzyme inhibiting action of DS which involves inhibition of mixed-function oxidase, aldehyde dehydrogenase activity, and, to a lesser extent, that of alcohol dehydrogenase (Zemaitis and Greene, 1976). The rate of elimination of radiolabeled EDC by 4-month-old rats was similar to that previously reported for 150 to 250 g Osborne-Mendel rats dosed at the same 150 mg/kg bw amount of EDC (Reitz et al., 1980, 1982). These investigators found that 85% of the radioactivity appeared in the urine, with 7-8% as CO₂, 4% in the carcass, and 2% in the feces at 48 hr. The presence of unchanged EDC in the breath was apparently not evaluated by those investigators since a recirculating chamber system was used. A more recent study by Mitoma et al., in which 4 to 6 week old Osborne-Mendel rats were dosed with EDC at 100 mg/kg bw, showed 69.5% of the dose excreted in the urine with lesser amounts, 11.5%, 8.2%, and 7.1%, appearing in the expired air, as CO₂, and

carcass, respectively. Older rats evaluated in the current study were shown to eliminate a lower amount of the radioactivity over a 24-hour period, a finding which may be related to the higher adipose tissue in these rats.

The chromatographic evaluation of urinary metabolites showed that the principal metabolite, thiodiglycolic acid, accounted for 54.3 to 71% of the radioactivity present in the urine. The sulfoxide of this compound accounted for an additional 18.7 to 32.7%, and 0.3 to 4.3% of urinary radioactivity was present as chloroacetic acid. Additionally, two unidentified urinary components accounted for 1.5 to 3.7% and 4.3 to 7.3% of the urinary activity. Metabolism of EDC by the long-term exposure Sprague-Dawley rats was similar to that reported previously for younger Osborne-Mendel rats, and a proposed scheme of metabolism is shown in Figure 8. For these younger rats, thiodiglycolic acid and its sulfoxide, amounting to 67% and 28.8%, respectively, were reported as urinary EDC metabolites by Reitz et al. (1982). Other investigators have reported the presence of S-carboxymethylcysteine, thiodiacetic acid, and chloroacetic acid as major metabolites in the urine of 4 to 6 week old rats (Mitoma et al., 1985) and in mice (Yllner, 1971). Additionally, the production of reactive intermediates, chloroacetaldehyde or S-(2-chloroethyl)glutathione, has been suggested to occur (Guengerich et al., 1980; Anders and Livesey, 1980). Such Metabolites would react covalently with cellular macromolecules and could be responsible for any carcinogenic or mutagenic effects of EDC (Banerjee, et al., 1980; Guengerich et al., 1980). Several investigators have examined the possible DNA binding by EDC, both in vitro and in vivo.

Guengerich and his coworkers (1980) demonstrated that the in vitro EDC binding to DNA appeared to be highest in the presence of GSH, but some activity was observed in the presence of microsomal protein and NADPH. The analysis of DNA isolated from rat liver after EDC exposure showed 2 to 5 times higher DNA alkylation after oral administration than after inhalation (Reitz et al., 1982). These investigators reported that liver DNA binding was 13.9 to 21.3 uM as EDC/M DNA after oral dosing at 150 mg/kg and 3.3 to 8.2 uM as EDC/M DNA after inhalation at 150 ppm/6 hr. In the present study, the binding of ¹⁴C to hepatic DNA was somewhat higher for the long-term male and female rats, ranging from 18.9 to 53.3 uM as EDC/M DNA after oral dosing with EDC at 150 mg/kg. Although this binding did not appear to be affected by DS or ET treatment, other factors such as possible saturation of binding sites by long-term EDC exposure cannot be ruled out.

In summary, the results of the present study indicate that rats exposed to EDC at 50 ppm and given DS in the diet showed increased numbers of tumors when compared with those exposed to filtered air and given standard diet. This combined EDC/DS treatment resulted in a high incidence of hepatic, testicular, and mammary tumors in the rat. DNA binding by EDC did not appear to be altered by DS treatment, and the metabolism of EDC was qualitatively the same as that of corresponding controls. However, a reduced rate of elimination of EDC, along with sustained blood levels of the unchanged EDC may be related to the observed carcinogenic effects of the combined treatment.

TABLE 1

AVERAGE 24-HOUR FOOD CONSUMPTION FOR MALE AND
FEMALE RATS DURING MONTHS 1 THROUGH 24.

GROUP ^a	MALE RATS		FEMALE RATS	
	GRAMS/DAY	%DIFFERENCE	GRAMS/DAY	%DIFFERENCE
CONTROL	24.9 ^b	-	18.1	-
DS	21.2	-14.9	14.2	-21.6
ET	20.7	-16.8	14.5	-19.9
EDC	23.6	-5.2	17.1	-5.5
EDC/DS	20.0	-19.7	14.4	-20.4
EDC/ET	19.6	-21.3	14.6	-19.3

^a Groups of 50 male and 50 female Sprague-Dawley rats were exposed to either 50 ppm 1,2-dichloroethane (EDC) or filtered air 7 hours/day, 5 day/week for 24 months. Certain groups recieved either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water throughout the exposure period.

^b Calculated as the average of 24-hour mean food consumption determined throughout the 24-month study.

TABLE 2

AVERAGE 24-HOUR WATER CONSUMPTION FOR MALE AND
FEMALE RATS DURING MONTHS 1 THROUGH 24.

GROUP ^a	MALE RATS		FEMALE RATS	
	GRAMS/DAY	%DIFFERENCE	GRAMS/DAY	%DIFFERENCE
CONTROL	42.3 ^b	-	40.3	-
DS	45.9	8.5	40.8	1.2
ET	48.2	13.9	45.0	11.7
EDC	48.4	14.4	45.5	12.9
EDC/DS	54.9	29.8	45.7	13.4
EDC/ET	51.8	22.4	47.9	18.9

^a Groups of 50 male and 50 female Sprague-Dawley rats were exposed to either 50 ppm 1,2-dichloroethane (EDC) or filtered air 7 hours/day, 5 day/week for 24 months. Certain groups recieved either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water throughout the exposure period.

^b Calculated as the average of 24-hour mean water consumption determined throughout the 24-month study.

TABLE 3

ABSOLUTE AND RELATIVE LIVER WEIGHTS OF MALE RATS.

DOSE GROUP ^a	TERMINAL BODY WT. ^b	ABSOLUTE LIVER WT. ^b	RELATIVE LIVER WT. ^c
CONTROL	607.9 ± 114.0	22.00 ± 5.08	3.69 ± 0.97
DS	605.8 ± 95.4	23.61 ± 3.51	3.89 ± 0.54
ET	624.0 ± 107.5	23.36 ± 7.47	3.74 ± 1.15
EDC	672.2 ± 121.5	25.15 ± 5.30	3.74 ± 0.83
EDC/DS	526.3 ± 61.8 ^d	24.36 ± 4.31	4.44 ± 1.09 ^d
EDC/ET	657.1 ± 105.0	23.60 ± 4.58	3.64 ± 0.76

^aGroups of 50 male Sprague-Dawley rats were exposed to either 50 ppm 1,2-dichloroethane (EDC) or filtered air 7 hours/day, 5 day/week for 24 months. Certain groups received either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water throughout the exposure period.

^bWeight in grams (mean ± S.D.)

^cWeight in grams/100 grams body weight (mean ± S.D.)

^dSignificantly different from control value ($p < 0.05$) by Dunnett's Multiple Comparison test.

TABLE 4

ABSOLUTE AND RELATIVE LIVER WEIGHTS OF FEMALE RATS.

DOSE GROUP ^a	TERMINAL BODY WT. ^b	ABSOLUTE LIVER WT. ^b	RELATIVE LIVER WT. ^c
CONTROL	417.6 ± 93.6	15.57 ± 3.45	3.79 ± 0.66
DS	331.0 ± 63.0	13.55 ± 2.99	4.11 ± 0.56
ET	470.1 ± 117.6	16.81 ± 4.60	3.65 ± 0.96
EDC	441.6 ± 87.7	16.53 ± 4.23	3.76 ± 0.69
EDC/DS	348.5 ± 74.7 ^d	18.07 ± 5.24	5.27 ± 1.49 ^d
EDC/ET	523.2 ± 155.4	17.36 ± 5.50	3.41 ± 0.92

^aGroups of 50 female Sprague-Dawley rats were exposed to either 50 ppm 1,2-dichloroethane (EDC) or filtered air 7 hours/day, 5 day/week for 24 months. Certain groups recieved either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water throughout the exposure period.

^bWeight in grams (mean ± S.D.)

^cWeight in grams/100 grams body weight (mean ± S.D.)

^dSignificantly different from control value ($p < 0.05$) by Dunnett's Multiple Comparison test.

TABLE 5

TUMOR INCIDENCE FOR MALE RATS^a.

	CONTROL	EDC	DS	ET	EDC/DS	EDC/ET
PRIMARY TUMORS	69	86	53	88	102	69
TOTAL RATS WITH TUMORS	42	45	32	42	45	42
RATS WITH MULTIPLE TUMORS	17	29	15	25	29	17
TOTAL BENIGN TUMORS	64	74	45	74	84	55
TOTAL MALIGNANT TUMORS	5	12	8	14	18	14
TOTAL MALIGNANT WITH METASTASIS	0	0	1	1	4	2

^aGroups of 50 male Sprague-Dawley rats were exposed to either 50 ppm EDC or filtered air 7 hours/day, 5 days/week for 24 months. Certain groups received either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water throughout that exposure period.

TABLE 6

TUMOR INCIDENCE FOR FEMALE RATS ^a.

	CONTROL	EDC	DS	ET	EDC/DS	EDC/ET
PRIMARY TUMORS	85	87	63	72	105	80
TOTAL RATS WITH TUMORS	47	47	46	41	46	47
RATS WITH MULTIPLE TUMORS	28	26	14	20	33	22
TOTAL BENIGN TUMORS	70	72	58	61	82	43
TOTAL MALIGNANT TUMORS	15	15	5	11	23	7
TOTAL MALIGNANT WITH METASTASIS	2	1	2	2	4	1

^aGroups of 50 female Sprague-Dawley rats were exposed to either 50 ppm EDC or filtered air 7 hours/day, 5 days/week for 24 months. Certain groups received either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water throughout that exposure period.

TABLE 7

TUMOR CLASSIFICATION AND NUMBER FOR MALE RATS^a.

	CONTROL	EDC	DS	ET	EDC/DS	EDC/ET
INTRAHEPATIC BILE DUCT CHOLANGIOMA	0	0	0	0	18 ^{**}	0
TESTIS INTERSTITIAL CELL	4	6	6	14	22 [*]	10
SUBCUTANEOUS FIBROMA	4	6	2	0	20 [*]	4
NEOPLASTIC NODULES	0	4	2	4	12 [*]	8
MAMMARY ADENOCARCINOMA	0	0	0	0	3 [*]	0
MAMMARY FIBROADENOMA	0	3	0	0	0	2
MAMMARY ADENOMA	0	0	2	0	0	1
HEPATOCELLULAR CARCINOMA	2	2	0	0	0	0
PITUITARY ADENOMA	63	50	47	63	55	61

^aGroups of 50 male Sprague-Dawley rats were exposed to either 50 ppm EDC or filtered air 7 hours/day, 5 days/week for 24 months. Certain groups received either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water throughout that exposure period.

* Significantly different from control ($p < 0.05$) by Fisher's exact probability test.

** Significantly different from control ($p < 0.001$) by Fisher's exact probability test.

TABLE 8

TUMOR CLASSIFICATION AND NUMBER FOR FEMALE RATS^a.

	CONTROL	EDC	DS	ET	EDC/DS	EDC/ET
INTRAHEPATIC BILE DUCT CHOLANGIOMA	0	0	0	0	34 ^{**}	0
MAMMARY ADENOCARCINOMA	8	10	4	13	25 [*]	4
MAMMARY FIBROADENOMA	30	42	11	27	40	32
HEPATOCELLULAR CARCINOMA	0	0	0	0	2	0
NEOPLASTIC NODULES	2	2	2	0	4	2
SUBCUTANEOUS FIBROMA	0	2	0	0	2	0
PITUITARY ADENOMA	74	71	81	67	58	83

^aGroups of 50 female Sprague-Dawley rats were exposed to either 50 ppm EDC or filtered air 7 hours/day, 5 days/week for 24 months. Certain groups received either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water throughout that exposure period.

* Significantly different from control ($p < 0.05$) by Fisher's exact probability test.

** Significantly different from control ($p < 0.001$) by Fisher's exact probability test.

TABLE 9

BLOOD LEVELS OF 1,2-DICHLOROETHANE (EDC)^a FOR MALE AND
FEMALE RATS FOLLOWING 2 YEARS OF INHALATION EXPOSURE TO EDC.

Group ^b	Male Rats		Female Rats	
	0.25 hr ^c	2.25 hr	0.25 hr	2.25 hr
EDC	0.28 ± 0.13	0.22 ± 0.08	0.26 ± 0.05	0.28 ± 0.04
EDC/DS	1.46 ± 0.48*	1.20 ± 0.28*	1.54 ± 0.49*	1.08 ± 0.08*
EDC/ET	0.36 ± 0.19	0.38 ± 0.15	0.30 ± 0.07	0.35 ± 0.13

^aCalculated as ug EDC/mL blood (mean ± SE, n=5).
Control male and female rats showed no detectable EDC.

^bGroups of 50 male and 50 female Sprague-Dawley rats were
exposed to 50 ppm 1,2-dichloroethane (EDC) 7 hours/day,
5 day/week for 24 months. Certain groups recieved either
0.05% disulfiram (DS) in the diet or 5% ethanol (ET)
in the drinking water throughout the exposure period.

^cSampling time after termination of exposure.

*Significantly different from EDC or EDC/ET ($p < 0.001$).

Table 10

TOTAL 24-HOUR RECOVERY OF 14-C FROM MALE RATS^a

SAMPLE	CONTROL	24-MONTH-OLD RATS					4-MONTH OLD
		DS	ET	EDC	EDC/DS	EDC/ET	
URINE	46.6	35.3 [*]	45.6	42.5	27.6 [*]	51.1	49.7
EXPIRED EDC	30.5	40.9 [*]	29.9	27.3	57.6 [*]	17.7	35.5
EXPIRED CO ₂	0.5	>0.1	0.1	0.1	>0.1	0.2	0.1
FECES	1.8	1.0	2.6	0.9	0.9	1.9	5.2
TOTAL	79.4	77.2	78.2	70.8	86.1	70.9	92.4

^a Animals received 150 mg/kg bw doses of [14C]-1,2-Dichloroethane after male Sprague-Dawley rats were exposed to either 50 ppm EDC or filtered air 7 hours/day, 5 days/week for 24 months. Certain groups received either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water throughout that exposure period.

^b Percent of Dose (mean, n=3)

^{*} Significantly different from Control (p<0.05)

Table 11

TOTAL 24-HOUR RECOVERY OF 14-C FROM FEMALE RATS^a

SAMPLE	CONTROL	24-MONTH-OLD RATS				4-MONTH	
		DS	ET	EDC	EDC/DS	EDC/ET	OLD
URINE	55.0	36.4*	41.6	34.0	24.9*	55.1	51.5
EXPIRED EDC	28.0	55.3*	30.0	40.4	57.9*	17.7	39.6
EXPIRED CO ₂	0.7	0.1	0.2	0.1	<0.1	0.2	0.3
FECES	1.1	0.2	2.4	0.9	0.2	0.9	4.4
TOTAL	84.7	91.9	74.0	75.3	82.9	73.9	97.1

^aAnimals received 150 mg/kg bw doses of [14C]-1,2-Dichloroethane after female Sprague-Dawley rats were exposed to either 50 ppm EDC or filtered air 7 hours/day, 5 days/week for 24 months. Certain groups received either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water throughout that exposure period.

^bPercent of Dose (mean, n=3)

*Significantly different from Control ($p < 0.05$)

Table 12

URINARY METABOLITES OF 1,2-DICHLOROETHANE IN MALE RATS^a

COMPOUND ^b	CONTROL	DS	ET	EDC	EDC/DS	EDC/ET
FRACTION I (9 min)	1.7 ^c	2.3	2.3	2.3	1.7	3.7
THIODIGLYCOLIC ACID SULFOXIDE (11 min)	26.7	32.7	28.7	28.7	28.0	31.3
FRACTION III (14 min)	5.0	6.3	4.7	7.0	5.0	7.3
THIODIGLYCOLIC ACID (16.5 min)	65.3	57.0	63.0	60.0	60.7	54.3
CHLOROACETIC ACID (19 min)	0.7	1.7	0.3	1.7	4.0	2.7

^aAnimals received 150 mg/kg bw doses of [14C]-1,2-Dichloroethane after male Sprague-Dawley rats were exposed to either 50 ppm EDC or filtered air 7 hours/day, 5 days/week for 24 months. Certain groups received either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water throughout that exposure period.

^bhplc analysis was performed using a Waters chromatograph equipped with a Model 6000A solvent pump and using a 30-cm-long X 4-mm-i.d. stainless steel column packed Bio-Rad Aminex HPX-87H ion exchange resin. Urinary metabolites were detected using a Flow-one radioactivity monitor.

^cPercent of urinary radioactivity (mean, n=3)

Table 13

URINARY METABOLITES OF 1,2-DICHLOROETHANE IN FEMALE RATS^a

COMPOUND ^b	CONTROL	DS	ET	EDC	EDC/DS	EDC/ET
FRACTION I (9 min)	2.0 ^c	1.5	2.0	3.0	1.7	1.7
THIODIGLYCOLIC ACID SULFOXIDE (11 min)	23.7	24.0	22.3	20.0	18.7	28.7
FRACTION III (14 min)	4.7	5.0	6.0	5.3	4.3	7.0
THIODIGLYCOLIC ACID (16.5 min)	68.3	65.5	67.3	69.3	71.0	60.7
CHLOROACETIC ACID (19 min)	1.0	3.5	1.3	1.7	4.3	1.3

^a Animals received 150 mg/kg bw doses of [14C]-1,2-Dichloroethane after female Sprague-Dawley rats were exposed to either 50 ppm EDC or filtered air 7 hours/day, 5 days/week for 24 months. Certain groups received either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water throughout that exposure period.

^b hplc analysis was performed using a Waters chromatograph equipped with a Model 6000A solvent pump and using a 30-cm-long X 4-mm-i.d. stainless steel column packed Bio-Rad Aminex HPX-87H ion exchange resin. Urinary metabolites were detected using a Flow-one radioactivity monitor.

^c Percent of urinary radioactivity (mean, n=3)

Table 14

LIVER DNA BINDING AFTER ORAL ^{14}C -EDC^a

DOSE GROUP	MICROMOLES OF EDC/MOLE DNA ^b		
	MALE	FEMALE	COMBINED
CONTROL	43.5 $\pm$ 1.2	36.4 $\pm$ 6.6	40.0 $\pm$ 3.4
DS	41.6 $\pm$ 8.3	29.0 $\pm$ 2.0	35.3 $\pm$ 4.8
ET	37.5 $\pm$ 3.0	26.9 ^c	33.2 $\pm$ 3.8
EDC	18.9 ^c	35.0 $\pm$ 3.9	28.6 $\pm$ 4.5
EDC/DS	35.6 $\pm$ 4.5	22.5 $\pm$ 4.6	29.0 $\pm$ 4.1
EDC/ET	53.3 $\pm$ 25.2	23.1 $\pm$ 7.1	38.2 $\pm$ 13.5

^a Animals received 150 mg/kg bw doses of [^{14}C]-1,2-Dichloroethane after female Sprague-Dawley rats were exposed to either 50 ppm EDC or filtered air 7 hours/day, 5 days/week for 24 months. Certain groups received either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water throughout that exposure period.

^b DNA was isolated and binding determined by scintillation counting (mean $\pm$ S.E., n=3).

^c Average of 2 rats.

Figure 1

Growth curves for male rats during the 24-month study. All groups consisted of 50 rats at the initiation of the study.

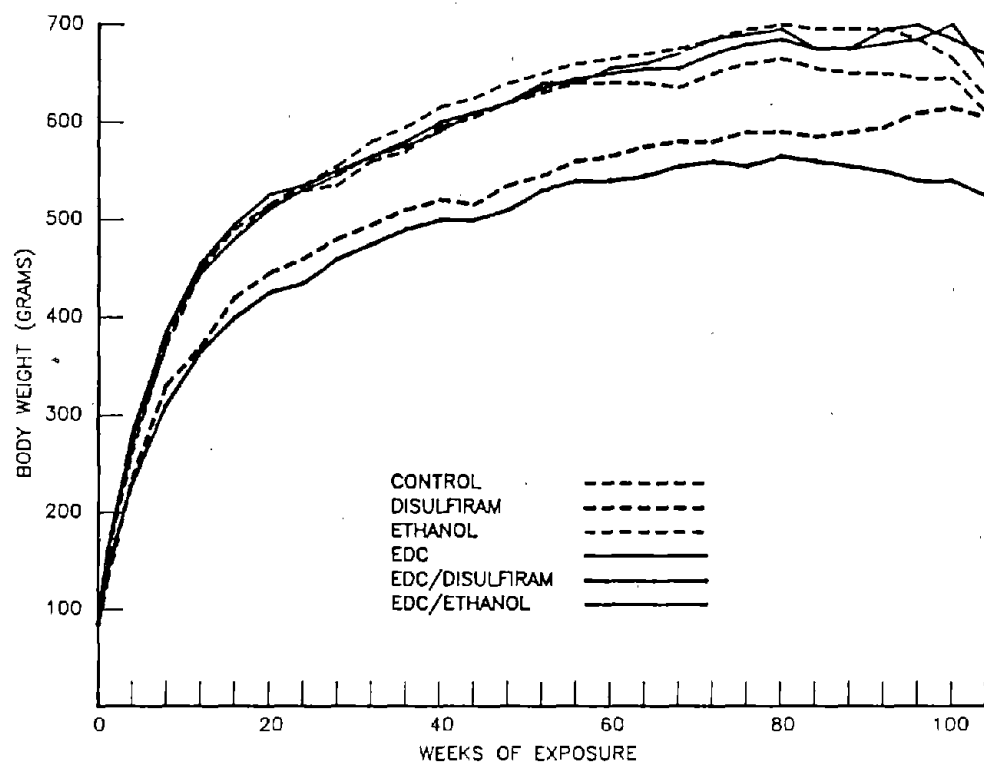


Figure 2

Growth curves for female rats during the 24-month study. All groups consisted of 50 rats at the initiation of the study.

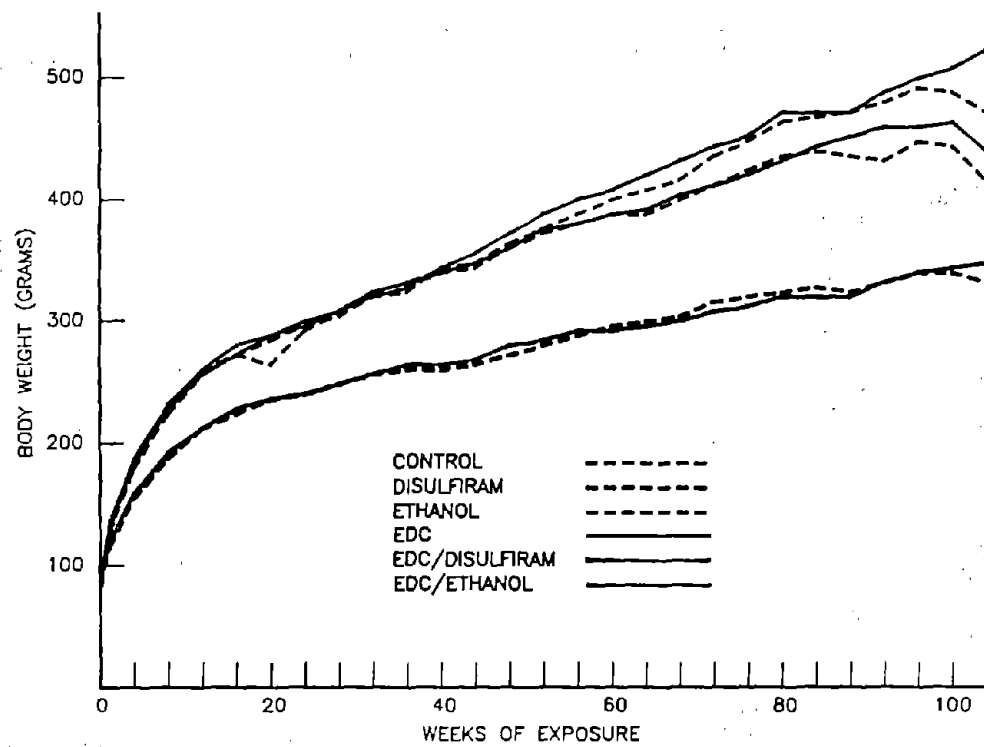


Figure 3

Cumulative mortality in male rats inhaling 50 ppm of 1,2-dichloroethane (EDC) with or without either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water.

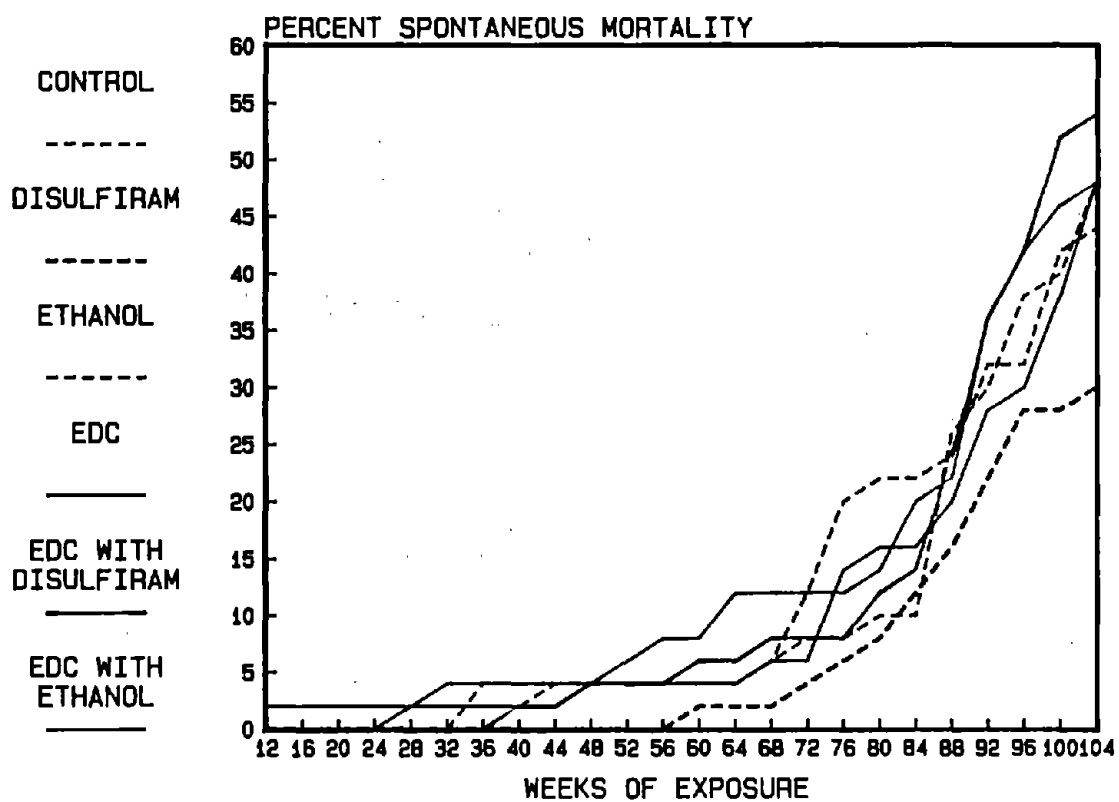


Figure 4

Cumulative mortality in female rats inhaling 50 ppm of 1,2-dichloroethane (EDC) with or without either 0.05% disulfiram (DS) in the diet or 5% ethanol (ET) in the drinking water.

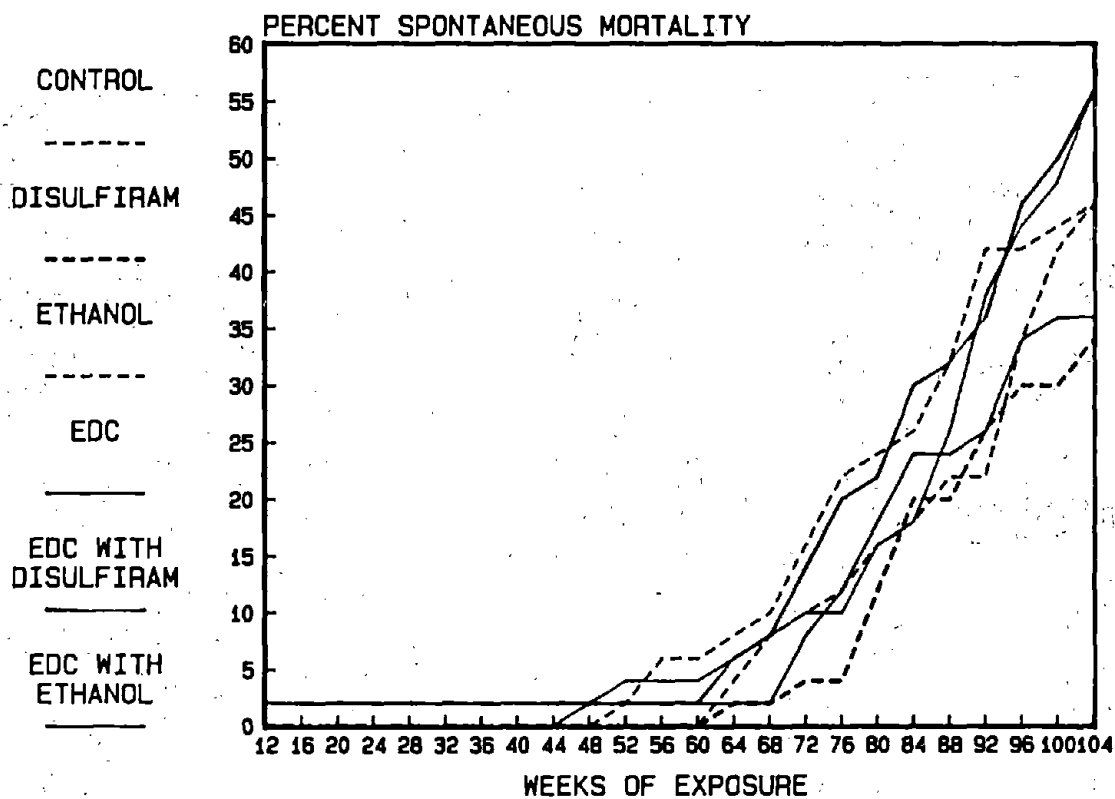


Figure 5

Liver cholangioma from rat exposed to 1,2-dichloroethane at 50 ppm (7 hours/day, 5 days/week for 24 months) in the air and 0.05% disulfiram in the diet. Note proliferation of bile duct epithelial cells, flattened or low cuboidal epithelium lining and noninvasive fine connective tissue border. Hematoxylin and eosin stain was used and magnification of X100 is shown.

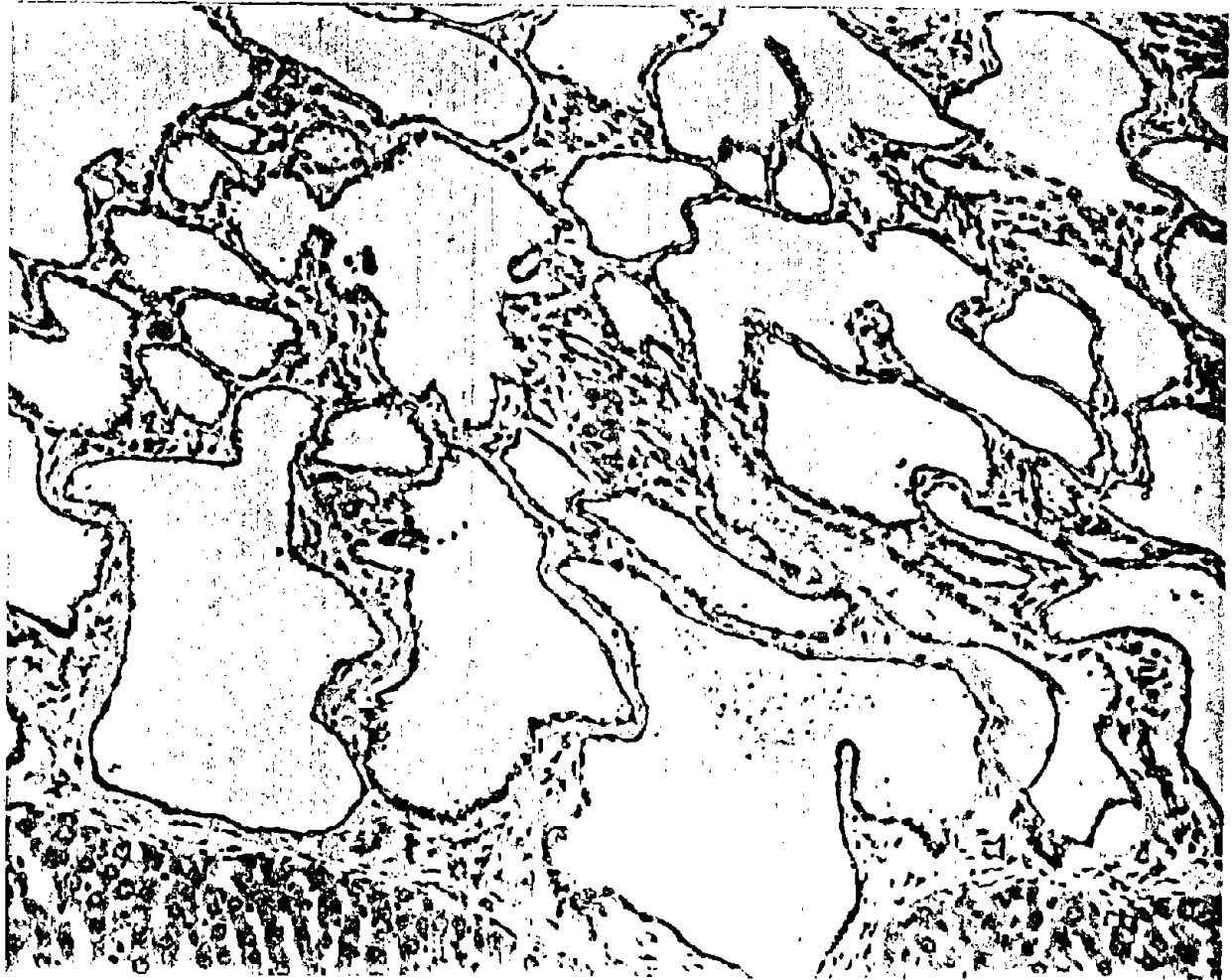


Figure 6

Gas chromatogram of headspaces drawn from rat blood spiked with EDC. A 1,1,2-trichloroethane internal standard is used and the headspace analysis was performed using a Varian Model 3700 gas chromatograph equipped with a 6.0-ft-long X 4-mm-i.d. glass column packed with 20% SP-2100/0.1% Carbowax on 100/120-mesh Supelcoport (Supelco, Inc.). The oven temperature was maintained at 65°C and a nitrogen carrier gas flow rate was 70 cm³/min and peaks were detected using a electron capture detector

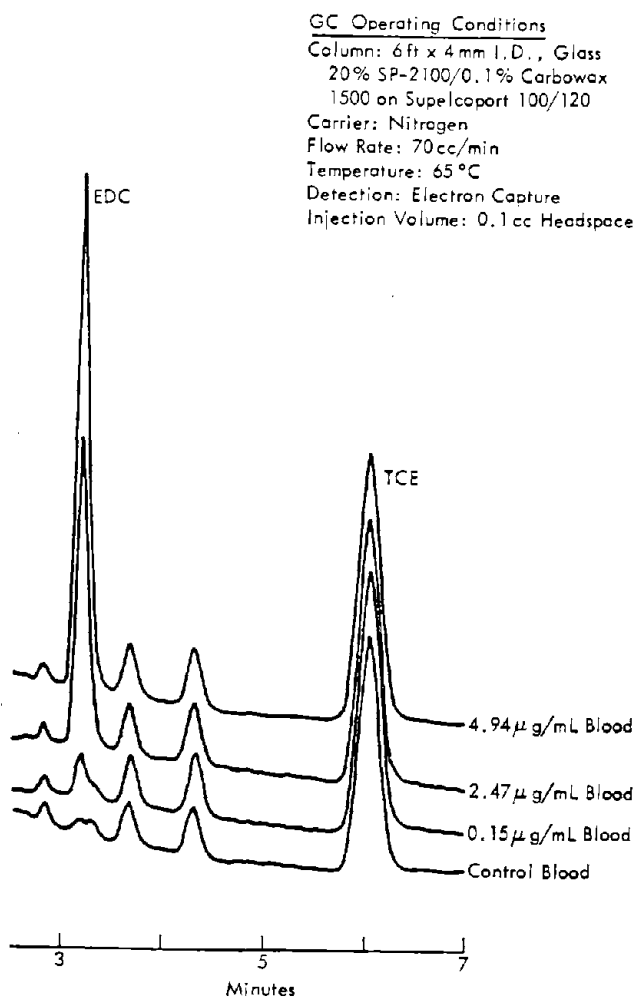


Figure 7

Cumulative excretion of radioactivity from rats dosed with ^{14}C -EDC at 150 mg/kg bw following chronic inhalation exposure to filtered air and untreated food and water.

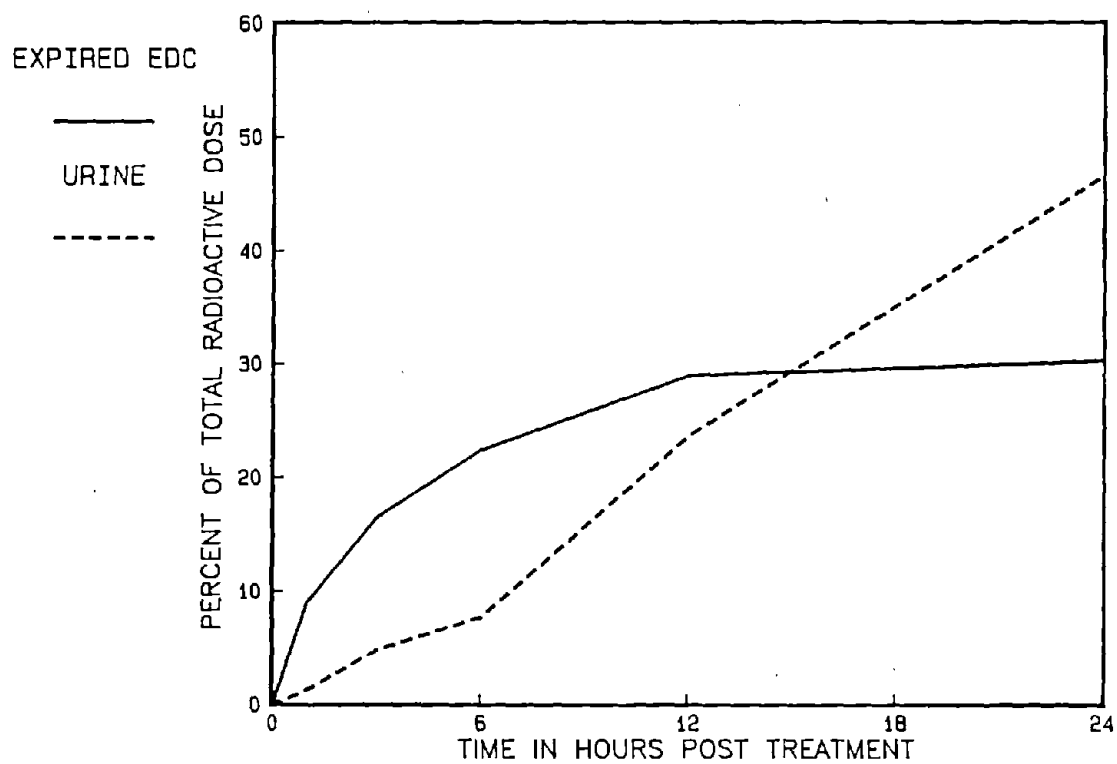


Figure 8

Cumulative excretion of radioactivity from rats dosed with ^{14}C -EDC at 150 mg/kg bw following chronic inhalation exposure to 50 ppm of EDC in the air and 0.005% DS in the diet.

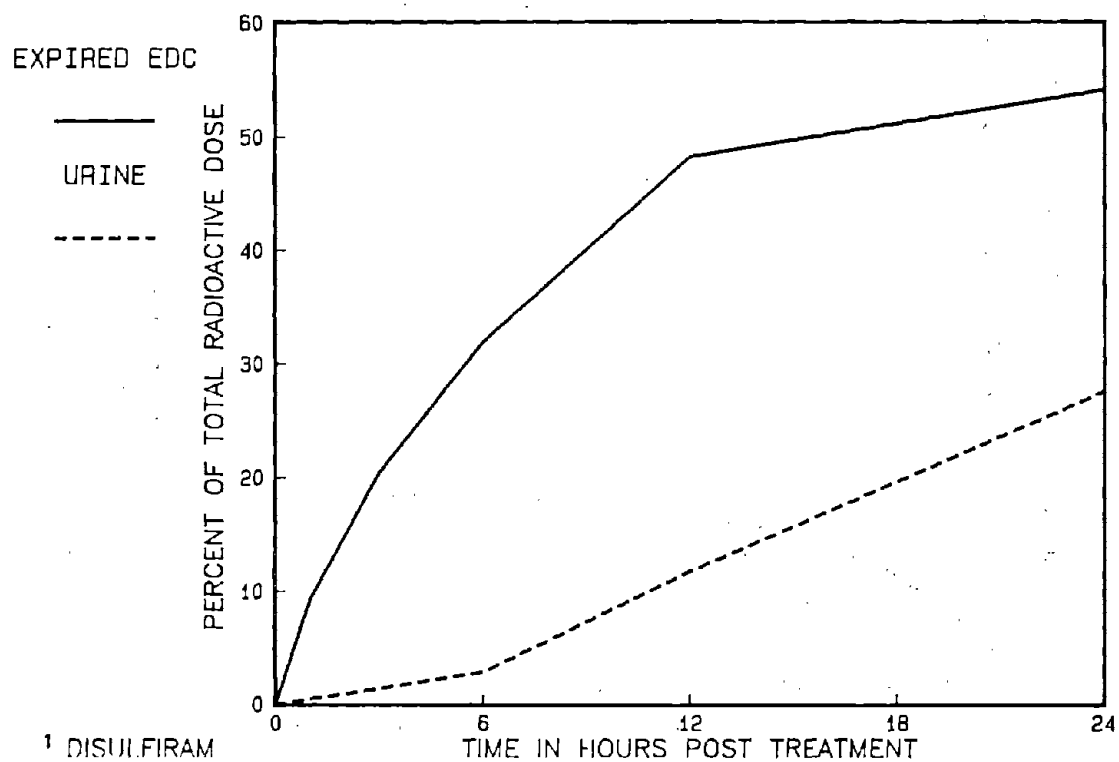


Figure 9

Cumulative excretion of radioactivity from rats dosed with ^{14}C -EDC at 150 mg/kg bw following chronic inhalation exposure to 50 ppm of EDC in the air and 5% ET in the drinking water.

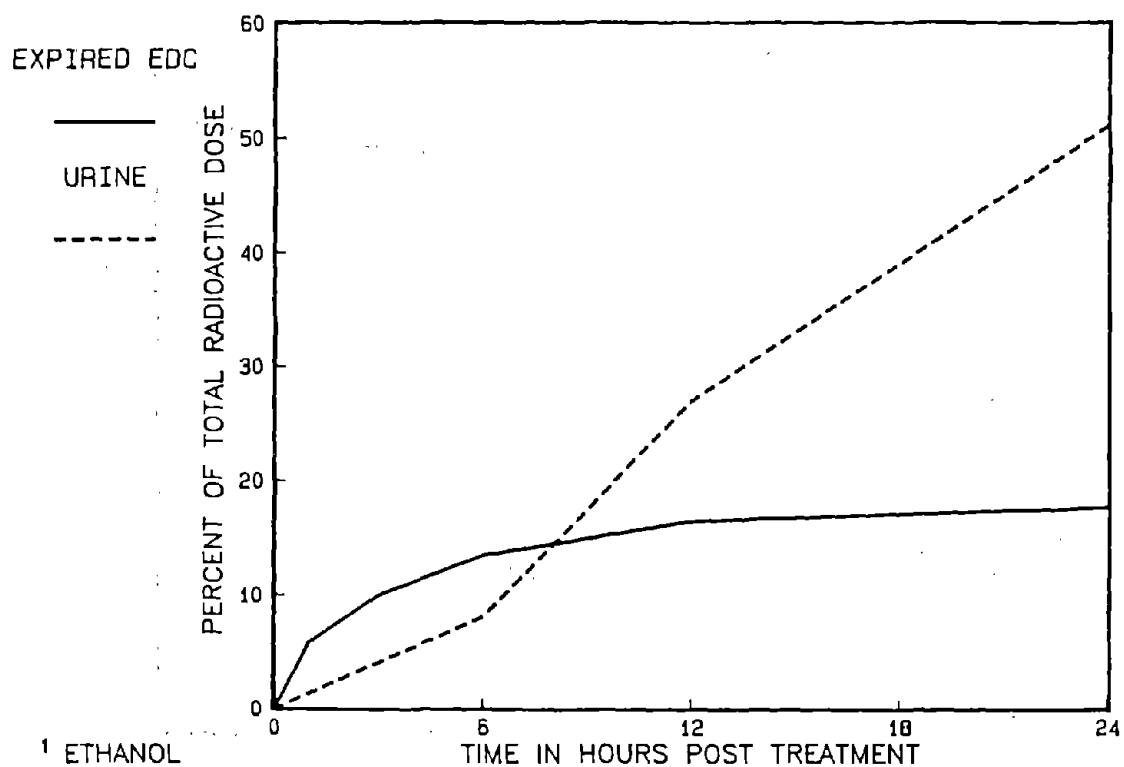


Figure 10

Radiographic profile of urine obtained from rats dosed with ^{14}C -EDC at 150 mg/kg bw following chronic inhalation exposure to 50 ppm of EDC. The hplc analysis was performed using a Waters chromatograph equipped with a Model 6000A solvent pump and using a 30-cm-long X 4-mm-i.d. stainless steel column packed Bio-Rad Aminex HPX-87H ion exchange resin. Metabolites were chromatographed using a mobile phase of 0.005 N H_2SO_4 at a flow rate of 0.5 mL/min and detected using a Radiomatic Flo-One radioactivity monitor.

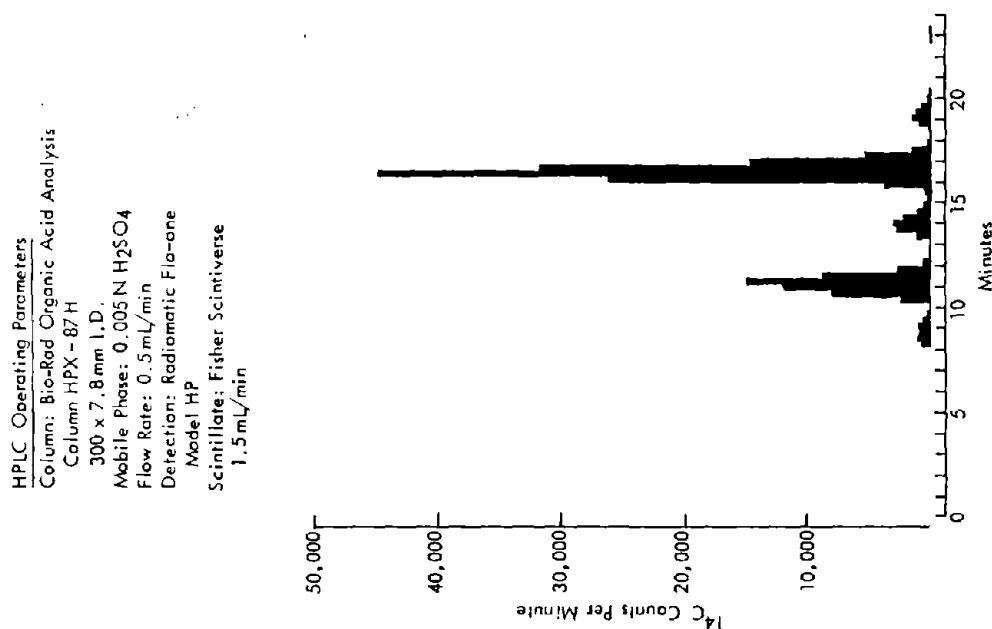


Figure 11

Proposed metabolic pathway for microsomal metabolism of EDC (a).

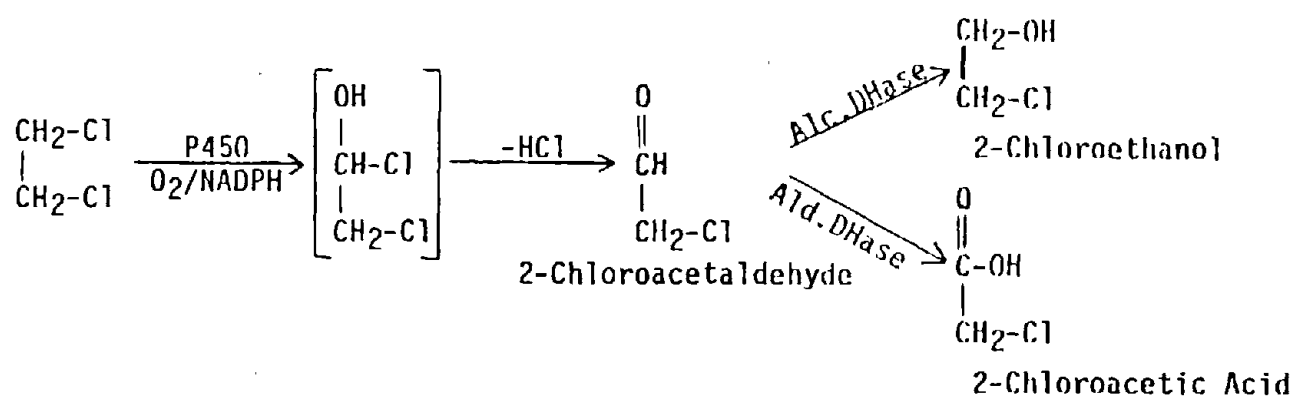


Figure 12

Proposed metabolic pathway for microsomal metabolism of EDC (b).

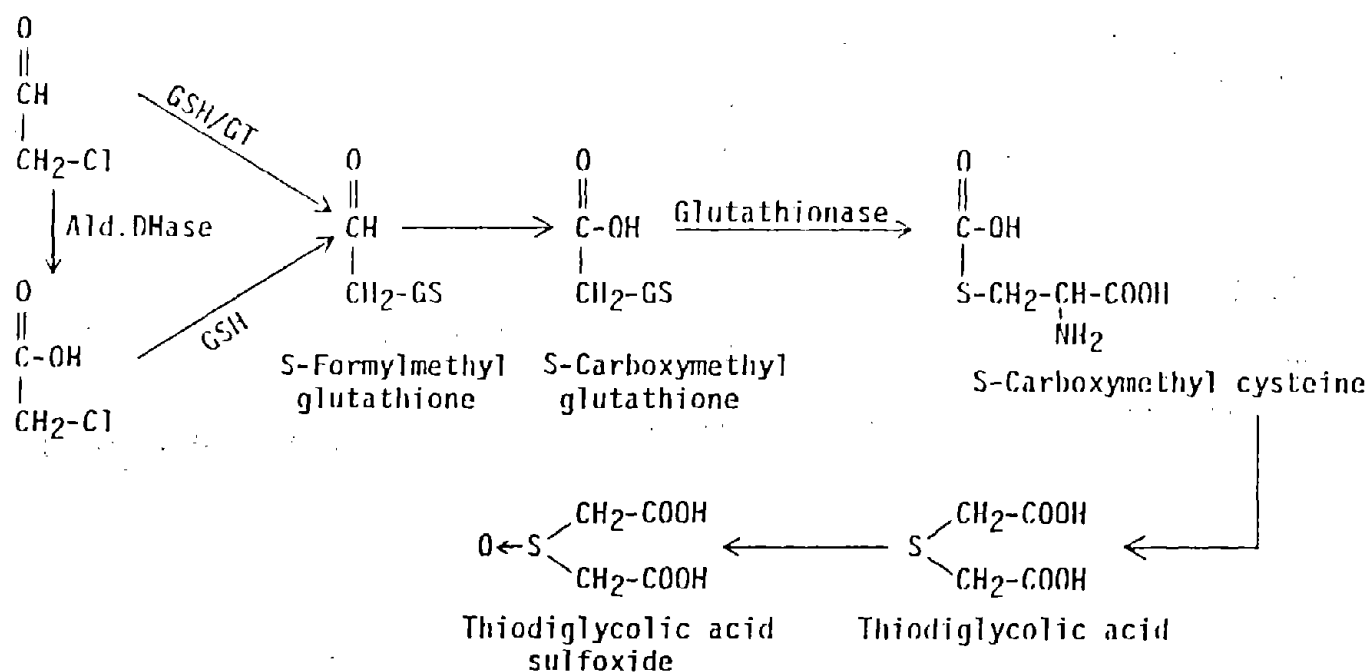
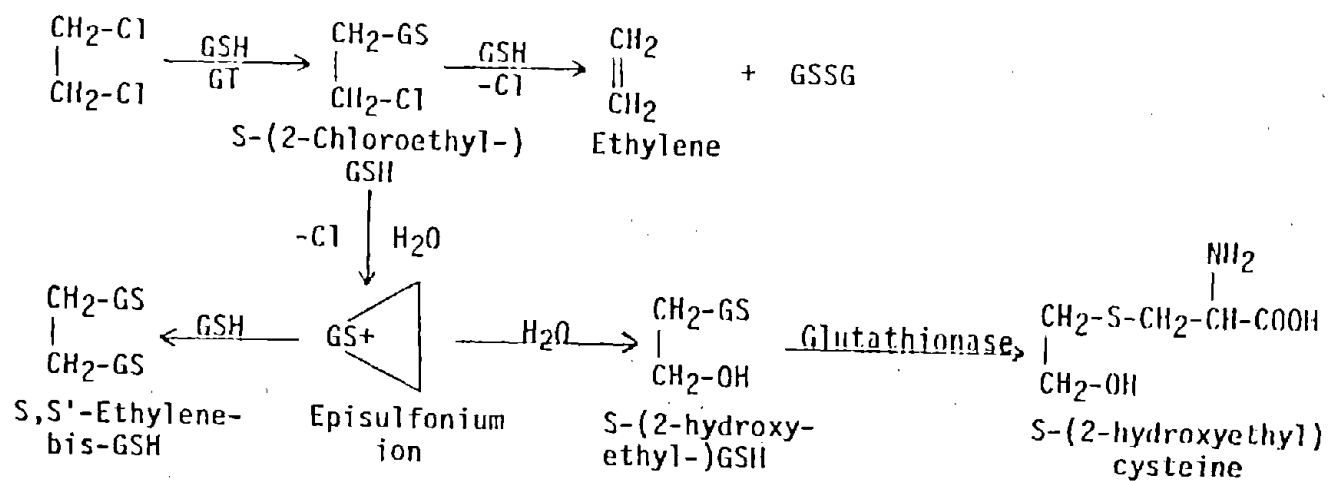


Figure 13

Proposed metabolic pathway for cytosolic metabolism of EDC.



FOOTNOTES

¹Presented in part at the 25th Annual meeting of the Society of Toxicology, March 3-7, 1986, New Orleans, LA.

²Mention of company or product names is not to be considered an endorsement by the National Institute for Occupational Safety and Health.

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QUESTIONS AND DISCUSSION

DR. BRESNICK: The paper is open for discussion.

There are two questions I'd like to ask. At the recent FASEB meeting, a fair amount of nutrition and cancer research was presented. One of the conclusions was that in diet studies, it's very, very important to control total calories.

Now I note that in your series, particularly with ethanol, the food consumption was depressed but water, and presumably ethanol consumption, was enhanced. So I wonder how the total caloric intake of those animals sort of balanced out?

MR. CHEEVER: We did not examine or try to adjust for the caloric intake. We assumed that the reason they consumed less was due to the increased caloric intake from the ethanol consumption. They did gain slightly more weight throughout the study, but it was not significantly greater.

DR. NEAL: Bob Neal, Chemical Industry Institute of Toxicology. One possible explanation for that higher blood level and, indeed, higher tissue levels of dichloroethane over a sustained period of time may have been because of the inhibition of the cytochrome P-450 enzymes in various tissues, mainly in the liver.

I wonder--did you, in fact, look in these animals under these conditions to see if, in fact, the P-450 mono-oxygenase system was inhibited under the conditions of feeding of the disulfiram?

MR. CHEEVER: No, we did not look at the tissue P-450 levels for these animals. However, Lang and his coworkers in 1976 indicated that disulfiram diminished the inductive effect of phenobarbital on cytochrome P-450.

DR. FEINMAN: Susan Feinman, CPSC. Are there any human studies--I'm not familiar with this--that indicate this interaction in workers who are taking Antabuse or who are exposed to these compounds through being alcoholics or through being in the rubber industry?

MR. CHEEVER: I'm not aware of any epidemiological studies on disulfiram.

DR. BRESNICK: Another question I have relates to the DNA binding studies. If I understand those studies correctly, the DNA isolated samples were counted and the specific activity determined. Is that right?

MR. CHEEVER: Yes.

DR. BRESNICK: What contribution--after all, you do get a fair amount of CO₂ generating from EDC--does the labeled CO₂ make to purine ring structure? I mean, I'm trying to see how much of the CO₂ actually wound up in the ring, and how much of it was truly adducted EDC or EDC metabolite?

MR. CHEEVER: This was only a six-hour binding period, and the animals were sacrificed at the end of that time, so we did not look for any N-etheno-cytidine adducts formed by reaction with chloroacetaldehyde.

DR. BRESNICK: No, I wasn't thinking so much of that. I was actually thinking of the CO_2 forming part of the one carbon pool, then going into the C8 position and C2 position of the purine, and then contributing, for example, metabolic CO_2 to the ring structure.

It has nothing to do with adducts. It really has to do with metabolism.

MR. CHEEVER: Well, by measuring it at the end of the study, it's possible that certain sites could have been already taken by previous EDC exposure. We did not examine that. But, as I say, the radiochemical EDC binding study that we conducted was a six-hour binding period.

DR. BRESNICK: George?

DR. MILO: George Milo, Ohio State University. I take it that this was total cellular DNA, this was not genomic DNA? You didn't separate out the mitochondrial DNA to show that relationship.

MR. CHEEVER: The DNA was isolated in accordance with the method of Joyce and Daniel of 1982.

DR. MILO: So it's total cellular DNA?

MR. CHEEVER: Right.

DR. BRESNICK: Time for a question or two more.

(No response)

Speculation. The last few slides went over pretty fast. At least with dibromoethane, Guengerich and others have shown that you can get adduct formation to the DNA actually after glutathione adduction. So the adduct you find is a glutathione-ethane DNA adduct.

Is it possible, in your disulfiram experiments, that you actually obtain a disulfiram-ethane DNA adduct?

MR. CHEEVER: I would hesitate to speculate on that, but I have been more or less convinced that this is more related to the glutathione adduct formation than the chloroacetaldehyde reaction that had been originally proposed.

I think that there's certainly a lot more work on this mechanism that needs to be done.

DR. NEAL: That was an interesting speculation on your part. Disulfiram is broken down by diethyldithio carbamate.

DR. BRESNICK: Right.

DR. NEAL: And so you would have a sulfhydryl-containing compound with considerable nucleophilicity, which would have some propensity to form a reactive product with the dichloroethane as would glutathione, although maybe not quite as active.

DR. BRESNICK: That's why I think it's very important to determine how much metabolic CO_2 is being incorporated, and that what you're measuring is not really metabolic CO_2 as opposed to adduct.

MR. CHEEVER: I thank you very much.

DR. BRESNICK: Thank you, Mr. Cheever.

**FRACTIONATION AND ANALYSIS OF
ASPHALT FUMES FOR CARCINOGENICITY TESTING**

**Presented by Dr. Barry R. Belinky
National Institute for Occupational Safety and Health**

FRACTIONATION AND ANALYSIS OF ASPHALT FUMES FOR CARCINOGENICITY TESTING

Belinky, B.R., Cooper, C.V., and Niemeier, R.W.; US Dept HHS, PHS, CDC, National Institute for Occupational Safety & Health; 4676 Columbia Pkwy; Cincinnati, Ohio 45226

Thank you, Dr. Bresnick, ladies and gentlemen.

The purpose of this report is to describe the current status of studies being undertaken in conjunction with an NCI/NIOSH interagency agreement to assess the carcinogenicity and the promoting activity of asphalt fumes.

Methods development and chemical analysis were done at the NIOSH research facility in Cincinnati. Liquid chromatographic fractionation and the animal studies have been and are being done under contract at Arthur D. Little, Inc. (ADL), in Cambridge, Massachusetts.

This interagency agreement is actually a follow-up to an earlier joint effort of NCI and NIOSH which evaluated the skin carcinogenicity of condensed roofing asphalt fumes and coal tar pitch (CTP) fumes. The importance of these studies is evident, first from the fact that over 150,000 workers are involved in the roofing industry and use large amounts of both of these materials, and secondly, that the cancer-related deaths for these workers are significantly in excess of the norm -- especially for skin cancer. Data for the ratios of observed to expected mortality rates are shown in Table I.

The objective of the earlier study was to assess the carcinogenic potential of various roofing fumes by means of a skin painting experiment. Two different grades of coal tar pitch, a Type I (regular coal tar pitch), and a Type III (no-burn coal tar pitch), and two different grades of asphalt, Type I (dead-level asphalt), and Type III (steep asphalt) were evaluated.

An evaluation was made of carcinogenic activity of fumes generated at two different temperatures (232 °C and 316 °C) to simulate the conditions to which the asphalt and CTP are exposed in the kettles on the roofs. The experiment addressed activity both in the presence and absence of simulated sunlight.

The solutions were applied cutaneously to CD-1 and C3HeJ mice. The results indicated that the C3HeJ mice were more susceptible to tumorigenic effects than the CD-1 mice, that carcinogenicity increased with increasing temperature, and that sunlight inhibited the occurrence of tumors. The most important information gleaned from that study, however, is that the asphalt fumes produced tumors in comparable quantity to the CTP fumes. This is somewhat in conflict with earlier work which indicated that asphalt itself, is not particularly tumorigenic. In the CTP, we found that benzo(a)pyrene (BaP) content could account for about 75% of the activity of the fumes. But the BaP content of asphalt is essentially negligible, and one cannot account for the carcinogenicity of the asphalt in terms of its specific BaP concentration or content of polynuclear aromatics (PNA) in general.

The natural continuation of that experiment was to try to separate the asphalt fumes into several chemically distinct fractions which could then be evaluated with respect to their individual carcinogenic effects and the effects when applied in various combinations with one another. This would presumably result in the identification of the active fraction or fractions of asphalt fume. Following chemical characterization, we would then expect to be able to identify some unique compound or compounds which would permit the assessment of asphalt fume in the presence of CTP fume, since they are often used in conjunction with one another. This assessment in turn would be used as an aid to the development of recommendations which would lead, hopefully, to the reduction of carcinogenic risk among roofing workers.

In order to generate experimental results which could be compared with those of the original experiment, an attempt was made to produce fume which was similar to that used in the earlier study. A Type III asphalt was purchased from the same supplier and was identified as an air-blown Arabian crude with properties similar to those of the original material.

The generation system consisted of a heated 12-L reaction flask containing about 10 kg of asphalt. Filtered laboratory air was pulled by means of a vacuum pump over the heated asphalt mixture (which was maintained at 316 ± 5 °C) through a succession of traps. The first trap was an ice water bath, the next two were dry-ice/acetone traps, and the fourth trap was an impinger containing a one-to-one mixture of cyclohexane/acetone. Over the course of several weeks, six kilograms of fume were generated.

The fumes, after collection, were combined and transferred to a separatory funnel and the condensed water removed. The organic phase was flash evaporated, the residual solids weighed, and cyclohexane/acetone (1/1) added to give a 50% (w/v) solution. The solutions were stored in brown glass bottles for later fractionation.

Prior to the fractionation, the fume was filtered, flash evaporated, and reconstituted in a 50/50 mixture of hexane and methyl tert-butyl ether (MTBE). This solvent exchange was performed since the acetone present in the original solution would undergo a Schiff base reaction with the chromatographic column which was used for the fractionation. The reconstituted solution was then filtered successively through 10-micron and 0.5-micron filters. Solids content was determined gravimetrically, and any losses of insoluble material during the filtration steps were measured so that mass balance calculations could be made after the fractionation was complete.

The fractionation was accomplished by means of preparative high performance liquid chromatography (PHPLC). The chromatographic method was developed in a stepwise procedure. First, an analytical scale was used wherein several unique fractions were obtained -- each containing a number of distinct chemical classes. During this phase of the development, approximately a milligram of fume was injected on the column. Solvent flow rate was 2 mL/min. After the separation of fractions was optimized (as determined by gas chromatography/mass spectroscopy (GC/MS) and ultraviolet (UV) spectrophotometry), the chromatographic scale was increased in both injection volume and mass of injected sample. A semipreparative column was used as an intermediate step between the analytical column and the preparative

column. The semipreparative column was one inch in diameter; several hundred milligrams were injected at a time; and a 50 mL/min flow rate was used. The final scale-up to the preparative column was then made. Optimum throughput was achieved at an injection size of 12 grams of fume in 24 mL of solution on a two and a half inch column with a flow rate of 250 mL/min.

The PHPLC system used was a Waters Associates Autoprep 500A. An amino-propylsilane column with 30 to 75 micron packing was used for high loading capacity. A stepwise solvent gradient was used which consisted of six solvents: hexane, a 90/10 mixture of hexane and MTBE, MTBE (100%), methylene chloride, methanol, and finally a column regeneration mixture of methanol/water/triethylamine (3600/400/2). This last solution was used to remove any strongly adsorbed carbonyl containing compounds from the column. The triethylamine was eliminated during subsequent flash evaporation steps. Chemical analysis of the collected fractions showed no contamination with triethylamine. Solvent changes and fraction collection times and volumes were as shown in Table II. The total sample collection time of 40.5 minutes was followed by a reverse gradient to reequilibrate the column, giving a total run time of 49 minutes for fractionation of 12 grams of fume. Including the reequilibration solvent, each chromatographic run required somewhat under ten liters of solvent.

As many as six chromatographic runs were made in a day. For each day's output, all 'A' fractions were pooled, as well as all 'B' fractions, all 'C' fractions, all 'D' fractions, and all 'E' fractions.

Now, since the stated goal of this project was to determine the carcinogenic effects of the fractions individually, and in combination with one another, it was critical that the integrity of each of the fractions be maintained, and that cross-contamination of one fraction with another be minimized. Towards this end, several types of analytical protocols were instituted, including infrared (IR) spectra and gravimetric analysis on a daily basis, and GC/FID and HPLC on a quarterly basis, to verify fraction stability. In addition GC/MS, HPLC/UV/Fluorescence, elemental analysis, and nuclear magnetic resonance spectroscopy (NMR) are being used to qualitatively characterize the various fractions.

Gravimetric analyses on the pooled, flash evaporated fractions were performed on a daily basis. Table III shows the results for the five runs made on 12/15/85. A total of 74.3 g raw fume was injected; 70.4 g, or 95% was recovered from the column. The daily percentages of each fraction collected were then tabulated on a spreadsheet for all runs. Fractions with recoveries that fell outside of the mean $\pm$ two standard deviations were identified.

IR spectra were generated on a daily basis for all fractions as well. Despite the fact that each fraction was a crude mixture containing thousands of individual species, the IR spectra easily identified significant amounts of cross-contamination. Evaluation of the IR spectra in conjunction with the statistical analysis of the gravimetric data identified 12 contaminated fractions (out of a total of 385 fractions). These were recombined and rechromatographed.

The pooled fractions were analyzed by gas chromatography with flame ionization detection (GC/FID). The chromatograms yielded unique fingerprints, and such chromatography will be repeated on a quarterly basis to assess the stability of the stored fractions during the animal study.

The pooled fractions were also analyzed by GC/MS. The mass spectra were able to characterize the general classes of compounds present in each fraction, as well as to identify numerous individual compounds. The general composition of each fraction is shown in Table IV.

Both proton and carbon NMR spectra have been obtained for the pooled fractions. The proton spectra are especially useful in determining the presence of methyl, methylene, methylene bonded to oxygen or sulfur atoms, and aromatic hydrogen atoms. By comparing the integration ratios of the aromatic/aliphatic protons, it is hoped that a better understanding of the amount of aromatic substitution will be obtained. The ^{13}C spectra can, in addition, detect carbonyl peaks and can distinguish between substituted and unsubstituted aromatic carbons. The NMR studies are ongoing with the collaboration of Dr. Elwood Brooks of the University of Cincinnati.

The next phase of this project is the animal painting study, which is presently underway. A total of 42 animal groups are involved. The experimental design provides for an evaluation of raw fume, reconstituted fume, individual fractions, fractions in various combinations with one another, fractions painted in conjunction with BaP to study cocarcinogenic and promoting activities, and a suitable number of controls.

Finally, I would like to acknowledge several people who have contributed significantly to this effort. At ADL, Jeff Worthington and Kevin Beltis generated the raw fume and supervised the preparative liquid chromatography, and Joe Moncini was saddled with the job of flash evaporating over 2000 liters of solvent from the collected fractions. At NIOSH, Michelle Bolyard and Jamie Grubbs generated over 500 infrared spectra, Dr. Gangadhar Choudhary and Karen Williams performed the GC/FID chromatographic analyses, and Charles Cooper and Dr. Alan Lunsford performed the GC/MS analyses. At the University of Cincinnati, Dr. Elwood Brooks produced the NMR spectra. Thank you.

TABLE I
OBSERVED VS EXPECTED NUMBER OF DEATHS
FOR SELECTED SITES OF CANCER IN
UNITED SLATE, TILE, AND COMPOSITION
ROOFERS, DAMP, AND WATERPROOF WORKERS ¹
(20+ YEARS)

<u>Site of Cancer</u>	<u>Ratio Obs/Exp</u>	<u>Significance²</u>
Lung	1.59	p < 0.005
Buccal-Pharyngeal, Larynx, Esophagus	1.95	p < 0.005
Stomach	1.67	p < 0.05
Skin	4.00	p < 0.01
All sites	1.45	p < 0.005

¹ Hammond, E.C., Selikoff, I.J., Lawther, P.L., and Seidman, H., Ann. N.Y. Acad. Sci. 271, 116-124 (1976).

² Significance calculated by NIOSH personnel using Fisher's Exact Test.

Table II

SOLVENT GRADIENT AND FRACTION
COLLECTION TIMES AND VOLUMES
FOR PHPLC FRACTIONATION
OF ASPHALT FUMES

Time (min:sec)	Solvent	Fraction	Volume (mL)
0:00	Hexane		
1:45 - 3:00		A	313
3:00 - 4:30		B	375
3:45	Hexane/MTBE		
4:30 - 12:30		C	2000
10:30	MTBE		
12:30 - 25:30		D	2500
14:30	Methylene chloride		
18:30	Methanol		
20:30*	MeOH/H ₂ O/Et ₃ N		
25:30 - 40:30		E	1875
40:30 - 49:00	Reequilibrate		

* Solvent flow rate reduced to 100 mL/min between 20.5 minutes and 38 minutes.

Table III

GRAVIMETRIC ANALYSIS OF
ASPHALT FUME FRACTIONS

Generation Nos. 342-346
12/16/85

74.3 g injected (150 mL)

<u>Fraction</u>	<u>Wt. (g)</u>	<u>% of Amount Injected</u>
A	42.8	57.6
B	4.9	6.6
C	8.9	12.0
D	11.0	14.8
E	2.8	3.8

Table IV

COMPOSITION OF FRACTIONS BY CHEMICAL CLASS
AS IDENTIFIED BY GC/MS

<u>FRACTION</u>	<u>COMPOSITION</u>
A	C9 to C35 Alkanes Alkylated benzenes Alkylated naphthalenes
B	Alkylated benzothiophenes Alkylated dibenzothiophenes Alkylated benzonaphthothiophenes Alkylated phenanthrenes C6 to C26 Olefins
C	Alkylated phenylethanones C2 to C11 Alkylated dihydrofuranones
D	Alkylated phenols Alkylated ketones
E	C6 to C22 Alkylated ketones Alkylated naphthols and phenols

QUESTIONS AND DISCUSSION

DR. BRESNICK: Dr. Belinky's paper is open for discussion and questions.

You mentioned you were going to search for azo arenes. Do you have any feeling that they actually are there?

DR. BELINKY: We anticipated them being there because in the raw asphalt fume, we had about a half percent nitrogen. But at no time, in a fairly exhaustive GC mass spec analysis, could we identify one single azo arene.

I felt better when the elemental analysis came in yesterday and, indeed, we do have some nitrogens present. And I suspect that what's happening is that the azo arenes are very polar and/or very high molecular weight. And we just can't get them off of GC mass spec columns. So they're there and we'll get them.

DR. BRESNICK: What about the tumorigenicity? You haven't started the tumor experiments, have you?

DR. BELINKY: No, sir. Once we do the tumorigenicity experiments and have identified active fractions, what we then plan to do is followup with, perhaps, taking one of these fractions--I'm leaning towards fraction B as being the culprit.

(Laughter)

That isn't very scientific. But I suspect, because of the large number of alkylated species present and some of the unknown toxicity of the sulfur heterocycles, that that may be a very interesting fraction.

We would then take and fractionate that even further so that we reduce the complexity of that one fraction and hopefully identify a larger number of compounds.

I would like to add one more thing if there is time.

DR. BRESNICK: Sure.

DR. BELINKY: We did some GC--quantitative GCFID analysis for N-dodecane, which is a known carcinogen--a co-carcinogen--and found it to be present 1.6 percent in the raw fume and 3.4 percent in the fraction A.

We expect to use that as a marker compound in the industrial hygiene assessment of roofing asphalt in the field. It has a high degree of interest primarily because of its known co-carcinogenicity and because of its unique presence in the asphalt as opposed to the coal tar pitch.

DR. BRESNICK: You mentioned reducing the complexity of the experiments by reducing the complexity chemically. In fact, you may have increased the complexity as to what the experiments actually mean. People are not exposed to the individual compounds, they're exposed to mixtures.

Maybe a mixture experiment is going to be more important.

Last question for Dr. Belinky?

(No response)

I think that this is a good time to end this morning's conference.

Thank you very much.

I think we should give all the speakers a good round of applause.

(Applause)

SESSION II—EPIDEMIOLOGY

**Chairman: Dr. Carl M. Shy
School of Public Health
University of North Carolina**

Tuesday Afternoon, April 22, 1986

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100.

CHAIRMAN'S REMARKS/INTRODUCTION

**Dr. Carl M. Shy
School of Public Health
University of North Carolina**

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CHAIRMAN'S REMARKS/INTRODUCTION

Dr. Carl M. Shy
University of North Carolina

The afternoon session, as you know, is on epidemiology, an important discipline that yields important results. And I can best illustrate that with a very brief story about the four persons who were traveling in an airplane, the pilot, a boy scout, a priest, and an epidemiologist.

(Laughter)

The airplane all of a sudden stopped, the engine stopped, and the pilot tried a few things. And after awhile, he turned around to the people and said, "I just can't get this plane started. And, unfortunately, I only brought three parachutes for the four of us. And I have to go and report this to the FAA to let them know what's going on."

So he grabbed one of the parachutes and jumped out. And then the epidemiologist said, "Well, I have some extremely important research going on that's going to be a great benefit to mankind but I've got to finish my work. So I have to take one of the chutes."

So he jumped out.

And then the priest turned to the boy scout and said, "Well, my son, you know, I've lived my life quite fully. I think I've made my contribution to society. Why don't you take the last chute and I'll just go down with the airplane."

And the boy scout said to the priest, "That is all right, father, there are still two chutes left for the two of us. That brain guy jumped out with my knapsack."

(Laughter)

So for the speakers who are about to come on, put on your knapsacks and let's get going.

The first presentation is by Dr. Genevieve Matanoski, who will talk about the mortality of workers in the painting trades.

MORTALITY OF WORKERS IN THE PAINTING TRADES

**Presented by Dr. Genevieve Matanoski
Johns Hopkins University
School of Hygiene and Public Health**

MORTALITY OF WORKERS IN THE PAINTING TRADES

Dr. Genevieve Matanoski
Johns Hopkins University

DR. MATANOSKI: Thank you.

Today, I would like to begin by discussing the design of this study because I think it had some unique features, which were worked out initially by not only ourselves but also the members of the team from the National Institute for Occupational Safety and Health. The people from the National Institute for Occupational Safety and Health who helped in the original design of this study are Marie Haring Sweeney, Dennis Zaebst, and Betsy Eagen.

The study is unique because we did not initially decide to study the painting union group, which was eventually our group for study. First, we determined the populations which were exposed to paints throughout the entire United States.

We reviewed the populations based upon the volume of paint consumed, the danger of the paint substance used in the various industrial settings, the number of people who were exposed, and the types of conditions under which painters were exposed.

The initial stages of this study actually consisted of a walk-through of 50 industrial sites where painting, or paint products and materials, were used. This included the airplane manufacturing industry, the auto manufacturing industry, and various other sites, which were also part of this contract.

It was decided in the end to look at people in construction and maintenance painting, not necessarily because they applied the majority of the paints--they do not; most of the paint used in the United States is actually used in industrial settings, where there are usually controlled conditions and the number of painters is very small. Nor were construction and maintenance painters selected on the basis of the highest risk from a paint product; many of the products used in industrial settings are potentially more dangerous because of the materials used in the paint product itself. They were selected because there were more people in that particular trade and group of painting trades than any other group that we looked at. They also had, because of the characteristics of their workplace, less opportunity for control of exposure in their workplace. So from that standpoint, we eventually elected to study workers in the painting trades, rather than industrial painters who apply paint in more controlled situations.

The painters who belong to the Painting Union of the United States, of the International Brotherhood of Painters and Allied Trades, constitute perhaps 70 percent of the painters within the United States. And it's relatively hard to get an estimate of that because we don't know precisely how many people in the United States apply paint.

The membership of this particular organization is approximately 200,000 ongoing. That is, there are people who join and leave, but approximately 200,000 painters belong to this union at any one time.

In this group, 60 percent are painters and 40 percent are workers in other kinds of trades associated with construction or maintenance, such as glaziers and tile-layers of various kinds.

We elected to study only four states out of the total painting union for the simple reason that it was such an enormous population. We felt that four states would provide a sufficient population base to look at.

The union group that was studied included Missouri, New York, California, and Texas. These states were selected because the types of paints that are used are different due to varying characteristics of the geographic and climatic conditions. Depending on the geographic location, the south or the north or the west or the east coast, there are different types of materials within the paints.

There were some differences in the configuration of the union membership. The union did not have complete records until about 1975. Therefore, we restricted our population base to those individuals who worked at some time in the period 1975 through 1979, and who had been born prior to 1940. So they had to be at least 35 years old before they were considered eligible for this study. It was, therefore, what might be termed a census population in epidemiological terms. The study group was anyone who was working during the period 1975 through 1979. The member had to be a member for at least one year in the painting union, and we included only white males. We included only white males for the simple reason that 95 percent of the union, born before 1940, were white males, from what we could determine.

The study population was followed by the usual means, including a life insurance file of the union. Since the union did provide life insurance benefits for members who continued to pay union dues, we included all records from the life insurance files. The union also had death certificates for all individuals who had life insurance claims administered through the union.

We also followed this population through the Social Security Administration's various methods of tracing individuals, as well as through the National Death Index, the Veterans Administration, and the Health Care Financing Administration.

Unfortunately, a trade like this that works in the outside and very often has a very loose association with a contractor, we found, had very poor records of Social Security numbers. Therefore, the usual means of follow-up were not completely adequate to follow all of this population. That is why we looked at this population from various sources which did not require a Social Security number to identify deaths, namely, the Veterans Administration, the National Death Index, and the Health Care Financing Administration.

There is within this group a second piece of the study, which is a case-control study within a cohort. That is, from the total cohort of 55,000 painters, we studied a group of individuals who had lung cancer and who were members of locals in New York State.

We found those cases by a review of all the lung cancer cases reported in the New York State Cancer Registry for painters in our cohort of workers from that state. All cancers and all lung cancers from the New York State Cancer Registry were matched against all painters who worked in the New York unions.

Unfortunately, the union did not keep a record of all of the characteristics of the work of a painter. But they did keep a record of the local union's characteristics.

Mixed unions are predominantly painting unions; that is, they consist primarily of painters. In addition, there are glaziers, glassworkers, sign writers, tile- and carpet-layers, paint makers, wood finishers, warehouse workers, and other industrial workers, very few of whom actually joined the painting union. But the majority of all painting unions--57 percent--are mixed unions.

Although this period of follow-up was relatively short in duration, there were 57,000 individuals who had worked at some time between 1975 and 1979. It was a very large population base and it was older. Therefore, we had a fairly high death rate among this group. We had a total of 5,313 deaths in a five-year period in this group of 57,000 people. So we gained a tremendous amount of efficiency by limiting our population to this size group.

About 2 percent of our population was lost to follow-up, which was a relatively small amount, and about 12 percent of the workers left the union and had to be followed by outside sources.

The age distribution was still predominantly towards the younger age group, although there were some individuals who were relatively old. And the total population gained us, as you might expect, about 257,00 person-years in that five-year period.

Now let us look at the results of this cohort study, specifically the records of the 5,313 deaths that occurred. The total SMR for this population is only 88 percent, and this is not surprising even with the large number of deaths, as we were dealing primarily with active workers. This is an active worker population and not a long-term follow-up at all.

However, there are several sites which show some high SMRs, at least in comparison to the overall SMR. Those include stomach cancer, large intestinal cancer, liver cancer, lung cancer, bladder cancer, and kidney cancer.

Lung cancer became a focal point, as a case-control study within this cohort, prior to the time that we looked at this data. So our case-control study was already ongoing at the time that we saw this data.

If we look further, we can divide the unions into those who are members of mixed locals which would be predominantly a painting union, and unions which had predominantly other special groups within them.

One can make some gross comparisons between painters and other kinds of trades; not that the other kinds of trades do not have exposures. They do; they have solvents and other kinds of exposures. But they were not all the same exposures.

In looking at the painters, it appears that the painters, alone, have a slightly higher SMR, and that, again, the risks--that I pointed out before--appear to be high. And in this case, significantly high; all cancers are certainly higher than in the combined trades. Bladder cancer is somewhat higher, and lung cancer is higher than it was in the past. And among the painters there seems to be a higher risk of leukemia, compared to the other groups.

As for the other groups of painting trades, although there are risks in the same categories, they certainly are nowhere near as high as they were in the painters alone. And there is absolutely no risk of leukemia in the allied trades.

So leukemia looks like it may have been an important site, one for which we did not do a case-control study. As we were starting this study, we elected to pick the cancer we thought was going to be high and started immediately on the case-control study at the very beginning, rather than waiting until we got the preliminary data from our SMR. So it's a relatively short duration of study, and a relatively small population base of follow-up.

In one particular analysis, the SMRs are presented as an internal comparison; that is, the painting unions versus other specialty unions compared to the overall group in a logistic regression analysis. There were significant differences between the mixed unions, the painting unions, and the specialty unions. For example, in all causes of death, the painters have a higher risk.

Malignant neoplasms and cancer of the lung are significantly higher with a $p < .01$. Bladder cancer also showed up very high in the painter, and leukemia is significantly higher among painters, compared to other trades within that union.

So there appear to be some important risks within this group. And the population base in general is fairly well divided between the two groups.

To continue this study as part of the general study, we looked at a case-control study within a cohort; that is, the lung cancer risk. The reason that we did this was because unfortunately, looking at mixed versus specialty unions doesn't tell you very much about the actual exposures, and it certainly doesn't tell you anything about smoking habits. However, if we were going to look at lung cancer, we needed some data on smoking habits.

To do it on the total population of 55,000 individuals was impossible. So the optimum way to allow for greatest efficiency appeared to be to do a case-control study to identify the confounding variables that we wanted to look at. This thesis, in fact, comprised a great deal of the work that was done here. But the case-control study in particular was the thesis work of Dr. Heather Stockwell, who was our project coordinator throughout this entire study.

The case-control study consists of 124 lung cancer cases, which were identified in the painting unions from New York by matching our population from that state with all of the cancer registry data for the same period of time. That is, we matched our cancer registry--our population base--to the New York State Cancer Registry. We identified all deaths from cancer and all cases of cancer in this population.

I will talk today only about the lung cancer cases. The total number of lung cancer cases that matched with our New York population was 124. For this group, we attempted to select three non-cancer controls from the same population base. They were stratified by birth date and by the location of the union local of the case, and they were selected to be a member of the union who had not been identified as having cancer, either on the death certificate or on the registry data.

Now, unfortunately, as in most studies in which you're trying to do a follow-up of populations in a cohort, you find that it's not that easy to get answers to questionnaires; therefore, our follow-up population results were not as satisfactory, perhaps, as we would have liked them to be. But, they do represent effort, a great deal of effort--up to the point of actually sending out a detective. We didn't go that far. But we went almost that far. We sent out emergency telegrams, we sent out mailgrams, we sent out five mailings to them, we called them on the telephone; in fact, we called them on the telephone five times.

It was, to us, very important that we try to get as many of these cases and controls as we possibly could. So just short of sending out the detective, we did everything else. We even had the union contact them. Nothing seemed to be successful in this population. I'll tell you what our success rate was. First, we could only locate 16 percent. We missed 16 percent of our lung cancer cases and 18 percent of our non-cancer case controls. That means we found about 80 percent. That is, within this short time period, painters who were painting in the locals in New York had disappeared. Because the union didn't know where they were, no one could trace them in the time period directly after they had been working for the union. So, within five years, it was as if they had disappeared from the face of the earth. They were no longer union members and they were no longer working for the union, and there was no way that we could trace them.

Part of this, of course, was that we were tracing cancer cases, and there were many older men among the controls. The cancer cases, many of them, had died. And so tracing the next of kin to get a questionnaire to them was very difficult. Even tracing the men who might be in custodial care was very difficult.

We found that all of those who actually got a questionnaire, 66 percent of the lung cancer cases and 59 percent of the controls returned it. Although it's not completely hopeless, it certainly isn't as good as we would have liked it to be.

Now, unfortunately, 94 percent of the lung cancer questionnaires were completed by proxy. Only 70 percent were completed by proxy among the non-cancer controls, and we have a problem of differences between the two groups in that regard.

Other than the fact that the educational levels of the lung cancer group and the non-cancer controls were different, in every other regard these two population bases were very similar to one another; that is, in terms of marital status, in terms of race, and in terms of birth date, the two populations were not significantly different. But the lung cancer cases did have a lower educational level than the other group.

We had asked questions, either directly to the lung cancer cases or their proxy respondents and to the controls, regarding their usual occupation, what trade they belonged to when they were part of the IBPAT, and whether they had ever worked as a painter.

If they ended up being a floor-coverer, we asked were they originally a painter. When we did this, we found that for any one of the designations as the usual occupation, the IBPAT trade area as a painter, and ever worked as a painter, showed that painters had a higher risk of lung cancer than did controls--about two- to three-fold higher. These are all significant differences.

Then we looked at the use of spackling compound, which, of course, in the past had been heavily contaminated with asbestos. We treated that as a history item in the questionnaire. And if they had ever used spackling compound, the risk of lung cancer increased five times; that is, there was a five times higher risk among lung cancer cases who had used spackling compound compared to controls.

If we looked at whether they had used either a mask or a respirator we found a 1.57 odds ratio. We've reduced the odds ratio quite substantially.

If we looked at whether they ever used any other protective equipment, or had never used other protective equipment, we found no increased risk. That is, whether you used protective equipment or not seemed to make very little difference in the risk of lung cancer.

The biggest problem here appeared to be the spackling compound. If you look at what the usual occupation was, smoking, wearing a mask, asbestos exposure, drinking of beer--which I'll get to in a minute--and then a painter who wore no mask, we find that if we use an adjusted odds ratio, adjusted for all of the factors, and looked at each factor independently, painters do not seem to be as high a risk as they were originally.

If we look at smoking, of course, it has a five-fold excess risk, much as we would expect in a population with lung cancer.

If you use asbestos exposure, you find that the risk is about three times higher for those who had asbestos exposure. The other peculiarity here was that if you drank beer on a weekly basis and, even corrected for smoking, painting, use of a mask, and asbestos exposure, you find there is a very high risk.

If you look at painters who use no mask versus painters who used masks, you find that the risk is five times higher.

If you take apart the crude and adjusted odds ratio just for smoking, spackling, and beer weekly, you find that cigarette smoking increases the risk substantially, as we would expect, even corrected for spackling and beer. If you correct by expected dose or number of years worked with spackling, you find that that risk ratio is about four times higher, even corrected for smoking and beer drinking.

If you look at beer drinking corrected for the amount of smoking and the amount of spackling compound exposure, you find that beer drunk weekly increases the risk of lung cancer. Now, this sounded very unusual to start with, except that Dr. Pollock presented a paper in 1984 on Japanese in Hawaii also showing that alcohol, not beer but alcohol, increased the risk of lung cancer independently of smoking. So this may not be a fallacious finding.

The bottom line is that we have found among painters, compared to other allied trades, very high rates of lung cancer and several other cancers, including leukemia, bladder cancer, and overall cancer rates.

In addition, in the case-control study, it is clear that although painting carries some risk, when one corrects for the presence or absence of spackling exposure among painters, you seem to have all of the risks among painters being associated with the use of spackling compound rather than paints.

However, it is clear that we could not expect a very good history from a proxy on all paints that a person might have been exposed to in their lifetime in our efforts to get a total overall solvent exposure versus any other types of painting exposure in any individual. We may not have controlled for type of painting correctly or sufficiently to show a risk from solvent-based paint exposures versus non-solvent-based paint exposures.

And beer, which we were not really looking for in terms of this, seems also to increase the risk of lung cancer.

Thank you.

QUESTIONS AND DISCUSSION

DR. SHY: Questions for Dr. Matanoski?

DR. BRESNICK: Edward Bresnick, University of Nebraska Medical Center.

I noticed in the first two slides that the reverse effect seemed to be true with brain and CNS disorders. What do you make of that?

DR. MATANOSKI: I really have no idea what to make of that. They just didn't show up in our group.

DR. MANDULA: Is there any chance that the beer effect could be a passive smoking effect? For example, are they drinking beer in smoke-filled taverns?

DR. MATANOSKI: That's very possible. They certainly are drinking beer in smoked filled taverns. But we did not take direct information on passive smoking.

DR. MANDULA: Also, could your data be used to look at possible other effects among painters? A friend of mine is concerned with exposure to isocyanates among people in the painting trades, and I just wondered if the information you have could somehow be used to see if those exposures are associated with ill effects in your population.

DR. MATANOSKI: Well, the best way to go now is we've got an established population base from which we can now cut off and do case control studies and identify specific exposures. But the painting union doesn't have those exposure data. That's the trouble with studying a trade like this out in the free world. And we don't have any captured information on industrial hygiene, no product information. Nobody buys materials and keeps all the records of it.

So the only way that we could actually find any information about exposures was to ask the person. Now, it turns out that with lung cancer, it's unfortunate. They're mostly dead.

But with any other population, any other cancer site, it's very possible you would find a high enough portion of the population alive to be able to look at specific exposures that this group had.

In addition, there are certain population groups who have exposure to specific kinds of paints, and those could be sifted out of this population base.

DR. MANDULA: And looked at for morbidity?

DR. MATANOSKI: And looked at for morbidity, yes. That would probably be the best way.

DR. MANDULA: Okay, thank you.

DR. SHY: Time for one more.

DR. BAYARD: Steven Bayard, Environmental Protection Agency. On the spackling compound association, is it possible that spackling is associated with the indoor painters versus the outdoor painters?

DR. MATANOSKI: We couldn't separate out indoor and outdoor painting. Most of these men did both kinds during their lifetime. There are a few unions, which we could sort out if we worked with a larger population base, who do only outdoor painting. Otherwise, they usually did both kinds. So they've had both indoor and outdoor exposures.

DR. BAYARD: So your analysis didn't take into account any indoor versus outside exposure?

DR. MATANOSKI: No, it did not. That's it.

DR. BAYARD: Thank you.

DR. SHY: Thank you, Dr. Matanoski.

**MORTALITY OF CHROME LEATHER TANNERY
WORKERS AND CHEMICAL EXPOSURES IN TANNERIES**

**Presented by Dr. Frank B. Stern
National Institute for Occupational Safety and Health**

**MORTALITY OF CHROME LEATHER TANNERY WORKERS
AND CHEMICAL EXPOSURES IN TANNERIES**

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ABSTRACT

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Mortality of Chrome Leather Tannery Workers and Chemical Exposures in Tanneries

A retrospective mortality analysis was conducted in a cohort of 9365 individuals employed as of 1940 at one of two chrome leather tanneries in the United States (U.S.) and followed forward to the end of 1982. Vital status as of the closing date was determined for over 95% of the cohort. Potential hazardous workplace exposures varied with department and included nitrosamines, chromate pigments, benzidine-based direct dyestuffs, formaldehyde, leather dust and aromatic organic solvents. Mortality from all causes combined was lower than expected for each tannery, the standardized mortality ratio (SMR) being 81 and 93, respectively. Deaths from each site of cancer, including lung, was also lower than expected compared to that of either the population of the U.S. or of the local state rates. A significant excess of deaths was observed, however, due to accidental causes in one tannery and cirrhosis of the liver, suicide, and alcoholism in the other tannery. These excesses did not appear to be causally associated with occupational exposures. The findings of this study are consistent with the only other mortality investigation of leather tannery employees.

Key terms: cancer, occupational exposures, retrospective study.

MORTALITY OF CHROME LEATHER TANNERY WORKERS

INTRODUCTION

Employment in the leather and leather manufacturing products industry, Standard Industrial Classification (SIC) 31 (43), has been associated with cancer of the bladder, lung, larynx, buccal cavity and pharynx, kidney, nasal cavity, lymphoma, and cirrhosis of the liver (1, 2, 6, 9, 10, 12, 13, 17, 18, 19, 22, 23, 26). These reports have included tanners, shoemakers, repairers, and other unspecified "leather workers" in their analyses. This report focuses specifically on that subgroup of employees in SIC 31 engaged in the tanning and finishing of leather (SIC 311) and excludes those employees converting finished leather into products. Employees engaged in the chrome tanning and finishing of leather have potential for exposure to numerous known or suspected occupational carcinogens including hexavalent chromium salts, benzidine-based azo dyes, aromatic organic solvents, formaldehyde, and airborne leather dust (21, 23). N-nitrosodimethylamine (NDMA), a liver and kidney animal carcinogen and one of the 13 occupational carcinogens regulated by the Occupational Safety and Health Administration (11), has also been detected in the work environment of various tanneries (13, 32).

Tanning is a process by which hides and skins are converted into finished leather by the removal of the epidermis and subcutaneous layer of the hide and the subsequent stabilization of the middle portion (derma). Two main processes for leather tanning are used in the United States (U.S.). Vegetable tanning extracts are utilized to produce firmer, thicker leathers for bags, cases, strap leathers and for shoe soles and heels; trivalent basic

chromic sulfate is used to produce softer, thinner leathers for personal leather goods such as handbags, gloves, garments, upholstery and for upper parts of shoes. Approximately 85 percent of all leather produced in the United States is chrome tanned (personal communication from Dr. Robert Lollar, Technical Director of the Leather Industries of America); therefore, employees involved in producing chromed leather were the focus of our investigation.

A description of the processes (21) and potential hazardous chemical exposures (14, 15) for the two chrome leather tanneries that were studied are presented in table 1. The processes in the two tanneries have remained relatively the same since the end of the nineteenth century; however, some mechanization and chemical technology have been introduced to reduce the amount of manual operations. For example, rotating drums and hide processors, in which chemicals are often introduced through fixed pipes, have replaced the paddle vats for soaking, dehairing and tanning of the hides. Various chemical changes have included, among others, the use of dimethylamine sulfate (DMAS) as an accelerating agent in the dehairing process, chlorophenols as a disinfecting agent, and trivalent instead of hexavalent chromium salts as the tanning material. Some of these chemicals (e.g. DMAS and the chlorinated phenolic biocides), however, have since been replaced in the past decade in many tanneries in response to hazardous chemical questions. With new technologies, the increased importation of finished leather goods (mainly from Italy), and the greater use of man-made materials the number of workers employed in the tanning industry in the U.S. has declined from a peak of 33,000 in 1965 (29) to approximately 20,000 as of 1982 (38).

MATERIALS AND METHODS

The study cohort included all production workers employed between 1 January 1940 and 11 June 1979 at tannery A (N = 2,807) or between 1 January 1940 and 1 May 1980 at tannery B (N = 6,558). Cohort members were identified from personnel records which generally contained name, social security number, sex, race, date of birth, date of hire, date of separation, and specific work history information (i.e. department and job assignment and dates of each new work assignment). No information on country of birth (emigration) was available from employment records.

Vital status of each member of the cohort was determined as of 31 December 1982 by utilizing records of the Social Security Administration, Internal Revenue Service, and the National Death Index (NDI), (34) among others. For each cohort member identified as deceased, a death certificate was obtained from the appropriate state vital statistics office and the underlying and contributory causes of death were coded by a qualified nosologist, according to the revision of the International Classification of Diseases (ICD) in effect at the time of death. Deceased employees for whom no death certificate could be obtained were assumed dead as of the date given by the reporting agency; the cause of death was classified as unknown.

A modified life table analysis system (41) was utilized to accumulate person-years at risk (PYAR) for each study member beginning with the first day of employment at either tannery after 1 January 1940 until either 31 December

1982 or the date of death, whichever came earlier. The PYAR were classified by sex, race, 5-year age groups and calendar-time periods, employment periods and latency periods (time periods since first employment). The PYAR were then multiplied by the appropriate sex-, race-, age-, and cause-specific mortality rates of the U.S. population to yield the number of expected deaths. Expected mortality was similarly computed using the state death rates of Minnesota for tannery A and Wisconsin for tannery B in order to control for possible variations in mortality patterns between state and national rates due to population composition and geographical location. For site-specific cancers, state death rates for the years 1950-1979 were utilized; for other causes, state death rates for the years 1962-1979 were used (According to the unpublished data of March GM, Mortality and Population Data System, University of Pittsburgh).

Duration of employment was used in the analysis as a surrogate for cumulative exposures since past environmental monitoring data from the tanneries were not available. Categories for analysis by duration of employment (less than 1 year, 1-9 years, and equal to or greater than 10 years) were established by examining the distribution of the person-years at risk. For cancers, a minimum latency period of 15 years was selected since most occupationally related cancers usually occur after an appropriate induction-latency period (8). Observed and expected numbers of deaths were also stratified according to "ever having been employed" within certain departments of the tannery. The departments were grouped according to process description and chemical exposures as presented earlier in table 1. For most employees, potential for exposures depended upon the department (area) in which they worked; although, it was realized that overlapping of exposures between departments could occur.

A standardized mortality ratio (SMR) value for each cause of death was computed by multiplying the ratio of the observed to the expected number of deaths by 100. A two-sided confidence limit (95%) for each SMR value was then calculated assuming a Poisson distribution for the observed deaths (31). When the confidence interval did not include 100, the SMR value was considered to be statistically significant.

RESULTS

Cohort description: Through 31 December 1982, 95% of the 9,365 members in the cohort were successfully traced; 7,316 individuals (78%) were alive, 1,582 individuals (17%) were deceased and 467 individuals (5%) were lost to follow-up and considered alive for purposes of our study (table 2). Death certificates were obtained for 96.8% of all deaths. Of the 9,365 individuals in the cohort, 5,827 (75.7%) were male and 2,280 (24.3%) were female. Approximately 18% of the cohort was nonwhite.

The mean age of the cohort at hire was 27 years. More than one half of the workers (52%) were hired prior to 1960 thus the majority of the workforce had a minimum of 22 years between date of hire and 1982 (the ending date of the study). For tannery A, 21% of the workers were employed for 10 years or longer; for tannery B, only 8% were employed for 10 years or longer. These percentages indicate a fairly substantial employment turnover rate at both tanneries, somewhat typical of the leather tanning industry in the U.S.

Total deaths: Although mortality from all sites of cancer was of concern based on knowledge from previous studies, all major causes of death were examined (table 3). The 1,582 deaths observed among workers from both tanneries combined were 89 percent of the number expected based upon U.S. age-adjusted mortality rates. Statistically significant deficits of deaths from all causes combined were observed for both tannery A (SMR = 81, CI = 74-88) and tannery B (SMR = 93, CI = 88-100). This reduction in mortality for each tannery was due to decreased risks of death from most causes. Significant elevations in mortality, however, were found for accidents in tannery A, and alcoholism, cirrhosis of the liver and suicide in tannery B.

Cancers: The number of deaths from all cancers combined was less than expected for both tannery A and tannery B whether U.S. rates or state death rates were used for comparison (table 4). [Discussion of the cancer results is limited to findings based on state, rather than U.S., death rates since regional rates were thought to control better for possible variations in mortality patterns] The "All Cancers" deficit was largely explained by a reduced number of deaths from cancers of the trachea, bronchus and lung. Analysis by duration of exposure for these subsites of cancer showed an absence of any positive correlation with excess mortality risk when examined after a time period of 15 years from first exposure; the SMR for longer than 10 years employment being the lowest (table 5). Deficits in mortality from cancers of the trachea, bronchus, and lung were also observed within each department of the tanneries (table 6).

All other primary sites of cancer mortality were also below expectation (table 4). Specifically, for cancers of the buccal cavity and pharynx, eight deaths were observed for both tanneries combined (with 8.5 expected), all resulted after a 15 year latency period (table 5). Three of these deaths were from cancer of the tongue, two each from cancer of the lip and pharynx, and one from cancer of the buccal cavity.

Ninety-four deaths were due to cancers of the digestive organs and peritoneum (with 90.2 expected) (table 4); none of the site-specific cancers in this grouping was in excess. Of eight deaths from cancer of the liver, seven occurred among employees of tannery B (with 6.9 expected). All seven of these deaths occurred after a latent period of 15 years and four of the seven deaths after 10 years of employment (with 2.7 expected) (table 5). Three of the deaths from liver cancer were among those employed in the tanyard of tannery B, a finding which was statistically significant (SMR = 720, CI = 126-1361). However, two of these deaths occurred among employees with less than one month of employment at the tannery.

Of the 40 deaths from genitourinary cancers (with 50.0 expected) (table 4), eight were due to cancer of the kidney (with 8.4 expected) and five were due to cancer of the bladder (with 6.8 expected). Seven of the eight kidney cancer deaths occurred after a period of 15 years latency (with 5.6 expected); four of these occurred after only one year of employment (table 5). Two deaths from cancer of the kidney occurred among employees of the beamhouse of tannery B (with 0.9 expected). Of the five deaths from cancer of the bladder,

four occurred among employees of tannery B (with 4.1 expected) (table 4) and two of these deaths occurred among those employed in the retan, color, and fat-liquoring department (with 1.0 expected).

Mortality from diseases of the lymphatic and hematopoietic system was lower than expected for tannery A and tannery B (table 4). Within this category, deaths from both leukemia and aleukemia and from malignant lymphomas were below those expected.

One death resulted from cancer of the nasal cavity (with 0.4 expected) in an employee with more than 18 years experience in the finishing department of tannery B.

Alcoholism and Cirrhosis of the Liver: Significant excesses of mortality from alcoholism (11 deaths; SMR = 192; CI = 101-344) and cirrhosis of the liver (45 deaths; SMR = 150; CI = 110-201) were observed among workers at tannery B, but not at tannery A (table 3). These statistically significant excess risks persisted when state rates were used as the comparison population. The majority (73%) of the deaths from cirrhosis occurred among those employed less than one year at the tannery. Nine of the 45 cirrhotic deaths occurred among employees of the beamhouse of tannery B (SMR = 138; CI = 62-203). Death certificate examination indicated that five of the nine cirrhotic deaths listed chronic alcoholism as a contributory cause.

Accidents: The excess of accidental deaths was of borderline statistical significance at tannery A (SMR = 128; CI = 100-163) but slightly less than expected at tannery B (SMR = 92; CI = 73-115) (table 3). Of the 65 accidental deaths among employees of tannery A, only 23 (with 22.2 expected) resulted from causes other than motor vehicle accidents, and only one (burned by flash fire) resulted from employment at the tannery. Most of the accidental deaths occurred among those employed in the beamhouse of tannery A (24 cases; SMR = 228; CI = 146-394); however, only eight (with 4.7 expected) of these deaths resulted from causes other than transportation and none occurred at the tannery. Only six of the 24 deaths occurred within one year of last employment at the tannery.

Suicide: Deaths from suicide were significantly elevated over those expected for employees of tannery B (42 cases; SMR = 171; CI = 123-232) (table 3). Half of the tanners who died from suicide worked at the tannery for less than one year and committed suicide, on the average, 8.5 years after termination of employment. Only three of the 42 suicides occurred within one year of last employment at tannery B.

DISCUSSION

The major findings of this study suggest, in general, that leather tanners and finishers are not at an increased risk for those causes of death that were of a priori concern, when compared to the mortality experience of either the population of the U.S. or of the state in which the tanneries are located.

These results seem to corroborate the only other mortality investigation focused specifically on employees of the leather tanning and finishing industry (30). This latter study, however, was limited by small numbers (N=833).

The outcomes of a priori concern in our investigation of tanners and finishers included cirrhosis of the liver, cancers of the liver, kidney, lung, bladder, nasal cavity, buccal cavity and pharynx, and larynx, and lymphoma. These diseases were based on adverse health effects previously reported by others for the leather and leather manufacturing products industry (as cited below) and chemicals known to have been used in the tannery environment.

Studies of employees from the broader category of the leather and leather manufacturing products industry have shown varying results. Houton et al., (18) reported increased risks for cancers of the bladder, buccal cavity and pharynx, and larynx among "operatives in the leather industry" compared to clerical workers. In a series of case-control analyses from the same population, DeCoufle (11) confirmed Houton's findings and also observed an increased risk for malignant lymphomas compared to noncancer cases. Dubrow (12) and Cole (8) both found statistically significant increased risks for bladder cancer and cirrhosis of the liver among "leather workers" which included tanners, as well as among employees involved in the cutting, assembling and buffing of leather. Increased risk of kidney cancer has been reported among "leather workers" (2, 6, 26) as has risks from lung cancer (17, 22), and nasal cancer (10, 30).

Of particular interest were mortality risks associated with exposure to N-nitrosodimethylamine (NDMA). NDMA is an extremely potent carcinogen (11) that can be formed by the interaction of oxides of nitrogen and dimethylamine sulfate, a chemical used to accelerate dehairing in the tannery process. NDMA has been shown to cause cancer in a variety of organs, especially the liver and kidney (4, 16), in virtually every animal species in which it has been tested (28), although the doses used far exceeded the maximum exposures tolerated by man (24, 25). The carcinogenic risk to humans has yet to be established. Barnes and Magee (4) have observed two cases of cirrhosis of the liver and liver damage among three men working in a research laboratory of a large industrial complex where NDMA had been used as a solvent.

In our study, an elevated mortality risk was observed from cirrhosis of the liver (9 deaths; SMR = 138; CI = 62-203) and kidney cancer (2 deaths; SMR = 222; CI = 25-802) among beamhouse employees of tannery B where dimethylamine sulfate had been used to accelerate the dehairing process. A significantly increased risk for liver cancer (3 deaths; SMR = 720; CI = 126-1361) was also observed in an adjacent (tanyard) department. The airborne levels of nitrosamines found ranged from 0.1 - 11 $\mu\text{g}/\text{m}^3$, which, while very low, appear to be typical of a tannery that uses dimethylamine sulfate. However, higher levels have been observed in another tannery (32).

Of the nine deaths from cirrhosis of the liver in our study, five of the death certificates listed chronic alcoholism as a contributing cause; and of the

three deaths due to liver cancer, two had been employed for less than one month at the tannery. Similarly, one of the two kidney cancer deaths had been employed for less than one month at the tannery. In addition, none of the deaths from non-malignant diseases of the genitourinary system, including nephritis and nephrosis and kidney disease, were elevated. Therefore, the increased risks cited here must be interpreted with caution.

In contradistinction to previous studies of workers in the leather and leather manufacturing products industry (10, 17, 22), this analysis did not find increased mortality from lung cancer or other non-malignant respiratory diseases. In the earlier years (prior to World War II), tanneries in the U.S. which tanned skins often used the two-bath tanning method in which hides were saturated with hexavalent chromium salts (potassium or sodium dichromate) and sulfuric acid, and then removed manually and placed in a bath that reduced the dichromate to trivalent chromium sulfate. Exposures to hexavalent chromium compounds, some of which have been shown to be respiratory carcinogens in both animals and humans (20, 27) were probable. Several explanations may account for the apparent discordance between our results and those of the previous studies. First, most chrome tanneries in the U.S. have switched to the one-bath tanning method since the early 1940's in which chromium [VI] had already been reduced to chromium [III]; most of these latter compounds have not been determined to be carcinogenic. Since fewer than eight percent of our cohort had been employed prior to 1940, worker exposure to chromium [VI] was minimal. Second, the three previous studies referenced above included employees from the broader occupational category of the leather

and leather manufacturing products industry. These employees may have been exposed to respiratory carcinogens not normally present in the tannery environment. Third, although the tobacco smoking habits of our cohort were unknown, the two tanneries under study had strict enforcement of anti-smoking rules and, therefore, employees probably did not smoke more than the comparison population. The only study of leather workers which has taken smoking status into account (40) also observed a lower than expected risk from lung cancer and other respiratory diseases among tannery workers.

Cancer of the bladder was of concern because of the use of some dyestuffs derived from benzidine and beta-naphthylamine in the dyeing of the chrome leather. Benzidine and beta-naphthylamine are dye intermediates which have been shown to be human bladder carcinogens (7, 19, 33). One of the two tanneries in our study, tannery B, showed detectable concentrations of benzidine in bulk dyes in the dye room. Of the four bladder cancer deaths that occurred in this tannery, two employees that died (with 1.0 expected) had once worked in the dye room. However, neither employee had worked for longer than two months at the tannery and, due to small numbers, the results could have been due to chance.

In the finishing department of tannery B, there was one death due to squamous cell carcinoma of the nasal cavity. Cancer of the nasal cavity is rare; the annual incidence for white males is approximately eight in one million in the U.S., with about one half of these being fatal within five years. Sixty percent of the fatal cases of nasal cancer are squamous cell carcinomas. Both

formaldehyde exposure and leather dust have been linked with squamous cell carcinoma of the nasal cavity in rodents (3, 36) although the findings in human epidemiological studies are still unclear (42). In the finishing department of tannery B, formaldehyde was used as a leather preservative and as a protein fixer on glazed leather, and leather dust originated from the buffing operation. The single fatal case of squamous cell carcinoma of the nasal cavity in our study had worked in the finishing department for more than 18 years and died 55 years after initial employment, an induction-latency period consistent with occupational nasal carcinoma. The observation of nasal cancer among leather tanners was also recently reported in England (1 obs. vs. 0.21 exp.) (30).

Deaths from accidental causes were found to be significantly elevated among employees of tannery A (SMR = 128, CI = 100-163) especially among those who had worked in the beamhouse. This was not unexpected since tanneries traditionally rank high in accidental injuries among all industries surveyed (39). However, only one accidental death occurred at this tannery. Almost all other accidental deaths resulted from fatal car or motorcycle accidents, accidental falls at home, or drownings. This result is consistent with data from the Bureau of Labor Statistics which found that, of 10,309 employment compensation claims filed during the years 1976 - 1983 for injuries in SIC 311, only 4 were for fatalities (37).

Our study had several limitations which should be noted. First, because detailed information concerning past environmental exposures was not

available, we had to define exposure by classifying workers according to 1) the duration of their employment, 2) the length of time interval since their first employment (latency), and 3) their assignments to various departments. These are crude surrogate measures of actual exposure. Had we been able to identify more precisely those members of the cohort with the heaviest cumulative exposures, we might have been able to define more precisely the effects of such exposures on mortality. Second, mortality is not always an adequate indicator of potential health risks associated with employment, particularly for those diseases such as bladder cancer which are readily treatable (35). Morbidity, which may be a better measurement of potential health risk in some cases, was beyond the scope of the present study. Third, ascertainment of vital status was only 95% complete. While this percentage of follow-up is well within the range generally considered acceptable for cohort mortality studies, the 5% deficit may have had the effect of inflating person-years at risk and thus lowering the SMRs. In addition, death certificates were not recorded for 50 (3.2 percent) of the 1582 known deaths. This had the effect of lowering the cause-specific SMRs by an additional 3.2 percent, on the average. Fourth, although there is no particular reason to assume so, there is always the possibility that personnel records may have been incomplete. Finally, the statistical power of this study to detect increases in mortality for diseases of a priori interest varied widely according to the background frequency of each condition (5) as shown by the confidence intervals.

In summary, although this study revealed some elevated risks for certain causes of death among tannery workers, no significantly increased risks were noted for any cause of death thought a priori to be occupationally related. Several limitations of this study, however, are discussed which may have accounted for our negative findings. Considering the limitations of this study, it would be improper to conclude that employment in the leather tanning and finishing industry presents no occupational health risks.

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Table 1. Process description and chemicals sampled in two leather tanning facilities.

| Process (by department) | Process description | Chemicals sampled in each department | No. of Samples | Range of Concentration (PPM) ^a |
|------------------------------|--|--|----------------|---|
| A. Beamhouse | | | | |
| Receive, sort, trim and side | Receive, sort, and trim the perimeter areas of the hide and split the hide in half (from tail along the backbone to center of neck). | | | |
| Soak and wash | Remove blood, dirt, manure, and excess salt and restore moisture to open up the contracted fibers so they are chemically active. | | | |
| Dehair | Destroy the hair using hydrated lime containing accelerating agents (e.g. sulfide chemicals and dimethylamine sulfate) | Hydrogen sulfide
N-nitrosodimethylamine (produced from dimethylamine sulfate) | 3
29 | 0-2.0
0-11 ^b |
| Flesh | Remove excessive flesh, fat, and muscle | | | |
| Delime and bate | Neutralize the high alkalinity of the limed hides with buffering salts (e.g. ammonium sulfate or ammonium chloride) | Ammonia
Sulfuric acid | 2
6 | 9-30
0-0.24 |
| Pickle | Place the hide in an acid environment ready to accept the tanning material using a solution of sodium chloride and sulfuric acid. | | | |
| B. Tanyard | | | | |
| Tan | Convert the raw collagen fibers of the hide into a stable product which is no longer susceptible to rotting by placing the hides in a drum containing basic trivalent chromic sulfate solutions. | Total chrome including chromium (VI) (air)

% Total Chrome (Bulk Sample) | 55

5 | 0.2-54 ^b

1.5-3.8 |
| Wring | Remove excess moisture to ease splitting. | | | |
| Split and shave | Adjust thickness of hide. | | | |

Table 1 (continued)

| Process
(by department) | Process description | Chemicals sampled
in each department | No of
Samples | Range of
Concentration
(PPM) |
|---|--|---|------------------|------------------------------------|
| <u>C. Retan, color, fat-liquoring</u> | | | | |
| Retan | Add tanning agents such as vegetable and synthetic tannins to modify the physical properties of the leather. | | | |
| Color | Color the leather using acid, metallic, basic, or direct dyes | Formic Acid | 19 | 1.3-6.7 |
| | | Benzidine in bulk dyes | 2 | 2.0-55 |
| | | Copper | 2 | - |
| Fat-liquoring | Lubricate the leather with animal oil to give it pliability and tensile strength | Cobalt | 2 | - |
| | | Manganese | 2 | - |
| | | Cadmium | | |
| Dry | Remove excess moisture | | | |
| Set out | Smooth and stretch the leather | | | |
| <u>D. Finishing</u> | | | | |
| Condition, stake, buff, finish, plate, measure, grade | Mechanical and chemical operations that soften, smooth, & help preserve the leather | N-butyl acetate | 27 | - |
| | | Butyl cellosolve | 69 | 0.2-12 |
| | | Methyl ethyl ketone | 26 | - |
| | | Methyl isobutyl ketone | 83 | 0.3-51 |
| | | Toluene | 83 | 0.2-206 |
| | | Xylene | 83 | 0-48 |
| | | Acetone | 83 | 0.3-105 |
| | | Formaldehyde | 5 | 0.5-7 |
| | | Airborne leather fibers | 22 | 0.2-3.9 ^c |
| | | Copper | 2 | - |
| | | Cobalt | 2 | - |
| | | Manganese | 2 | - |
| | | Cadmium | 2 | - |
| | | Respirable Dust | 3 | 70% |

E. All other
(Mainly maintenance and support)

a = PPM, parts per million unless otherwise specified.

b = $\mu\text{g}/\text{m}^3$, micrograms per cubic meter

c = mg/m^3 , milligrams per cubic meter

Table 2. Vital status of cohort members of two leather tanneries by sex and race, 31 December 1982.

| VITAL STATUS | Males | | | | Females | | | | Total Cohort | |
|-----------------------------------|---------|-------|----------|-------|---------|-------|----------|-------|--------------|-------|
| | White | | Nonwhite | | White | | Nonwhite | | | |
| | N | (%) | N | (%) | N | (%) | N | (%) | N | (%) |
| Alive | 4,376 | (75) | 1,037 | (82) | 1,549 | (82) | 354 | (92) | 7,316 | (78) |
| Deceased | 1,219 | (21) | 137 | (11) | 216 | (11) | 10 | (3) | 1,582 | (17) |
| Death certificates obtained | 1,184 | . | 125 | . | 213 | . | 10 | . | 1,532 | . |
| Death certificates
outstanding | 35 | . | 12 | . | 3 | . | 0 | . | 50 | . |
| Lost to follow-up | 232 | (4) | 84 | (7) | 132 | (7) | 19 | (5) | 467 | (5) |
| Total Cohort | 5,827 | (100) | 1,258 | (100) | 1,897 | (100) | 383 | (100) | 9,365 | (100) |
| Person-years at risk | 129,654 | | 19,741 | | 49,324 | | 5,543 | | 204,262 | |

Table 3. Mortality experience for selected causes among employees of two leather tanneries, 1940-1982^a

| Cause of death ^b | Both tanneries | | | Tannery A | | | Tannery B | | |
|---|----------------|------------------|-----------------|-----------|------|---------|-----------|------|---------|
| | Deaths | | | Deaths | | | Deaths | | |
| | Observed | SMR ^c | CI ^d | Observed | SMR | CI | Observed | SMR | CI |
| All cancers (140-205) | 282 | 79* | 70- 89 | 104 | 78* | 64- 95 | 178 | 80* | 69- 93 |
| Alcoholism (322) | 12 | 148 | 76-259 | 1 | . | . | 11 | 192* | 101-344 |
| Nervous system diseases (330-334) | 109 | 80* | 66- 97 | 48 | 91 | 67-121 | 61 | 74 | 56- 96 |
| Circulatory system diseases (400-468) | 620 | 85* | 78- 92 | 239 | 76* | 67- 87 | 381 | 91 | 82-101 |
| Respiratory system diseases (470-527) | 89 | 93 | 74-114 | 35 | 87 | 61-122 | 54 | 99 | 73-127 |
| Cirrhosis of the liver (581) | 52 | 114 | 85-150 | 7 | 44* | 18- 92 | 45 | 150* | 110-201 |
| Genitourinary system diseases (590-652) | 19 | 81 | 49-126 | 7 | 83 | 33-172 | 12 | 79 | 41-139 |
| Accidents (E800-E959) | 144 | 105 | 89-124 | 65 | 128* | 101-163 | 79 | 92 | 73-115 |
| Suicide (E963,E970-E979) | 58 | 143* | 109-185 | 16 | 99 | 56-161 | 42 | 171* | 123-232 |
| Homicide (E964,E980-E985) | 22 | 76 | 46-111 | 1 | . | . | 21 | 85 | 53-130 |
| All causes (001-998) ^e | 1582 | 89* | 85- 94 | 568 | 81* | 74- 88 | 1014 | 93 | 88-100 |

a. Based upon United States mortality rates.

b. ICD, Code of the International Classification of Diseases, (seventh revision).

c. SMR, standardized mortality ratio (observed/expected) x 100; not calculated for observed number of deaths less than 2.

d. CI, 95% confidence interval (two-sided test).

e. Includes unknown causes.

* $p < 0.05$.

Table 4. Mortality experience for selected causes of cancer among employees of two leather tanneries, 1950-1982

- Comparison with U.S. and with Minnesota (Minn.) — Tannery A and Wisconsin (Wisc.) — tannery B state death rates.

| Cause of death ^a | Tannery A | | | | Tannery B | | | |
|--|-----------|------------------|---------|-----------------|-----------|--------|---------|--------|
| | Observed | SMR ^b | | CI ^c | Observed | SMR | | |
| | Deaths | (U.S.) | (Minn.) | | Deaths | (U.S.) | (Wisc.) | CI |
| All Cancers (140-205) | 99 | 78 | 88 | 72-107 | 177 | 84* | 82 | 73-98 |
| Buccal cavity & pharynx (140-148) | 3 | 78 | 102 | 21-301 | 5 | 83 | 88 | 29-207 |
| Digestive organs & peritoneum (150-159) | 38 | 104 | 107 | 76-148 | 56 | 96 | 94 | 71-124 |
| Liver (155-156a) | 1 | . | . | . | 7 | 123 | 101 | 28-269 |
| Respiratory (160-164) | 19 | 46 | 66 | 40-104 | 45 | 78 | 95 | 70-128 |
| Trachea, bronchus, lung (162-163) | 18 | 47 | 67 | 40-106 | 42 | 77 | 93 | 67-126 |
| Genitourinary tract (171-182) | 16 | 84 | 89 | 51-145 | 24 | 75 | 75 | 48-112 |
| Kidney (180) | 4 | 133 | 119 | 33-307 | 4 | 93 | 79 | 22-202 |
| Bladder (181) | 1 | . | . | . | 4 | 83 | 98 | 27-253 |
| Other and unspecified (190-199) | 15 | 102 | 116 | 65-192 | 21 | 83 | 71 | 45-107 |
| Lymphatic & hematopoietic system (200-205) | 8 | 65 | 58 | 25-116 | 14 | 72 | 74 | 42-123 |
| Leukemia & aleukemia (204) | 4 | 77 | 70 | 19-180 | 6 | 75 | 75 | 28-164 |
| Lymphoma (200-203,205) | 4 | 57 | 51 | 14-129 | 8 | 70 | 66 | 29-131 |

a. ICD, Code of the International Classification of Diseases, (seventh revision).

b. SMR, standardized mortality ratio (observed/expected) x 100, not calculated for observed deaths less than 2.

c. CI, 95% confidence interval (two-sided test).

* $p < 0.05$

Table 5. Mortality experience for selected causes of cancer by cumulative years of employment and fifteen year latency interval since first employment among workers of two leather tanneries, 1940-1982^a

| Cause of death ^b | Cumulative years of employment | | | | | | | | | | | |
|-------------------------------------|--------------------------------|------------------|-----------------|----------|-----|--------|----------|-----|--------|-----------------------|-----|--------|
| | < 1 | | | 1-9 | | | ≥ 10 | | | Total 15 yr + Latency | | |
| | Observed | | | Observed | | | Observed | | | Observed | | |
| | Deaths | SMR ^c | CI ^d | Deaths | SMR | CI | Deaths | SMR | CI | Deaths | SMR | CI |
| All cancers (140-205) | 111 | 84 | 70-102 | 58 | 101 | 77-131 | 72 | 75* | 59-94 | 241 | 84* | 74-96 |
| Buccal cavity & pharynx (140-148) | 3 | 79 | 20-215 | 3 | 187 | 48-510 | 2 | 71 | 12-236 | 8 | 97 | 42-191 |
| Digestive organs & | | | | | | | | | | | | |
| peritoneum (150-159) | 31 | 90 | 61-128 | 21 | 135 | 84-207 | 31 | 114 | 77-162 | 83 | 103 | 82-128 |
| Liver (155-156A) | 2 | 66 | 11-213 | 1 | . | . | 4 | 148 | 47-357 | 7 | 96 | 39-199 |
| Respiratory system (160-164) | 31 | 77 | 52-109 | 15 | 89 | 50-146 | 9 | 33* | 15-63 | 55 | 66* | 50-86 |
| Trachea, bronchus, lung (162-163) | 30 | 78 | 53-112 | 15 | 93 | 52-154 | 7 | 27* | 11-57 | 52 | 65* | 49-85 |
| Genitourinary tract (171-182) | 17 | 94 | 55-151 | 5 | 60 | 19-140 | 12 | 75 | 39-131 | 34 | 80 | 55-111 |
| Kidney (180) | 4 | 141 | 45-345 | 1 | . | . | 2 | 97 | 16-315 | 7 | 114 | 46-235 |
| Bladder (181) | 3 | 100 | 26-273 | - | - | - | 1 | . | . | 4 | 51 | 14-130 |
| Other & unspecified (190-199) | 15 | 100 | 56-165 | 5 | 90 | 29-208 | 7 | 72 | 29-149 | 27 | 87 | 57-125 |
| Lymphatic & hematopoietic (200-205) | 9 | 82 | 37-156 | 5 | 103 | 33-240 | 8 | 104 | 45-205 | 22 | 93 | 58-141 |
| Leukemia & aleukemia (204) | 2 | 45 | 5-168 | 2 | 100 | 11-361 | 6 | 170 | 63-373 | 10 | 101 | 48-186 |
| Lymphoma (200-203,205) | 7 | 105 | 42-219 | 3 | 105 | 21-305 | 2 | 48 | 5-173 | 12 | 87 | 45-153 |

a. Based upon United States mortality rates

b. ICD, Code of the International Classification of Diseases, (seventh revision)

c. SMR, standardized mortality ratio (observed/expected) x 100; not calculated for observed deaths less than 2

d. CI, 95% confidence interval (two-sided test)

* p < 0.05

Table 6. Mortality experience for selected causes by department among employees of two leather tanneries, 1940-1982^a

| Causes of death ^b | Breakhouse | | | Tannery | | | Retan, Color, Fat-liquor | | | Finishing | | | All other dept. | | |
|---|-------------------|------------------|-----------------|---------|------|---------|--------------------------|-----|--------|-----------|-----|--------|-----------------|-----|--------|
| | Obs. ^c | SMR ^d | CI ^e | Obs. | SMR | CI | Obs. | SMR | CI | Obs. | SMR | CI | Obs. | SMR | CI |
| All causes (140-205) | 52 | 73* | 55-96 | 36 | 74 | 52-103 | 54 | 72* | 54-94 | 118 | 80* | 66-96 | 71 | 82 | 64-103 |
| Oral cavity & pharynx (140-148) | 2 | 82 | 14-275 | - | - | - | 2 | 79 | 23-264 | 1 | . | . | 4 | 156 | 49-371 |
| Liver (155-156A) | 1 | . | . | 3 | 230 | 59-628 | 2 | 105 | 18-348 | 1 | . | . | 2 | 87 | 15-287 |
| Trachea, bronchus, lung (162-163) | 16 | 68 | 42-119 | 5 | 36* | 13-80 | 12 | 51* | 26-90 | 24 | 70 | 45-105 | 144 | 59 | 33-100 |
| Kidney (180) | 2 | 126 | 21-413 | 1 | . | . | 1 | . | . | 3 | 102 | 26-273 | 2 | 103 | 18-348 |
| Bladder (181) | 0 | - | - | - | - | - | 2 | 96 | 16-315 | 3 | 89 | 23-247 | 1 | . | . |
| Leukemia & aleukemia (204) | 2 | 77 | 9-278 | 1 | . | . | 3 | 100 | 20-292 | 7 | 125 | 50-258 | 5 | 138 | 45-324 |
| Lymphomas (200-203, 205) | 3 | 83 | 17-243 | 1 | . | . | 1 | . | . | 7 | 92 | 37-190 | 2 | 45 | 5-164 |
| Alcoholism (322) | 3 | 138 | 35-371 | 1 | . | . | 2 | 88 | 15-287 | 3 | 121 | 31-327 | 3 | 182 | 48-510 |
| Nervous system diseases (330-334) | 16 | 54* | 31-88 | 20 | 94 | 57-145 | 29 | 105 | 70-151 | 40 | 80 | 57-109 | 29 | 81 | 54-117 |
| Circulatory system diseases (400-468) | 119 | 74* | 62-89 | 85 | 75* | 59-91 | 132 | 79* | 67-95 | 205 | 78* | 68-90 | 199 | 103 | 90-119 |
| Respiratory system diseases (470-527) | 23 | 106 | 67-159 | 12 | 79 | 41-139 | 16 | 73 | 42-120 | 32 | 91 | 63-129 | 26 | 107 | 70-157 |
| Cirrhosis of the liver (581) | 11 | 111 | 55-199 | 3 | 50 | 13-136 | 10 | 88 | 42-163 | 21 | 122 | 76-187 | 17 | 166 | 97-267 |
| Genitourinary system diseases (590-652) | 1 | . | . | 7 | 80 | 33-168 | 3 | 61 | 16-170 | 4 | 76 | 24-186 | 6 | 104 | 38-229 |
| Accidents (E800-E995) | 44 | 148 | *108-199 | 25 | 127 | 82-187 | 37 | 101 | 72-171 | 41 | 88 | 63-120 | 28 | 92 | 62-134 |
| Suicide (E963, E970-979) | 6 | 77 | 28-170 | 12 | 207* | 107-361 | 17 | 165 | 97-267 | 15 | 97 | 54-160 | 14 | 150 | 82-253 |
| Homicide (E964, E980-E985) | 8 | 84 | 36-166 | 3 | 76 | 20-209 | 5 | 54 | 20-119 | 5 | 72 | 27-161 | 3 | 62 | 16-170 |
| All causes ^f (001-998) | 325 | 83* | 75-94 | 219 | 81* | 72-94 | 352 | 87* | 87-97 | 539 | 81* | 75-89 | 443 | 99 | 91-109 |

a. Ever worked in that department - Based upon United States mortality rates.

b. ICD, Code of the International Classification of Diseases, (seventh revision).

c. Obs, Observed Deaths.

d. SMR, Standardized Mortality Ratio (observed/expected) x 100; not calculated for observed deaths less than 2.

e. CI, 95% Confidence Interval (two-sided test).

f. Includes unknown causes.

* p < 0.05.

QUESTIONS AND DISCUSSION

DR. SHY: Time for questions.

MR. MARCUS: Sam Marcus, NCHS. Was there any attempt made to rank the employees by degree of exposure?

DR. STERN: No industrial evaluations had been conducted on any tannery that I know of before we took our industrial hygiene sampling. So we don't know what the previous exposures were.

However, we did take industrial hygiene samples and we did evaluate and analyze the cohorts by department in which certain substances were known to have been used. And that's the only industrial hygiene or evaluation of industrial hygiene we were able to do.

MR. MARCUS: Well, weren't there certain employees who were exposed at a higher rate to, say, nitrosamines and other toxic substances?

DR. STERN: Well, persons that work around the power vacs are all exposed to nitrosamines. There were some persons that were exposed slightly more than other persons. But we didn't have previous exposures; just looking at the exposures in 1981 or 1982 would not have shown what the previous exposures were.

And so we just grouped them together per department. And that's, I think, the best we could have done with what we had.

MR. MARCUS: I'm sorry. But could you get back data, say, 20, 30 years ago at all on what the exposures were to some of these employees?

DR. STERN: Nobody that I know has any exposure data for leather tanneries. We visited between 20 and 25 tanneries out of the 250 in the United States.

**MORTALITY AMONG INDUSTRIAL WORKERS
EXPOSED TO FORMALDEHYDE**

**Presented by Dr. Aaron Blair
National Cancer Institute**

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Mortality Among Industrial Workers Exposed to Formaldehyde¹

Aaron Blair,^{2,3} Patricia Stewart,² Maureen O'Berg,^{4,5} William Gaffey,^{5,6} Judy Walrath,^{2,4} John Ward,⁷ Ron Bales,⁷ Samuel Kaplan,⁷ and Dewey Cubit^{8,9}

ABSTRACT—A historical cohort study evaluated the mortality experience of 26,561 workers employed in 10 formaldehyde-producing or -using facilities. Approximately 600,000 person-years of follow-up accrued as workers were followed to January 1, 1980. Estimates of historical exposure to formaldehyde by job were developed by project industrial hygienists using monitoring data available from participating plants, comments from long-term workers, and comprehensive monitoring data specifically collected for this study. Mortality from all causes combined was about as expected [standardized mortality ratio (SMR)=96] based on mortality rates of the general U.S. population. Significantly fewer deaths occurred from infective and parasitic diseases (SMR=51) and from accidents (SMR=72) than expected. Cancer overall was not related to formaldehyde exposure. Workers exposed to formaldehyde had slight excesses for Hodgkin's disease and cancers of the lung and prostate gland, but these excesses were not consistently related to duration of or average, cumulative, or peak formaldehyde exposure levels. Recent animal studies found nasal cancer among rats exposed to formaldehyde, but no excess of this tumor occurred in this study. Mortality from brain cancer and leukemia among these industrial workers was not excessive in contrast to reported excesses among professional groups (e.g., anatomists, embalmers, and pathologists) with exposure to formaldehyde. Although there was a deficit for cancer of the buccal cavity and pharynx, mortality from certain subsites, i.e., the nasopharynx and oropharynx, was elevated. These subsites did not, however, show a consistently rising risk with level of exposure. These data provide little evidence that mortality from cancer is associated with formaldehyde exposure at levels experienced by workers in this study.—*JNCI* 1986; 76:1071-1084.

Animal bioassays conducted by the Chemical Industry Institute of Toxicology (1) and the New York University (2) indicate that formaldehyde causes nasal tumors in rats. The potential for continuous occupational exposure to formaldehyde in some industries, opportunity for residential and ambient background exposures in the general population, and evidence of its carcinogenicity in laboratory animals raised serious public health issues regarding this substance. In addition, epidemiologic studies of persons exposed to formaldehyde have noted some associations with leukemia and cancer of the brain, lung (3), and buccal cavity and pharynx (4, 5). To assess the risk of these cancers and other causes of death among persons exposed to formaldehyde, the National Cancer Institute with the collaboration of the FI conducted an epidemiologic study of industrial workers exposed to formaldehyde.

SUBJECTS AND METHODS

From the FI, trade organizations, directories of chemical producers and users, and other resources, we identi-

fied approximately 200 companies reported to use or to produce formaldehyde. From 80 companies meeting the eligibility criteria (workers exposed to formaldehyde before 1966 and necessary personnel records), we selected for investigation the 10 plants with the largest numbers of employees and longest use of formaldehyde.

Cohort design and follow-up.—The cohort is composed of all workers first employed at the selected plants before January 1, 1966. Subjects were traced to January 1, 1980, to determine vital status. The complete personnel record of each worker was abstracted to obtain date of birth, race, sex, address, Social Security number, name and address of dependents, and information on each job held at the plant, including job titles, work areas, and dates jobs were held. The vital status of subjects was ascertained through the Social Security Administration, Health Care Finance Administration, Veterans Administration, credit bureaus, motor vehicle departments, and telephone directories. For those deceased, death certificates were obtained and underlying causes of death were determined by an experienced nosologist using the rules in effect at the time of death and using assigned rubrics of the Eighth Revision of the International Classification of Diseases.

We obtained Social Security Quarterly Earnings reports to verify the completeness of the cohort. This

ABBREVIATIONS USED: FI = Formaldehyde Institute; RR = rate ratio(s); SMR = standardized mortality ratio(s); TWA = time-weighted average.

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approach has proved to be an effective method for evaluating the completeness of personnel records (6). This verification process indicated that records were incomplete for plant 05 before 1957 and for plant 06 before 1951. Consequently, persons from these plants terminating employment before 1957 and 1951, respectively, were excluded from the cohort.

Exposure estimates.—Historical exposures to formaldehyde were estimated for each unique job title, work area, and calendar-year combination, with the use of three independent lines of evidence (7).

First, some 300,000 job titles abstracted from company personnel files were standardized to 6,700 titles. The industrial hygienists prepared exposure matrices for each job-work area-calendar-year combination. For creation of these matrices, visits were made by the industrial hygienists to each plant to conduct walk-through surveys, to obtain monitoring data from the company, and to discuss plant operations with experienced company personnel. The amount and quality of the monitoring data varied among the plants. Some plants had made several operational changes that affected exposure levels, while others had made few. An exposure form was completed for each unique job that included the following information: estimated 8-hour TWA exposure to formaldehyde, level and frequency of peak exposures, other potential chemical exposures, potential for exposure to formaldehyde in the particulate or liquid form, use of personal protective equipment, and confidence in the exposure estimates. A peak was defined as an excursion of short duration (generally <15 min) that exceeded the upper level of the TWA category to which the job was assigned. Separate forms were created for as many time periods as necessary. The particulate exposures could be from solid formaldehyde; a formaldehyde-containing product, such as a resin or molding compound; or a particulate onto which formaldehyde could be adsorbed. An evaluation of the concentrations or sizing of the particulates was not made. Actual TWA ppm estimates of formaldehyde exposures were assigned to five ranked categories: 1) trace (having worked at a plant using or producing formaldehyde but having no known exposure), 2) <0.1 ppm, 3) 0.1–<0.5 ppm, 4) 0.5–<2.0 ppm, and 5) ≥ 2.0 ppm.

Second, a listing of all jobs by relative level of formaldehyde exposure was sent to participating plants for review by their industrial hygienists.

Third, monitoring of formaldehyde levels was conducted at each plant during the summer and the winter of 1983–84 by using du Pont and 3M passive dosimeter badges and a standard National Institute for Occupational Safety and Health method (8). Although historical exposure estimates cannot be absolutely validated, the comparison of results from the three independent lines of evidence provided a measure of reliability. A fuller description of the industrial processes, jobs, and the exposures will be presented in a later paper.

Analytic methods.—SMR and directly standardized RR were used to compare the mortality experience of formaldehyde workers with that of the total U.S. popu-

lation, local population, and nonexposed workers. Person-year accumulation in the cohort began on January 1 of the initial year of cohort identification (see table 1), when subjects started employment at a plant (if after the cohort identification date) or upon the subjects' first achieving a specified level or type of exposure, depending upon the particular analysis being undertaken. Person-year accumulation ceased at the closing date of the study, at the last date known alive, at the date of death, or at the date of reaching a higher exposure level (whichever was appropriate). Expected numbers for SMR were calculated by applying 5-year age and calendar-year mortality rates from the appropriate race-sex group of the referent population for the years 1930 to 1980 to the person-year distribution of the formaldehyde workers by using a program developed by Marsh and Preininger (9). County cancer rates were obtained from the 10 counties where the plants were located (10) and used for a local comparison. Cancer rates for the time period preceding 1950 were not available for individual counties and were assumed to be the same as in 1950–54. Rates for 1970–79 are now available in the resource described in (10). Mortality rates for various exposure subgroups were directly adjusted to the age- and calendar-time person-year distribution of the entire cohort to provide direct comparisons among selected exposure categories. SMR and confidence intervals were not presented when observed and expected numbers were less than five, except when exposure trends were evaluated. Ninety-five percent confidence intervals were calculated by using the method of Bailer and Ederer (11). A chi-square test was used to evaluate statistical significance of SMR trends (12).

Measures of exposure to formaldehyde used in the analyses included level or intensity; duration; average, cumulative, or peak exposure; presence of particulates; and measures that excluded recent exposures from the exposure index. Estimated TWA level of the job, by rank category, was the basis for intensity. Duration was the time spent in formaldehyde-exposed jobs. Cumulative exposure was calculated by multiplying the time spent in each job by the midpoint of the TWA category to which the job was assigned (for category 5, 2.5 ppm was used) and summed across all jobs held by the subject. Cumulative exposure categories were created to allow sufficient numbers for analysis in the highest category. Among white men, cumulative exposure in the highest category ranged from 5.5–89 ppm-year and was comprised of 3,767 persons. Cumulative exposure was divided by the time in exposed jobs, to obtain a measure of average exposure. For analysis, peak level was defined as the highest exposure level experienced whether as TWA or peak excursion above the rank category. For each of these measures of exposure, SMR were calculated for three categories of time since first exposure: <10 years, 10–19 years, and ≥ 20 years. Cumulative exposure analyses using other approaches were also performed, including more categories to evaluate potential effects of: higher exposures and longer latency, age at first exposure, lagged exposures, time since last expo-

sure, and calendar-time-specific exposures. Selected analyses were performed for each plant separately to capitalize on the independence of the data from each plant. Regression of SMR on exposure categories was used to evaluate consistency of trends among the 10 plants. The mean of these regression coefficients was compared to zero by a *t*-test. Analyses were also performed on groupings of plants producing similar products.

RESULTS

Characteristics of each plant and its employees are shown in table 1. The 26,561 persons included in this study comprised 20,714 white men, 1,839 black men, 3,104 white women, 26 black women, and 878 workers of unknown race or sex. Plant 03 had a large number of black male employees and plant 06 had many white female employees. Many subjects first entered the cohort after 1946, with fewer entering the cohort earlier (12%). Most workers (64%) were less than 30 years old at initiation, although 4,027 (16%) were over 40 years of age. About 5% of the cohort could not be successfully traced. Tracing was more successful for men (96%) than for women (83%) and for whites (96%) than for blacks (92%). Losses to follow-up ranged from 1% in plant 05 to 6% in several plants. Of 4,396 subjects reported deceased, death certificates were found for 4,059 (92%). In the analysis, persons presumed dead but lacking death certificates were included in total deaths, but they were not assigned to any specific cause-of-death category. The proportion of deaths without death certificates did not vary by exposure level. Approximately 600,000 person-years of risk were accumulated by all subjects with a range from 15,000 for plant 05 to 140,000 for plant 01. Analyses included 24,717 persons after excluding those of unknown race, sex, or date of birth or those holding only jobs of unknown exposure to formaldehyde (black women were also excluded because of small numbers). Eleven percent of the subjects had their highest TWA exposure assigned to rank 1 (nonexposed), 12% to rank 2 (<0.1 TWA ppm), 34% to rank 3 (0.1 – <0.5 TWA ppm), 40% to rank 4 (0.5 – <2.0 TWA ppm), and 4% to rank 5 (≥ 2.0 TWA ppm). Results from industrial hygiene monitoring led to a change in historical estimates for 91 jobs. Historical estimates were changed one rank for 37 jobs and two ranks for five jobs, while 50 jobs remained in the same rank.

For survey of all causes of death, SMR were calculated for persons ever holding a job where the 8-hour TWA estimated exposure to formaldehyde was greater than 0.1 ppm (exposed), and the SMR were calculated for all others (nonexposed) (table 2). The cut point of greater than 0.1 ppm was chosen for the exposed, to exclude persons with minimal exposures. Mortality from all causes combined was significantly depressed among nonexposed white women (SMR=83) and exposed black men (SMR=80) but was about as expected among white men. Significant deficits were seen for infective and parasitic disease and for accidents among both exposed and nonexposed white men. Deficits for most of these

conditions were also seen among black men and white women, but they were generally not statistically significant. Mortality from emphysema and cirrhosis of the liver was less than expected among white men, regardless of exposure.

No statistically significant excesses occurred for specific cancers, although numbers of deaths for most cancers were small among black men (46 deaths) and white women (92 deaths). Among white men, larger SMR occurred among the formaldehyde exposed than among those not exposed to formaldehyde, for leukemia and Hodgkin's disease and cancers of the buccal cavity and pharynx, liver, larynx, lung, bone, skin, prostate gland, bladder, kidney, and eye. For these cancers, the SMR among the exposed were greater than 100 for Hodgkin's disease (SMR=142) and for cancers of the liver (SMR=102), larynx (SMR=142), lung (SMR=111), bone (SMR=123), prostate gland (SMR=115), and kidney (SMR=123), while SMR of less than 100 occurred among the nonexposed. Two deaths from nasal cancer occurred (both among the exposed), whereas about three were expected. No unusual cancer mortality patterns were seen for white women or black men.

Of the 17 deaths from brain cancer among formaldehyde-exposed white men, 3 were due to gliomas, 5 to astrocytomas, and 1 to a medulloblastoma and 8 were identified only as brain tumors or brain cancer. Thus, according to information on the death certificates, 47% of the brain cancers were specifically noted as gliomas, which is similar to a series of 718 brain cancer deaths from Louisiana, New Jersey, and Pennsylvania where 51% were due to gliomas (Thomas T: Personal communication). The cell types for the 23 leukemias were acute lymphatic (1 vs. 2.2 expected), chronic lymphatic (6 vs. 5.4 expected), acute myeloid (8 vs. 11.4 expected), and chronic myeloid (3 vs. 5.5 expected); the remaining 5 included 2 lymphatic leukemias unspecified and 3 leukemias unspecified. Special mortality rates from the general U.S. population were used to provide expected numbers for subsites of cancer of the buccal cavity and pharynx (7). Deaths occurred from cancer of the: lip (2 observed, 0.6 expected), tongue (2 observed, 5.6 expected), other parts of the mouth (2 observed, 1.6 expected), nasopharynx (7 observed, 2.2 expected—a statistically significant excess), oropharynx (5 observed, 2.9 expected), hypopharynx (2 observed, 1.9 expected), and other unspecified oral and pharyngeal sites (1 observed, 4.4 expected).

Mortality among white men by intensity of exposure is shown in table 3 for selected cancers. Only Hodgkin's disease showed a significant rising trend with intensity of exposure. SMR for most sites were <100 in the 0- and >0 – <0.1 -ppm categories. RR from direct age-adjusted rates generally showed patterns similar to those of the SMR.

Relative risks of death from selected cancers by cumulative exposure to formaldehyde (table 4) were similar to cancer risks by intensity of exposure. No cause of death showed significant excesses or a consistent upward trend in cancer risk with level of cumulative exposure.

TABLE 1.—Demographic characteristics of the cohort

| Demographic characteristic | No. of subjects with demographic characteristics; plant identification No.'s & their products ^a | | | | | | | | | | |
|-----------------------------|--|-----------------------|--------------|-------------------------|-------------------------|-----------------------------------|-------------------------------------|--|----------------------------------|------------------------------|-------------|
| | 01 & resins, MC | 02 & formaldehyde, MC | 03 & plywood | 04 & photo-graphic film | 05 & photo-graphic film | 06 & resins, decorative laminates | 07 & resins, MC, formaldehyde, Hexa | 08 & Hexa, MC, resins, molded products | 09 & resins, MC, molded products | 10 & formaldehyde MC, resins | Total (%) |
| Yr of cohort identification | | | | | | | | | | | |
| | 1943 | 1945 | 1949 | 1958 | 1957 | 1951 | 1958 | 1954 | 1956 | 1941 | Total yrs |
| Subjects, No. | | | | | | | | | | | |
| White men | 3,664 | 783 | 1,337 | 1,617 | 565 | 3,988 | 3,578 | 1,365 | 1,517 | 2,300 | 20,714 (78) |
| Black men | 184 | 0 | 972 | 9 | 38 | 120 | 157 | 0 | 67 | 292 | 1,839 (7) |
| White women | 413 | 3 | 82 | 78 | 133 | 1,154 | 496 | 313 | 349 | 83 | 3,104 (12) |
| Black women | 1 | 0 | 1 | 0 | 9 | 12 | 1 | 1 | 1 | 0 | 26 (1) |
| Other | 127 | 24 | 53 | 29 | 18 | 240 | 172 | 90 | 69 | 56 | 878 (3) |
| Total | 4,389 | 810 | 2,445 | 1,733 | 763 | 5,514 | 4,404 | 1,769 | 2,003 | 2,731 | 26,561 |
| Yr of entry into cohort | | | | | | | | | | | |
| ≤1945 | 614 | 43 | | | | | 1,357 | 537 | | 726 | 3,277 (12) |
| 1946-55 | 2,374 | 533 | 773 | | | 3,370 | 2,028 | 815 | | 1,577 | 11,470 (43) |
| 1956-65 | 1,400 | 232 | 1,654 | 1,717 | 762 | 2,081 | 1,015 | 417 | 2,002 | 428 | 11,708 (44) |
| Unknown | 1 | 2 | 18 | 16 | 1 | 63 | 4 | 0 | 1 | | 106 (4) |
| Age at entry, yr | | | | | | | | | | | |
| ≤30 | 2,667 | 533 | 1,665 | 910 | 311 | 3,414 | 3,116 | 1,085 | 1,548 | 1,837 | 17,086 (64) |
| 31-40 | 1,004 | 211 | 480 | 468 | 175 | 1,118 | 794 | 344 | 270 | 584 | 5,448 (21) |
| 41-50 | 513 | 58 | 234 | 310 | 183 | 663 | 360 | 215 | 120 | 219 | 2,875 (11) |
| 51-60 | 182 | 8 | 56 | 37 | 77 | 236 | 108 | 97 | 58 | 81 | 940 (4) |
| ≥61 | 23 | 0 | 10 | 8 | 17 | 83 | 26 | 28 | 7 | 10 | 212 (8) |
| Vital status | | | | | | | | | | | |
| Alive | 3,239 | 684 | 1,923 | 1,575 | 656 | 4,317 | 3,313 | 1,272 | 1,766 | 2,063 | 20,808 (78) |
| Deceased | | | | | | | | | | | |
| Death certificate | 799 | 92 | 337 | 127 | 103 | 819 | 777 | 364 | 107 | 534 | 4,059 (15) |
| Presumed dead | 87 | 9 | 34 | 6 | 1 | 51 | 78 | 23 | 7 | 41 | 337 (1) |
| Unknown (%) | 264 (6) | 25 (3) | 151 (6) | 25 (1) | 3 (1) | 327 (6) | 236 (5) | 110 (6) | 123 (6) | 93 (3) | 1,357 (5) |

^a MC = molding compounds; Hexa = hexamethylenetetramine.

TABLE 2.—Mortality from cancer and other major causes of death by race-sex and formaldehyde-exposure category, <0.1 ppm and ≥0.1 ppm^a

| Cause (ICD) ^b of death | White men | | | | White women | | | | Black men | | | |
|---|-----------|-------|------------------|---------|-------------|-----|-----------------|--------|-----------|-----|-----------------|--------|
| | OBS | EXP | SMR | 95% CI | OBS | EXP | SMR | 95% CI | OBS | EXP | SMR | 95% CI |
| All causes (1-999) | | | | | | | | | | | | |
| Exposed | 2,836 | 2,930 | 97 | 93-100 | 200 | 220 | 91 | 79-104 | 232 | 291 | 80 ^c | 70-91 |
| Nonexposed | 867 | 874 | 99 | 93-106 | 112 | 135 | 83 ^c | 68-100 | 105 | 103 | 102 | 84-124 |
| All cancer (140-209) | | | | | | | | | | | | |
| Exposed | 570 | 566 | 101 | 93-109 | 50 | 63 | 79 | 59-104 | 31 | 46 | 67 ^c | 46-96 |
| Nonexposed | 158 | 168 | 94 | 80-110 | 42 | 40 | 105 | 76-142 | 15 | 16 | 92 | 51-151 |
| All infective and parasitic disease (1-139) | | | | | | | | | | | | |
| Exposed | 24 | 39 | 61 ^c | 39-91 | 0 | 3 | — | — | 5 | 9 | 58 | 19-135 |
| Nonexposed | 6 | 16 | 38 ^c | 14-83 | 0 | 2 | — | — | 2 | 4 | — | — |
| Benign neoplasms (210-239) | | | | | | | | | | | | |
| Exposed | 7 | 9 | 82 | 33-169 | 2 | 1 | — | — | 0 | 1 | — | — |
| Nonexposed | 2 | 3 | — | — | 1 | 1 | — | — | 0 | 0 | — | — |
| Atherosclerotic heart disease (410-413) | | | | | | | | | | | | |
| Exposed | 954 | 993 | 96 | 90-102 | 48 | 54 | 89 | 66-118 | 46 | 55 | 84 | 62-112 |
| Nonexposed | 334 | 297 | 112 ^c | 101-125 | 27 | 30 | 89 | 59-129 | 26 | 20 | 131 | 85-192 |
| All vascular lesions of the CNS (430-438) | | | | | | | | | | | | |
| Exposed | 170 | 169 | 100 | 86-117 | 18 | 21 | 84 | 50-133 | 10 | 22 | 45 ^c | 22-83 |
| Nonexposed | 60 | 53 | 113 | 86-146 | 8 | 12 | 67 | 29-131 | 9 | 9 | 104 | 48-197 |
| Emphysema (492) | | | | | | | | | | | | |
| Exposed | 35 | 37 | 94 | 66-131 | 2 | 1 | — | — | 0 | 2 | — | — |
| Nonexposed | 11 | 11 | 98 | 49-176 | 1 | 1 | — | — | 0 | 1 | — | — |
| Cirrhosis of liver (571) | | | | | | | | | | | | |
| Exposed | 74 | 88 | 85 | 66-106 | 3 | 5 | 57 | 12-166 | 2 | 11 | 19 ^c | 2-68 |
| Nonexposed | 21 | 24 | 86 | 53-132 | 2 | 4 | — | — | 5 | 3 | 154 | 50-360 |
| Accidents (800-949) | | | | | | | | | | | | |
| Exposed | 207 | 283 | 73 ^c | 64-84 | 9 | 9 | 99 | 45-188 | 25 | 32 | 79 | 51-117 |
| Nonexposed | 46 | 77 | 60 ^c | 44-80 | 6 | 7 | 84 | 31-184 | 9 | 10 | 90 | 41-171 |
| Motor vehicle accidents (810-827) | | | | | | | | | | | | |
| Exposed | 105 | 144 | 73 ^c | 60-88 | 5 | 5 | 110 | 36-258 | 13 | 14 | 93 | 50-159 |
| Nonexposed | 24 | 37 | 64 ^c | 41-96 | 4 | 4 | — | — | 3 | 4 | — | — |
| Cancers | | | | | | | | | | | | |
| Buccal cavity and pharynx (140-149) | | | | | | | | | | | | |
| Exposed | 18 | 19 | 96 | 57-152 | 0 | 1 | — | — | 3 | 2 | — | — |
| Nonexposed | 3 | 6 | 54 | 11-157 | 0 | 1 | — | — | 3 | 1 | — | — |
| Digestive organs (150-159) | | | | | | | | | | | | |
| Exposed | 136 | 153 | 89 | 75-105 | 11 | 15 | 72 | 36-128 | 10 | 14 | 71 | 34-131 |
| Nonexposed | 57 | 47 | 121 | 92-157 | 7 | 9 | 77 | 31-159 | 5 | 5 | 96 | 31-224 |
| Esophagus (150) | | | | | | | | | | | | |
| Exposed | 13 | 13 | 99 | 53-169 | 0 | 0 | — | — | 1 | 3 | — | — |
| Nonexposed | 8 | 4 | 202 | 88-400 | 0 | 0 | — | — | 2 | 1 | — | — |
| Stomach (151) | | | | | | | | | | | | |
| Exposed | 24 | 29 | 83 | 53-124 | 1 | 2 | — | — | 2 | 3 | — | — |
| Nonexposed | 8 | 9 | 85 | 37-167 | 1 | 1 | — | — | 0 | 1 | — | — |
| Colon (153) | | | | | | | | | | | | |
| Exposed | 42 | 48 | 87 | 63-117 | 7 | 7 | 106 | 43-218 | 5 | 3 | 186 | 60-434 |
| Nonexposed | 20 | 15 | 137 | 84-212 | 5 | 4 | 127 | 41-295 | 1 | 1 | — | — |
| Rectum (154) | | | | | | | | | | | | |
| Exposed | 16 | 17 | 93 | 53-151 | 1 | 2 | — | — | 1 | 1 | — | — |
| Nonexposed | 9 | 5 | 166 | 76-315 | 0 | 1 | — | — | 1 | 0 | — | — |
| Liver (155-156) | | | | | | | | | | | | |
| Exposed | 11 | 11 | 102 | 51-182 | 1 | 1 | — | — | 0 | 1 | — | — |
| Nonexposed | 1 | 3 | — | — | 0 | 1 | — | — | 0 | 0 | — | — |
| Pancreas (157) | | | | | | | | | | | | |
| Exposed | 23 | 30 | 76 | 48-114 | 1 | 3 | — | — | 1 | 2 | — | — |
| Nonexposed | 9 | 9 | 100 | 46-189 | 0 | 2 | — | — | 1 | 1 | — | — |
| Respiratory System (160-163) | | | | | | | | | | | | |
| Exposed | 215 | 192 | 112 | 97-128 | 8 | 7 | 121 | 52-238 | 11 | 16 | 68 | 34-122 |
| Nonexposed | 53 | 56 | 95 | 71-124 | 7 | 4 | 163 | 66-335 | 3 | 5 | 55 | 11-161 |
| Nose (160) | | | | | | | | | | | | |
| Exposed | 2 | 2.2 | — | — | 0 | 0 | — | — | 0 | 0 | — | — |
| Nonexposed | 0 | .6 | — | — | 0 | 0 | — | — | 0 | 0 | — | — |

^a OBS=observed No.; EXP=expected No.; CI=confidence interval. —=SMR not presented when observed and expected numbers <5.^b Eighth Revision codes of the International Classification of Disease (ICD). CNS=central nervous system.^c P≤.05.

TABLE 2 (continued).—Mortality from cancer and other major causes of death by race-sex and formaldehyde-exposure category, <0.1 ppm and ≥0.1 ppm^a

| Cause (ICD) ^b of death | White men | | | | White women | | | | Black men | | | |
|---|-----------|-----|-----|--------|-------------|-----|-----|--------|-----------|-----|-----|--------|
| | OBS | EXP | SMR | 95% CI | OBS | EXP | SMR | 95% CI | OBS | EXP | SMR | 95% CI |
| Cancers | | | | | | | | | | | | |
| Larynx (161) | | | | | | | | | | | | |
| Exposed | 12 | 8 | 142 | 73-248 | 0 | 0 | — | — | 0 | 1 | — | — |
| Nonexposed | 4 | 3 | — | — | 0 | 0 | — | — | 0 | 0 | — | — |
| Lung (162-163) | | | | | | | | | | | | |
| Exposed | 201 | 182 | 111 | 96-127 | 8 | 6 | 127 | 55-250 | 11 | 15 | 73 | 37-131 |
| Nonexposed | 49 | 53 | 93 | 69-123 | 7 | 4 | 170 | 68-351 | 3 | 5 | 59 | 12-173 |
| Bone (170) | | | | | | | | | | | | |
| Exposed | 4 | 3 | — | — | 0 | 0 | — | — | 0 | 0 | — | — |
| Nonexposed | 0 | 1 | — | — | 0 | 0 | — | — | 0 | 0 | — | — |
| Skin (172-173) | | | | | | | | | | | | |
| Exposed | 10 | 12 | 80 | 38-147 | 1 | 1 | — | — | 0 | 0 | — | — |
| Nonexposed | 2 | 3 | — | — | 1 | 1 | — | — | 0 | 0 | — | — |
| Breast (female) (174) | | | | | | | | | | | | |
| Exposed | — | — | — | — | 12 | 15 | 81 | 42-142 | — | — | — | — |
| Nonexposed | — | — | — | — | 10 | 10 | 104 | 50-192 | — | — | — | — |
| Cervix (180) | | | | | | | | | | | | |
| Exposed | — | — | — | — | 3 | 3 | — | — | — | — | — | — |
| Nonexposed | — | — | — | — | 5 | 2 | 219 | 71-512 | — | — | — | — |
| Uterus (181-182) | | | | | | | | | | | | |
| Exposed | — | — | — | — | 3 | 2 | — | — | — | — | — | — |
| Nonexposed | — | — | — | — | 2 | 1 | — | — | — | — | — | — |
| All uterus (180-182) | | | | | | | | | | | | |
| Exposed | — | — | — | — | 6 | 6 | 98 | 36-214 | — | — | — | — |
| Nonexposed | — | — | — | — | 7 | 4 | 184 | 74-379 | — | — | — | — |
| Other genital organs, female (183-184) | | | | | | | | | | | | |
| Exposed | — | — | — | — | 6 | 5 | 120 | 44-262 | — | — | — | — |
| Nonexposed | — | — | — | — | 3 | 3 | — | — | — | — | — | — |
| All genital organs, female (180-184) | | | | | | | | | | | | |
| Exposed | — | — | — | — | 12 | 11 | 108 | 56-188 | — | — | — | — |
| Nonexposed | — | — | — | — | 10 | 7 | 143 | 68-263 | — | — | — | — |
| Prostate gland (185) | | | | | | | | | | | | |
| Exposed | 33 | 29 | 115 | 79-162 | — | — | — | — | 2 | 3 | — | — |
| Nonexposed | 4 | 9 | 44 | 12-113 | — | — | — | — | 2 | 1 | — | — |
| Testis (186-187) | | | | | | | | | | | | |
| Exposed | 3 | 5 | 56 | 12-164 | — | — | — | — | 0 | 0 | — | — |
| Nonexposed | 2 | 1 | — | — | — | — | — | — | 0 | 0 | — | — |
| Bladder (188) | | | | | | | | | | | | |
| Exposed | 14 | 15 | 96 | 52-161 | 1 | 1 | — | — | 0 | 1 | — | — |
| Nonexposed | 2 | 5 | 44 | 5-158 | 0 | 0 | — | — | 0 | 0 | — | — |
| Kidney (189) | | | | | | | | | | | | |
| Exposed | 18 | 15 | 123 | 73-194 | 0 | 1 | — | — | 0 | 1 | — | — |
| Nonexposed | 3 | 4 | — | — | 0 | 1 | — | — | 0 | 0 | — | — |
| Eye (190) | | | | | | | | | | | | |
| Exposed | 2 | 1 | — | — | 0 | 0 | — | — | 0 | 0 | — | — |
| Nonexposed | 0 | 0 | — | — | 0 | 0 | — | — | 0 | 0 | — | — |
| Brain and CNS (191-192) | | | | | | | | | | | | |
| Exposed | 17 | 21 | 81 | 47-130 | 0 | 2 | — | — | 1 | 1 | — | — |
| Nonexposed | 6 | 6 | 102 | 38-223 | 0 | 1 | — | — | 1 | 0 | — | — |
| Thyroid (193) | | | | | | | | | | | | |
| Exposed | 0 | 1 | — | — | 0 | 0 | — | — | 0 | 0 | — | — |
| Nonexposed | 0 | 0 | — | — | 0 | 0 | — | — | 0 | 0 | — | — |
| Lymphosarcoma and reticulosarcoma (200) | | | | | | | | | | | | |
| Exposed | 7 | 14 | 52 | 21-106 | 0 | 1 | — | — | 0 | 1 | — | — |
| Nonexposed | 4 | 4 | — | — | 0 | 1 | — | — | 0 | 0 | — | — |
| Hodgkin's disease (201) | | | | | | | | | | | | |
| Exposed | 14 | 10 | 142 | 78-238 | 1 | 1 | — | — | 2 | 1 | — | — |
| Nonexposed | 0 | 3 | — | — | 0 | 0 | — | — | 0 | 0 | — | — |
| Leukemia (204-207) | | | | | | | | | | | | |
| Exposed | 19 | 24 | 80 | 48-124 | 0 | 2 | — | — | 1 | 1 | — | — |
| Nonexposed | 4 | 7 | 58 | 16-148 | 1 | 1 | — | — | 0 | 0 | — | — |

^a OBS=observed No.; EXP=expected No.; CI=confidence interval. —=SMR not presented when observed and expected numbers <5.^b Eighth Revision codes of the International Classification of Disease (ICD). CNS=central nervous system.

TABLE 2 (continued).—Mortality from cancer and other major causes of death by race-sex and formaldehyde-exposure category, <0.1 ppm and ≥0.1 ppm^a

| Cause (ICD) ^b of death | White men | | | | White women | | | | Black men | | | |
|---|-----------|-----|---------|--------|-------------|-----|--------|--------|-----------|-----|--------|--------|
| | OBS | EXP | SMR | 95% CI | OBS | EXP | SMR | 95% CI | OBS | EXP | SMR | 95% CI |
| Cancers | | | | | | | | | | | | |
| Other lymphatic tissue (202, 203, 208) | | | | | | | | | | | | |
| Exposed | 15 | 13 | 112 | 62-184 | 1 | 1 | — | — | 0 | 1 | — | — |
| Nonexposed | 5 | 4 | 132 | 43-308 | 1 | 1 | — | — | 0 | 0 | — | — |
| All lymphatic and hematopoietic cancers (200-209) | | | | | | | | | | | | |
| Exposed | 56 | 62 | 91 | 68-118 | 2 | 5 | 38 | 5-137 | 3 | 4 | — | — |
| Nonexposed | 14 | 18 | 80 | 44-134 | 2 | 4 | — | — | 0 | 1 | — | — |
| No. of persons | | | | | | | | | | | | |
| Exposed | | | 16,962 | | | | 1,604 | | | | 1,467 | |
| Nonexposed | | | 5,562 | | | | 1,755 | | | | 555 | |
| No. of person-yr | | | | | | | | | | | | |
| Exposed | | | 378,497 | | | | 38,087 | | | | 28,437 | |
| Nonexposed | | | 100,237 | | | | 33,032 | | | | 8,884 | |

^a OBS=observed No.; EXP=expected No.; CI=confidence interval. —=SMR not presented when observed and expected numbers <5.^b Eighth Revision codes of the International Classification of Disease (ICD). CNS=central nervous system.

The SMR for cancer of the prostate gland, however, were 76 among the nonexposed, 80 and 93 among the lower exposure categories, and 138 among those in the highest exposure category. For Hodgkin's disease, SMR were 0, 58, 180, and 154 for the cumulative exposure categories of 0, <.5, .5-5.4, and ≥5.5 ppm-year, respectively. The risk of lung cancer was greater in each exposure category than that among the nonexposed, due to the deficit in risk among the nonexposed; but no trend of rising lung cancer risk was seen with increasing level of cumulative exposure. Likewise, no trend was seen for duration of exposure where SMR for the 20-year latency

group were 130 (52 deaths) for <1 year of exposure, 103 (32 deaths) for 1-9 years, 157 (23 deaths, $P \leq .05$) for 10-19 years, and 106 (40 deaths) for ≥20 years. Lung cancer mortality, by cumulative exposure for individual plants, showed no consistent pattern. Regression of SMR on the level of cumulative exposure by plant resulted in regression coefficients ranging from -0.15 to 0.14, with four plants having negative slopes and six having positive slopes. The mean of these coefficients did not differ significantly from 0 (t -statistic=0.73). A similar evaluation of regression coefficients for lung cancer SMR for the greater than 20-year latency group produced a t -statistic

TABLE 3.—Mortality for selected cancers by intensity of exposure, white men^a

| Cancer (ICD) ^b | Mortality after intensity of formaldehyde exposure at ppm: | | | | | | | | | | | | | | | χ^2 for
SMR trend |
|--|--|------------------|-----------------|---------|------------------|-----------------|---------|------------------|-----------------|---------|------------------|-----------------|------------|------------------|-----------------|---------------------------|
| | 0 | | | >0-<0.1 | | | 0.1-0.4 | | | 0.5-1.9 | | | ≥ 2.0 | | | |
| | OBS | SMR ^c | RR ^d | OBS | SMR ^c | RR ^d | OBS | SMR ^c | RR ^d | OBS | SMR ^c | RR ^d | OBS | SMR ^c | RR ^d | |
| Buccal cavity and
pharynx (140-149) | 2 | 89 | 100 | 1 | 30 | 34 | 4 | 59 | 65 | 14 | 130 | 141 | 0 | — | — | 0.98 |
| Liver (155-156) | 0 | — | — | 1 | 49 | 100 | 5 | 126 | 258 | 5 | 80 | 155 | 1 | 170 | 612 | 0.33 |
| Lung (162-163) | 14 | 68 | 100 | 35 | 110 | 166 | 70 | 106 | 159 | 125 | 119 | 178 | 6 | 55 | 79 | 0.05 |
| Skin (172-173) | 1 | 76 | 100 | 1 | 48 | 46 | 4 | 83 | 77 | 5 | 72 | 77 | 1 | 146 | 117 | 0.15 |
| Prostate gland (185) | 3 | 76 | 100 | 1 | 19 | 26 | 14 | 130 | 190 | 16 | 97 | 139 | 3 | 211 | 307 | 1.36 |
| Bladder (188) | 0 | — | — | 2 | 75 | 100 | 5 | 93 | 141 | 9 | 107 | 162 | 0 | — | — | 0.13 |
| Kidney (189) | 3 | 176 | 100 | 0 | — | — | 4 | 75 | 66 | 13 | 154 | 130 | 1 | 117 | 81 | 0.91 |
| Brain (191-192) | 2 | 89 | 100 | 4 | 110 | 105 | 4 | 51 | 43 | 11 | 93 | 74 | 2 | 165 | 111 | 0.76 |
| Hodgkin's disease (201) | 0 | — | — | 0 | — | — | 3 | 76 | 100 | 10 | 185 | 245 | 1 | 196 | 226 | 5.47 |
| Leukemia (204-207) | 0 | — | — | 4 | 95 | 100 | 4 | 44 | 46 | 14 | 104 | 103 | 1 | 78 | 63 | 1.96 |
| No. of persons | 2,676 | | | 3,140 | | | 9,166 | | | 9,004 | | | 747 | | | |
| No. of person-yr | 37,005 | | | 63,237 | | | 157,177 | | | 202,706 | | | 18,618 | | | |

^a OBS=observed No. —=no deaths.^b Eighth Revision codes of the International Classification of Disease (ICD).^c SMR based on national rates.^d RR based on direct age-adjusted rates among exposed compared to those of nonexposed or to those of the lowest exposure category with deaths.

TABLE 4.—Mortality from selected causes of death by cumulative exposure to formaldehyde, white men^a

| Cause (ICD) ^b of death | Formaldehyde exposure, ppm-yr | OBS | EXP | SMR | 95% CI |
|---|-------------------------------|-------|-------|------------------|---------|
| All causes (1-999) | 0 | 292 | 353 | 83 ^c | 74-93 |
| | ≤5 | 1,435 | 1,254 | 114 ^c | 109-120 |
| | .51-5.5 | 1,299 | 1,414 | 92 ^c | 87-97 |
| | >5.5 | 677 | 783 | 86 ^c | 80-93 |
| All cancer (140-209) | 0 | 54 | 67 | 80 | 60-105 |
| | ≤5 | 261 | 231 | 113 | 100-128 |
| | .51-5.5 | 249 | 272 | 92 | 80-104 |
| | >5.5 | 164 | 164 | 100 | 85-117 |
| Atherosclerotic heart disease (410-413) | 0 | 117 | 120 | 97 | 80-116 |
| | ≤5 | 452 | 392 | 115 ^c | 105-126 |
| | .51-5.5 | 460 | 488 | 94 | 86-103 |
| | >5.5 | 259 | 289 | 90 | 79-101 |
| Emphysema (492) | 0 | 4 | 5 | 88 | 24-225 |
| | ≤5 | 18 | 14 | 133 | 79-210 |
| | .51-5.5 | 17 | 18 | 92 | 54-147 |
| | >5.5 | 7 | 12 | 60 | 24-123 |
| Cirrhosis of liver (571) | 0 | 8 | 9 | 86 | 37-169 |
| | ≤5 | 45 | 39 | 116 | 84-155 |
| | .51-5.5 | 32 | 40 | 80 | 54-112 |
| | >5.5 | 10 | 23 | 43 ^c | 20-78 |
| Accidents (800-949) | 0 | 5 | 29 | 17 ^c | 6-40 |
| | ≤5 | 139 | 159 | 88 | 74-103 |
| | .51-5.5 | 74 | 123 | 60 ^c | 47-75 |
| | >5.5 | 35 | 49 | 72 ^c | 50-100 |
| Motor vehicle accidents (810-827) | 0 | 3 | 14 | 22 ^c | 4-64 |
| | ≤5 | 78 | 84 | 92 | 73-116 |
| | .51-5.5 | 35 | 61 | 57 ^c | 40-80 |
| | >5.5 | 13 | 22 | 59 | 31-100 |
| Cancers | | | | | |
| Buccal cavity and pharynx (140-149) | 0 | 2 | 2 | 89 | 11-323 |
| | ≤5 | 10 | 8 | 132 | 63-242 |
| | .51-5.5 | 5 | 9 | 56 | 18-131 |
| | >5.5 | 4 | 5 | 73 | 20-186 |
| Digestive organs (150-159) | 0 | 13 | 19 | 67 | 36-115 |
| | ≤5 | 77 | 61 | 126 | 100-158 |
| | .51-5.5 | 62 | 75 | 82 | 63-106 |
| | >5.5 | 41 | 44 | 93 | 67-126 |
| Esophagus (150) | 0 | 0 | 2 | — | — |
| | ≤5 | 8 | 5 | 155 | 67-305 |
| | .51-5.5 | 7 | 6 | 110 | 44-228 |
| | >5.5 | 6 | 4 | 151 | 55-328 |
| Stomach (151) | 0 | 3 | 4 | 76 | 16-221 |
| | ≤5 | 14 | 12 | 120 | 65-200 |
| | .51-5.5 | 8 | 15 | 55 | 24-108 |
| | >5.5 | 7 | 8 | 88 | 35-181 |
| Colon (153) | 0 | 7 | 6 | 118 | 47-242 |
| | ≤5 | 19 | 19 | 99 | 60-155 |
| | .51-5.5 | 22 | 24 | 93 | 58-141 |
| | >5.5 | 14 | 14 | 99 | 54-166 |
| Rectum (154) | 0 | 3 | 2 | 134 | 28-392 |
| | ≤5 | 10 | 7 | 145 | 70-267 |
| | .51-5.5 | 7 | 9 | 82 | 33-168 |
| | >5.5 | 5 | 5 | 102 | 33-238 |
| Liver (155-156) | 0 | 0 | 1 | — | — |
| | ≤5 | 6 | 4 | 138 | 51-300 |
| | .51-5.5 | 4 | 5 | 74 | 20-189 |
| | >5.5 | 2 | 3 | 65 | 8-236 |
| Pancreas (157) | 0 | 0 | 4 | — | — |
| | ≤5 | 16 | 12 | 134 | 76-217 |
| | .51-5.5 | 11 | 15 | 75 | 37-134 |
| | >5.5 | 5 | 9 | 56 | 18-130 |
| Respiratory system (160-163) | 0 | 17 | 22 | 77 | 45-124 |
| | ≤5 | 95 | 76 | 124 ^c | 101-152 |
| | .51-5.5 | 92 | 91 | 101 | 82-124 |
| | >5.5 | 64 | 59 | 109 | 84-139 |

^a OBS=observed No.; EXP=expected No.; CI=confidence interval. —=no deaths.^b Eighth Revision codes of the International Classification of Disease (ICD). CNS=central nervous system.^c $P \leq .05$.

TABLE 4 (continued).—Mortality from selected causes of death by cumulative exposure to formaldehyde, white men^a

| Cause (ICD) ^b of death | Formaldehyde exposure, ppm-yr | OBS | EXP | SMR | 95% CI |
|--|-------------------------------|---------|-----|-----|--------|
| Cancers | | | | | |
| Nasal (160) | 0 | 0 | 0.2 | — | — |
| | ≤5 | 1 | 1 | 100 | — |
| | .51-5.5 | 0 | 1 | — | — |
| | >5.5 | 1 | 1 | 100 | — |
| Larynx (161) | 0 | 3 | 1 | 292 | 60-853 |
| | ≤5 | 6 | 3 | 180 | 66-391 |
| | .51-5.5 | 6 | 4 | 147 | 54-320 |
| | >5.5 | 1 | 3 | 39 | 1-218 |
| Lung (162-163) | 0 | 14 | 21 | 68 | 37-113 |
| | ≤5 | 88 | 72 | 122 | 98-150 |
| | .51-5.5 | 86 | 86 | 100 | 80-124 |
| | >5.5 | 62 | 56 | 111 | 85-143 |
| Skin (172-173) | 0 | 1 | 1 | 76 | 2-425 |
| | ≤5 | 4 | 6 | 70 | 19-178 |
| | .51-5.5 | 5 | 6 | 87 | 28-203 |
| | >5.5 | 2 | 3 | 65 | 8-235 |
| Prostate gland (185) | 0 | 3 | 4 | 76 | 16-222 |
| | ≤5 | 8 | 10 | 80 | 35-159 |
| | .51-5.5 | 14 | 15 | 93 | 51-155 |
| | >5.5 | 12 | 9 | 138 | 71-240 |
| Bladder (188) | 0 | 0 | 2 | — | — |
| | ≤5 | 4 | 5 | 74 | 20-189 |
| | .51-5.5 | 9 | 7 | 121 | 56-230 |
| | >5.5 | 3 | 4 | 68 | 14-199 |
| Kidney (189) | 0 | 3 | 2 | 176 | 36-516 |
| | ≤5 | 6 | 6 | 100 | 37-218 |
| | .51-5.5 | 6 | 7 | 86 | 32-188 |
| | >5.5 | 6 | 4 | 141 | 52-306 |
| Brain and CNS (191-192) | 0 | 2 | 2 | 89 | 11-323 |
| | ≤5 | 12 | 10 | 124 | 64-216 |
| | .51-5.5 | 6 | 10 | 63 | 23-136 |
| | >5.5 | 3 | 5 | 56 | 12-165 |
| Lymphoma and reticulosarcoma (200) | 0 | 3 | 1 | 203 | 42-592 |
| | ≤5 | 2 | 6 | 34 | 4-121 |
| | .51-5.5 | 4 | 6 | 63 | 17-160 |
| | >5.5 | 2 | 4 | 56 | 7-202 |
| Hodgkin's disease (201) | 0 | 0 | 1 | — | — |
| | ≤5 | 3 | 5 | 58 | 12-170 |
| | .51-5.5 | 8 | 4 | 180 | 78-355 |
| | >5.5 | 3 | 2 | 154 | 32-449 |
| Leukemia (204-207) | 0 | 0 | 3 | — | — |
| | ≤5 | 9 | 11 | 84 | 38-160 |
| | .51-5.5 | 9 | 11 | 79 | 36-151 |
| | >5.5 | 5 | 6 | 83 | 27-193 |
| Other lymphatic tissue (202, 203, 208) | 0 | 2 | 1 | 135 | 16-487 |
| | ≤5 | 8 | 5 | 148 | 64-291 |
| | .51-5.5 | 6 | 6 | 95 | 35-207 |
| | >5.5 | 4 | 4 | 100 | 27-255 |
| All lymphatic and hematopoietic cancer (200-209) | 0 | 6 | 7 | 88 | 32-191 |
| | ≤5 | 22 | 28 | 80 | 50-120 |
| | .51-5.5 | 28 | 29 | 97 | 64-140 |
| | >5.5 | 14 | 16 | 88 | 48-148 |
| Number | 0 | 2,638 | | | |
| | ≤5 | 17,769 | | | |
| | .51-5.5 | 10,484 | | | |
| | >5.5 | 3,767 | | | |
| Person-yr | 0 | 37,002 | | | |
| | ≤5 | 209,890 | | | |
| | .51-5.5 | 164,531 | | | |
| | >5.5 | 67,310 | | | |

^a OBS=observed No.; EXP=expected No.; CI=confidence interval. —=no deaths.^b Eighth Revision codes of the International Classification of Disease (ICD). CNS=central nervous system.

TABLE 5.—Mortality from subsites of cancer of the buccal cavity and pharynx by cumulative exposure, ppm-years, to formaldehyde^a

| Cancer | Mortality after formaldehyde exposure at: | | | | | | | | | | | |
|-------------------------------|---|-----|-----|------------|-----|------------------|-----------------|-----|-----|-------------|-----|-----|
| | 0 ppm-yr | | | ≤.5 ppm-yr | | | 0.51-5.5 ppm-yr | | | >5.5 ppm-yr | | |
| | OBS | EXP | SMR | OBS | EXP | SMR | OBS | EXP | SMR | OBS | EXP | SMR |
| Lip | 0 | 0.1 | — | 1 | 0.2 | 477 | 0 | 0.2 | — | 1 | 0.1 | 764 |
| Tongue | 0 | 0.5 | — | 0 | 1.8 | — | 2 | 2.1 | 96 | 0 | 1.3 | — |
| Salivary glands | 0 | 0.2 | — | 0 | 0.5 | — | 0 | 0.6 | — | 0 | 0.3 | — |
| Gum, floor, other mouth sites | 0 | 0.4 | — | 1 | 1.5 | 66 | 0 | 1.8 | — | 1 | 1.1 | 88 |
| Nasopharynx | 1 | 0.2 | 530 | 2 | 0.7 | 271 | 2 | 0.8 | 256 | 2 | 0.5 | 433 |
| Oropharynx | 0 | 0.3 | — | 4 | 0.9 | 443 ^b | 1 | 1.0 | 95 | 0 | 0.7 | — |
| Hypopharynx | 1 | 0.2 | 594 | 1 | 0.6 | 172 | 0 | 0.7 | — | 0 | 0.4 | — |
| Other pharynx | 0 | 0.4 | — | 1 | 1.4 | 73 | 0 | 1.6 | — | 0 | 1.0 | — |

^a OBS=observed No.; EXP=expected No. —=no deaths.^b $P \leq .05$.

of 0.65. Although numbers were too small for similar analyses for Hodgkin's disease and prostate cancer, inspection of observed and expected numbers revealed no consistency by exposure categories among the plants.

Mortality by cumulative exposure is shown for subsites of buccal cavity and pharynx in table 5. SMR for cancer of the nasopharynx were elevated in all categories of exposure, including the nonexposed. For cancer of the oropharynx, there was a significant excess (SMR=443) in the lowest formaldehyde exposure category (≤0.5 ppm-yr) but not in higher exposure categories. Observed and expected numbers for cancer of the nasopharynx by cumulative exposure ≥20 years after first exposure were for 0 ppm-year (1 observed, 0.1 expected), <0.5 ppm-year (0 observed, 0.3 expected), 0.5-5.5 ppm-yr (1 observed, 0.4 expected), and >5.5 ppm-yr (2 observed, 0.3 expected).

Mortality was also examined by peak exposure and average exposure. Patterns were similar to those for intensity and cumulative exposure; for brevity they are not presented here. Calculation of rate ratios for lung cancer by intensity and peak exposure categories directly adjusted to the person-year distribution of the entire cohort revealed patterns similar to those for SMR.

The nonexposed (to formaldehyde) category included a higher proportion of salaried workers than the propor-

tion of those in the exposed categories, and socioeconomic differences between wage and salaried workers could confound certain comparisons. To evaluate this issue, we analyzed separately wage and salaried workers (table 6). SMR for all causes combined were significantly depressed among nonexposed salaried workers (SMR=64) but not among wage workers (SMR=96). Among the nonexposed, salaried workers had a lower SMR for lung cancer than did wage workers (SMR of 50 and 86, respectively), while the converse occurred for prostate cancer (SMR of 119 and 60). SMR for lung cancer among those exposed to formaldehyde ranged from 115 to 128 for wage workers and from 43 to 94 among salaried workers, with no evidence of rising cancer risks with increasing level of cumulative exposure in either group. SMR for prostate cancer were slightly elevated among the highest exposure categories for both wage (SMR=135) and salaried (SMR=155) workers but did not rise consistently with increasing exposure. Mortality by cumulative exposure and by time since first exposure to formaldehyde is shown for white male wage workers in table 7. The SMR of 132 for lung cancer was statistically significant for persons with wage jobs after 20 or more years since first exposure, but no consistently rising risk with cumulative exposure was seen. The chi-square test for trend was significant for

TABLE 6.—SMR by cumulative exposure, ppm-years, for wage or salaried workers, white men^a

| Cause of death | Pay category | Mortality after formaldehyde exposure at: | | | | | | | | | | | |
|-----------------------|--------------|---|-----|-----------------|------------|-------|------------------|---------------|-------|-----------------|-------------|-----|-----------------|
| | | 0 ppm-yr | | | ≤.5 ppm-yr | | | .5-5.5 ppm-yr | | | >5.5 ppm-yr | | |
| | | OBS | EXP | SMR | OBS | EXP | SMR | OBS | EXP | SMR | OBS | EXP | SMR |
| All causes | Wage | 142 | 148 | 96 | 1,279 | 1,070 | 120 ^b | 1,138 | 1,150 | 99 | 595 | 640 | 93 |
| | Salary | 101 | 158 | 64 ^b | 46 | 75 | 61 ^b | 136 | 241 | 56 ^b | 81 | 142 | 57 ^b |
| Lung cancer | Wage | 7 | 8 | 86 | 79 | 61 | 128 ^b | 78 | 68 | 115 | 55 | 45 | 123 |
| | Salary | 5 | 10 | 50 | 4 | 4 | 94 | 7 | 16 | 43 ^b | 7 | 11 | 64 |
| Prostate gland cancer | Wage | 1 | 2 | 60 | 8 | 8 | 95 | 12 | 13 | 95 | 10 | 7 | 135 |
| | Salary | 2 | 2 | 119 | 0 | 0 | — | 2 | 2 | 92 | 2 | 1 | 155 |
| Hodgkin's disease | Wage | 0 | 0 | — | 2 | 4 | 45 | 5 | 4 | 140 | 2 | 2 | 128 |
| | Salary | 0 | 0 | — | 0 | 0 | — | 2 | 1 | 246 | 1 | 0 | 260 |

^a Numbers of deaths are less than those of other tables because all subjects could not be assigned to wage or salary categories. OBS=observed No.; EXP=expected No. —=no deaths.^b $P \leq .05$.

TABLE 7.—SMR by cumulative exposure, ppm-years, to formaldehyde and time since first exposure among wage workers, white men^a

| Time, yr since
first exposure | Cancer | Mortality after exposure to formaldehyde at: | | | | | | | | | | | | | | |
|----------------------------------|----------------|--|-----|-----|------------|-----|------------------|---------------|-----|------------------|-------------|-----|-----|-------|-----|------------------|
| | | 0 ppm-yr | | | ≤ 5 ppm-yr | | | .5-5.5 ppm-yr | | | >5.5 ppm-yr | | | Total | | |
| | | OBS | EXP | SMR | OBS | EXP | SMR | OBS | EXP | SMR | OBS | EXP | SMR | OBS | EXP | SMR |
| <10 | Lung | 1 | 1 | 105 | 7 | 7 | 100 | 8 | 7 | 114 | 4 | 2 | 229 | 20 | 17 | 120 |
| | Prostate gland | 0 | 0 | — | 0 | 1 | — | 0 | 1 | — | 0 | 0 | — | 0 | 2 | — |
| 10-19 | Lung | 1 | 2 | 49 | 23 | 18 | 126 | 17 | 22 | 78 | 7 | 10 | 72 | 48 | 52 | 93 |
| | Prostate gland | 1 | 0 | 250 | 3 | 2 | 138 | 2 | 3 | 60 | 2 | 1 | 152 | 8 | 7 | 111 |
| ≥20 | Lung | 5 | 5 | 97 | 49 | 36 | 135 ^b | 53 | 39 | 135 ^b | 44 | 33 | 132 | 151 | 114 | 132 ^b |
| | Prostate gland | 0 | 1 | — | 5 | 5 | 95 | 10 | 8 | 120 | 8 | 6 | 136 | 23 | 21 | 112 |
| Total | Lung | 7 | 8 | 86 | 79 | 61 | 128 ^b | 78 | 68 | 115 | 55 | 45 | 123 | 219 | 182 | 120 ^b |
| | Prostate gland | 1 | 2 | 60 | 8 | 8 | 95 | 12 | 13 | 95 | 10 | 7 | 135 | 31 | 30 | 103 |

^a OBS=observed No.; EXP=expected No. —=no deaths.^b $P \leq .05$.

the <10-year category where SMR for lung cancer rose from 105 among nonexposed to 229 among the >5.5-ppm-year exposure category. Twenty years after first exposure, the SMR for prostate cancer rose with cumulative exposure to 136 in the highest exposure category. No clear pattern was seen for Hodgkin's disease, although few deaths occurred.

For evaluation of the effect of exposure to formaldehyde-containing particulates, SMR by cumulative exposure were calculated separately for workers employed in dusty areas and those never employed in such areas. No significant excesses were found in either group. The SMR were higher among those with particulate exposure than among those never exposed to dusts, for cancer of the buccal cavity and pharynx (SMR=98 and 71), for cancer of the brain (SMR=107 and 55), and for Hodgkin's disease (SMR=163 and 38). Analysis of causes of death by level of exposure did not reveal any consistent associations among either the particulate- or nonparticulate-exposed workers.

Creation of longer latency and higher cumulative exposure categories failed to show further increases in risk in the extended strata. For all workers, the SMR in these extended categories for latency were 104 (21 deaths) for the category <10 years since first exposure, 85 (53 deaths) for 10-19 years, 126 (119 deaths, significant at $P \leq .05$) for 20-29 years, and 101 (57 deaths) for ≥30 years. For cumulative exposure, the SMR for lung cancer were 106 (213 deaths) for <10 ppm-years, 128 (31

deaths) for 10-24 ppm-years, and 63 (6 deaths) for ≥25 ppm-years.

The 1950-79 cancer mortality rates for the 10 U.S. counties where these plants were located were also used to generate the expected number based on local rates (table 8). The mortality patterns with local rates by cumulative exposure were similar to those seen when mortality rates from the total U.S. population were used as the referent. SMR based on local rates generally were smaller than those with the national population as the referent. No cancer showed a significant trend, although SMR for prostate cancer did rise with exposure. Risks of cancer among workers who were continuously exposed to formaldehyde were similar to those among workers whose exposures had ceased.

DISCUSSION

Formaldehyde is a highly reactive chemical having the potential to cause local irritation and skin sensitization following repeated exposures (13, 14). It is a major industrial chemical with an annual production in the United States that exceeds 9 billion pounds (15). Inhalation studies in laboratory animals have demonstrated that formaldehyde causes squamous cell carcinomas of the nasal cavity in rats (1, 2). Mortality from cancer of the nasal cavity was not excessive either in the total cohort (2 deaths vs. 3.3 expected) or among workers exposed to formaldehyde (2 deaths vs 2.8 expected).

TABLE 8.—SMR for selected cancers by cumulative exposure, ppm-years, on the basis of local mortality rates, white men^a

| Cancer | Mortality after exposure to formaldehyde at: | | | | | | | | χ^2 for SMR trend |
|---------------------------|--|-----|-----------------|-----|--------------|-----|----------------|-----|------------------------|
| | 0 ppm-yr | | ≤ 5 ppm-yr | | 5-5.5 ppm-yr | | > 5.5 ppm-yr | | |
| | OBS | SMR | OBS | SMR | OBS | SMR | OBS | SMR | |
| All cancers | 54 | 78 | 261 | 112 | 249 | 89 | 164 | 98 | .12 |
| Buccal cavity and pharynx | 2 | 74 | 10 | 112 | 5 | 46 | 4 | 60 | .69 |
| Lung | 14 | 61 | 88 | 112 | 86 | 91 | 62 | 102 | .07 |
| Prostate gland | 3 | 71 | 8 | 76 | 14 | 87 | 12 | 129 | 1.90 |
| Hodgkin's disease | 0 | — | 3 | 56 | 8 | 177 | 3 | 155 | 1.69 |

^a OBS=observed No. —=no. deaths.

Other cohort studies of formaldehyde-exposed populations have also failed to detect an excess of nasal cancer (3). In fact, no deaths from nasal cancer have been noted in any previously reported cohort or proportionate mortality study (4, 5, 16-25). Cancer of the nose and nasal cavities is, however, very rare with an age-adjusted mortality rate in the United States of 4 per million among white men and 2 per million among white women (10); so the power to detect an excess risk in most cohort studies is small. Despite having nearly 600,000 person-years of observation, this study has only 80% power to detect a 4.0-fold increase in nasal cancer. For other cancers, however, the study can detect with 80% power the relative risks of: 1.2 for lung cancer, 2.5 for prostate cancer, 1.5 for brain cancer, 1.7 for Hodgkin's disease, and 1.7 for kidney cancer. Recent case-control studies of nasal cancer from Denmark (26) and The Netherlands (27) have noted elevated odds ratios of approximately 2 among workers exposed to formaldehyde (a relative risk smaller than this study could detect). No explanation for the discrepancy among the case-control studies from Denmark and The Netherlands and this study and other cohort or proportionate mortality studies is obvious, although the case-control studies included formaldehyde-exposed occupations that undoubtedly differed from those in the cohort studies.

Mortality from all causes combined was about as expected in the total cohort, although deficits occurred among white women and black men. Significant deficits occurred for infective and parasitic disease and for accidents among formaldehyde-exposed and nonexposed alike. Such deficits, however, are common when the mortality experience of a working population is compared to that of the general population (28, 29). Mortality from all cancers combined was about as expected among white men, although deficits occurred among exposed white women and black men. Previous reports have noted excesses of the prostate and bladder cancers among industrial workers exposed to formaldehyde (21, 30). This study showed a weak association of prostate cancer, but not bladder cancer, with indices of exposure to formaldehyde. SMR for prostate cancer were greater among salaried workers than among wage workers, suggesting socioeconomic influences. Finally, neither SMR nor tests for trends by various formaldehyde-exposure measures were statistically significant for prostate cancer.

Elevated risks for leukemia and brain cancer have been noted among embalmers, pathologists, and anatomists (16, 17, 23-25). We did not see an association between these cancers and any estimate of formaldehyde exposure (cumulative exposure, duration, time since first exposure, peak level, or presence of particulates) or any latency category and formaldehyde exposure.

Although no association between Hodgkin's disease and formaldehyde has been suggested from previous studies, we found a significantly rising SMR trend with exposure. No individual SMR for Hodgkin's disease, however, differed significantly from 100, and the SMR peaked in intermediate exposure categories. The small number of deaths from this tumor ($n=14$) precluded

detailed analyses by latency and exposure, complicating the interpretation of results.

Since inhalation is the primary route of exposure to formaldehyde among humans, tissues in the nose and nasal sinuses, buccal cavity and pharynx, and lung may all come into direct contact with formaldehyde. Formaldehyde appears to be rapidly metabolized to formate when it encounters body tissues (3, 13, 14, 31). This rapid metabolism further suggests that the tissues of primary contact may be most susceptible. The possibility of contact with remote tissues, however, cannot be entirely discounted. Among white men (the only race-sex group with sufficient numbers of deaths for detailed analysis), the SMR for cancer of the buccal cavity and pharynx were not significantly elevated at any exposure level. Workers exposed to particulates had a higher SMR for cancer of the buccal cavity and pharynx than those not exposed to particulates, but there was no increase with cumulative exposure. Analysis by subsites for cancer of the buccal cavity and pharynx indicated that the risk of cancer of the nasopharynx was significantly elevated among formaldehyde-exposed persons. The overall risk did not rise consistently with increasing intensity of exposure for this or any other subsite. The excess of nasopharyngeal cancer is, however, being evaluated further.

The relative risk of lung cancer, although slightly higher among exposed workers (SMR=111) than among nonexposed (SMR=93), was not significantly correlated with intensity or duration of exposure; cumulative, average, or peak exposure; or presence of particulates. The statistically significant excess of lung cancer among wage workers after 20 years since first exposure was unrelated to the level of exposure to formaldehyde, to the presence of particulates, or to peak exposures. In addition, neither SMR for all subjects nor the >20-year latency group showed consistent rising risks by cumulative exposure among the individual plants; and no test for trend of the SMR by exposure was statistically significant. In addition, the SMR in the ≥ 30 -year latency category fell to 101 and the SMR in the cumulative exposure category of ≥ 25 ppm-years was 63. This lack of arising risk of lung cancer with increasing levels of formaldehyde exposure is consistent with the finding of previous studies of the chemical industry in the United Kingdom (18) and the United States (30) where cumulative exposure was not related to risk of lung cancer. The largest plant from the British study did show a rising risk for lung cancer with level of formaldehyde exposure, although the relative risk in the highest level (SMR=118) was not statistically significant (19).

Limitations of this study should be considered. Despite approximately 600,000 person-years of follow-up, the power to detect moderate increases in relative risks for rare tumors, such as nasal cancer, is weak. The power for more common cancers, however, is better. As can be expected from analysis of many death causes and of exposure measures, some statistically significant excesses would occur by chance. Analyses by cumulative exposure, peak exposure, latency, and other measures,

however, failed to reveal any consistent patterns that would clearly suggest formaldehyde-related risks.

In studies of this design, estimating historical exposure levels is a crucial but difficult task. Monitoring data were available for only recent years, and exposures from earlier years had to be extrapolated from current levels. Procedures to estimate exposures used in this study were carefully designed to fully utilize available monitoring data from the plants and information on changes in plant operations and controls and to supplement these data with monitoring conducted during this study. Because of this effort, we feel the relative ranking of the jobs by formaldehyde exposure is sound. We also evaluated mortality risks by duration of exposure, a measure unaffected by estimates of exposure levels, and found no associations. We do, however, recognize the uncertainty associated with our quantitative estimates of formaldehyde levels. Furthermore, information on tobacco use was not available. The lack of a consistent elevation for tobacco-related causes of death, however, suggests that the smoking habits among this cohort did not differ substantially from those of the general population. The SMR for cancer of the lung and larynx were greater than 100, while SMR for emphysema, for cancer of the buccal cavity and pharynx, and for cancer of the bladder were less than 100. A survey of U.S. veterans employed in industries included in this study also reported patterns of tobacco use that were not dramatically different from those of the general U.S. population (32). Finally, certain cancers are unreliably reported on death certificates. For cancers of the nose and buccal cavity and pharynx, considerable underreporting occurs (33). There is no reason to suspect, however, that the degree of underreporting was associated with the level of formaldehyde exposure or that the degree of underreporting differed between the cohort and the general U.S. population.

In summary, deaths from all causes were about as expected among white men, indicating little, if any, "healthy worker" effect. Excesses seen for Hodgkin's disease and cancers of the lung and prostate gland did not show convincing rising cancer risks with cumulative formaldehyde exposure. Laboratory studies suggest that inactivation of mucous barriers may be critical in the carcinogenic process (13), so that peak exposures to formaldehyde may be particularly relevant. Analyses by peak exposures did not, however, show evidence of excess mortality from cancer. This large multiplant cohort study provided little evidence to suggest that formaldehyde exposures affected the mortality experience of these industrial workers.

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QUESTIONS AND DISCUSSION

DR. FEINMAN: Susan Feinman, CPSC. You indicated that small numbers were exposed at the highest levels. Would that possibly confound the data? Would the number exposed at greater than 2 ppm be so low that results might not be valuable?

DR. BLAIR: There were a few people exposed at high categories and the numbers are small which would lower the power.

We have included the range of exposure that exists in the workplace. We are looking at what exposure levels are actually experienced by workers.

DR. FEINMAN: But I mean statistically, is it such that it is a valid number to reach a conclusion?

DR. BLAIR: Yes, I think so. We looked at exposure in many different ways. There were 3,000 person-years up in the highest category, or something like that, which is not big.

We also looked at duration of exposure, where we had 20,000 person-years in the highest category, and at cumulative exposure, where we also had large numbers.

All of those measures of exposure give you the same answer.

DR. VAUGHAN: Tom Vaughan from the Fred Hutchinson Cancer Research Center. Are you aware of any evidence that formaldehyde is transported away from the respiratory epithelium and might, therefore, have an effect at nonrespiratory sites?

DR. BLAIR: No. Do you know of any?

DR. VAUGHAN: No, I'm fishing.

DR. BLAIR: The half-life is measured in minutes. In animal studies you really don't get in much of any increases in the blood level when they are exposed at high levels.

That's why it's hard to imagine how Hodgkin's disease and cancer of the prostate might be involved. But you don't want to dismiss something just because you don't know what happened metabolically.

DR. FLEMING: Roy Fleming from NIOSH. Given the relatively narrow ranges of exposure categories, the possibility that historical measurements contain some inaccuracies, and that analytical methods have variability, what is your sense about the possibility of misclassification of exposures and what would be the impact of that in terms of lack of a trend?

DR. BLAIR: Well, it's clear what effect misclassification on exposure has; it flattens a response gradient.

The key thing here, however, is the care with which we attempted to estimate exposures; we are confident we have ranked these properly.

That doesn't mean that there isn't some misclassification, but to totally eliminate a dose gradient you have to make classification completely at random.

I have confidence in the relative rankings of the jobs, and that's the key thing for the epidemiologic analysis.

DR. NEWELL: Gordon Newell, Electric Power Research Institute. Is Dr. Neal still here? Could I ask Bob to comment on the excess incidence of other tumors in the formaldehyde rat study in relation to what Dr. Blair said about the incidence of other tumors? It's a beautiful opportunity to combine the findings of the two studies.

DR. NEAL: Well, other than the squamous carcinoma of the nasal turbinates and a few adenocarcinomas that weren't statistically significant, we didn't see any other kind of malignant tumor. There were some benign tumors, but they were only statistically significant if you combined the data from both male and female rats. The benign tumors that we saw were papillomas. As noted before, if you combine the male and female rat incidence of papillomas there seemed to be a significant increase in those papillomas with increasing doses of formaldehyde, but if you look at each sex separately, there was not a significant increase in papillomas. Squamous cell carcinoma of the nasal turbinates was the only significant finding.

Relative to a question that was asked before concerning transport of formaldehyde away from the nasal cavity, we at CIIT did look in human volunteers for evidence of increase in formaldehyde in the blood measured in volunteers exposed by inhalation for an hour to 2 ppm, using themselves as controls. We couldn't see any evidence of an increase in blood formaldehyde. That is consistent with our evidence in animals that, in fact, you don't get transport of formaldehyde away from the nasal cavity in any amount that we can detect.

DR. MATANOSKI: Genevieve Matanoski, Johns Hopkins University. Aaron, having agonized several years ago over the beryllium question, could I ask you to think about perhaps the fact that beryllium also showed no dose-gradient for many things. The lung cancer was the main one that we were interested in.

And formaldehyde is also a highly irritative substance to individuals who are intolerant of it. Maybe you actually shouldn't be looking for a dose-gradient if people who are intolerant cannot stay in the area.

Would you comment on that?

DR. BLAIR: We were concerned about people moving in and out of jobs we looked at when people reach their highest exposed job by plant to see if they tend to get thrown into the dirty jobs early, causing them to leave the industry. That is not what we found to be the case.

In two plants, it looked like workers go into heavier exposure jobs first. In some plants they either get strung across the jobs or actually go into higher jobs in later years, probably because these are prestige jobs and higher paying jobs.

Workers, of course, can do things to moderate their exposures where possible. In some jobs, however, there isn't really much they can do about exposure. They have to stay in a particular area to work.

I relied on a dose-gradient as the primary criterion for causality.

Just because you don't see a dose-gradient, doesn't mean the exposure estimates are incorrect. You need some independent estimate to determine if there's something wrong with your exposure ranking. It should not be based on results.

The point that formaldehyde is an irritant is well taken, but we do not see evidence that workers are immediately placed in high exposure jobs.

DR. BEAUBIER: Jeff Beaubier, EPA. What happens if you combine the three groups of exposure? Because the SMRs are above 100 for all three categories, if you combine exposure groups, is it . . .

DR. BLAIR: There is an excess among the exposed.

DR. BEAUBIER: You can see statistical significance, then.

DR. BLAIR: No, not overall. There is a significant excess for the greater than 20-year category, for wage workers.

I believe that it is important to look at exposure gradients and it doesn't make sense to collapse back down.

**MORTALITY ANALYSES USING UNITED AUTO
WORKERS' COHORTS**

**Presented by Robert M. Park
United Auto Workers**

MORTALITY ANALYSES USING UNITED AUTO WORKERS' COHORTS

**Robert Park, Michael Silverstein, Neil Maizlish, Franklin Mirer
International Union, UAW, Detroit, Michigan**

April 22, 1986

**Presented at Fourth NCI/EPA/NIOSH Collaborative Workshop:
Progress on Joint Environmental and Occupational Cancer Studies**

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In 1981 the UAW received a NIOSH contract⁽¹⁾ to do six mortality studies using union-based information. Final reports have been produced for four studies and draft reports for the last two. Each study was a proportional mortality design, with analyses based on standardized proportional mortality ratios (PMR), crude and stratified mortality odds ratios (MOR), and logistic regression-based mortality odds ratios. Because proportional mortality studies are occasionally denounced as worthless, I will be making some comments in their defense.

In our belief that the ascertainment of deaths for a cohort is as important in a PMR study as in an SMR study, we have implicitly or explicitly defined a population at risk in our studies. Usually the implicit definition is related to information sources, for example, pension benefits files covering all workers with 10 or more years' service. The cohort at risk is then all workers over some time period who were ever pensioners, deferred vested employees (those who quit after at least 10 years' service and are eligible for a pension at retirement age), or active employees with 10 or more years.

Because occupational mortality studies on populations lacking work history have limited interpretability, we have placed emphasis on acquiring and configuring work histories to maximize their epidemiological value. One of our studies that did not involve work histories followed an industry-sponsored study that found lung cancer and lymphoma excesses in 10 vehicle assembly plants. Our study in four assembly plants offered only weak support for these findings and was not able to examine broad work categories, not to mention the myriad exposures that have occurred in these plants over time.⁽²⁾ Our other study lacking work history detail involved a relatively more homogeneous workplace — auto maintenance and repair workers employed by auto dealers and repair shops in New York City. In this study statistically significant associations were observed for cancer of the esophagus and nonmalignant respiratory disease with a latency-weighted duration of employment. In three of our studies we constructed work histories from old seniority and check-off lists. These are lists generated at points in time showing all active union members; their date of hire; and, typically, their current department, job classification or some other work descriptor. These valuable resources, while not always available, sometimes go back to the 1950s or before as in two of our studies. In the absence of such systematic sources, dues cards are often available, requiring a labor-intensive extraction. Sometimes employer personnel files are available. Beyond constructing the sequence of jobs or department assignments held by a worker, it is essential to collapse such categories where possible to fewer having common exposures of interest. Typically, hundreds of categories might be collapsed to say 30, and these variously grouped for particular analyses. This task of course involves considerable judgment. It requires probing discussions with groups from union and management who have long experience at the plant. It must be done prior to examining outcomes.

In a study of a grey iron foundry in Illinois, using personnel records and defining exposure groups on major department of longest duration, we found a significant excess of nonmalignant respiratory disease (PMR=1.8) consisting largely of emphysema: 10 observed for 3.5 expected.⁽³⁾ Lung cancer was also significantly elevated, PMR=1.5. The excess nonmalignant disease was concentrated in the finishing department (crude OR=3.7, $p=.004$) where castings

are chipped, ground and cleaned. Stratification on smoking, prior coal mining, or zinc foundry work did not account for this association. This study confirmed for us the continuing hazards of foundry work probably related to silica exposure and highlighted the lung cancer issue which may also pertain to silica. However, multiple other inhalation hazards exist in foundries, including aliphatic amines, aldehydes, diisocyanates, and PAHs. In studies such as this, where there were fewer than 250 deaths (in white men), traditional analytical methods lack the power to examine important distinctions in exposures.

In a study of 600 deaths in a Connecticut ball bearing plant, using seniority list and dues cards sources, we found considerable stability in workers' exposure categories over their employment career.⁽⁴⁾ Despite rather sparse work histories (most workers had departments identified at 2-4 points in time), we found a clear association between working in grinding categories 15 years prior to death and stomach cancer. A PMR of 3.8 was observed for grinders ($p=.006$), and an age-adjusted Mantel-Haenszel mortality odds ratio of 6.9 was observed for the association of stomach cancer with work in water-based cutting fluids which were used largely in grinding, with a p -value of 0.02. Fortunately, there was no association between the important potential stomach cancer confounder, birth in Central Europe, and the exposure; otherwise further stratification would have resulted in statistical nonsignificance.

A second bearing plant study involved 2,000 deaths.⁽⁵⁾ The cohort at risk was explicitly defined, and seniority list job assignments since 1946 were available at four-year intervals. This study provided the opportunity to confirm hypotheses on stomach cancer and other outcomes but also to develop more sensitive methods. First I would like to digress a moment on PMRs and SMRs.

The value of using external reference populations is that an enormous mortality experience is made available, particularly the age and chronologic dependence of cause-specific mortality. The disadvantage is that selection factors are at work, such as selection for health status at hire, selection for survival in industrial employment, and socio-economic status, itself an important mortality determinant. A key task of occupational epidemiology is to distinguish exposure and selection-related effects. Because the selection factors tend to operate in the same direction across causes of death, e.g., diminishing absolute rates of mortality across most causes, not uniformly of course, PMR studies which examine relative rates could be less sensitive to selection confounding bias than SMR studies. To investigate this, I compiled SMR estimates from studies published in Br J Ind Med, Amer J Ind Med, and JOM over the past two-three years.⁽¹⁰⁻²⁶⁾ All studies that involved greater than 200 deaths were collected. Those studies which calculated only certain cause-specific SMRs and one study using social class stratified reference rates were excluded. (Table 1) The purpose here is to examine the selection effects manifest in unexposed industrial populations, those having little overall exposure-attributable mortality. In several studies, unexposed subpopulations were identified, and these are presented here; in others there is little evidence of attributable disease in the entire cohort. I have indicated those cohorts having subgroups with statistically significant exposure-associated fatal malignant disease with an asterisk and those with attributable nonmalignant respiratory disease (NMRD) with two asterisks. The first column gives the all-causes SMR, typically less than 1.0 and ranging .72-.99. The second column shows the all-cancer SMRs. In many studies the all-cancer SMR was almost as small or smaller than the all-causes SMR, e.g., Nos. 1,3,5,11,12,13,15. The third

column gives the SMR for all NMRD; this category is one of the most sensitive indicators of healthy worker selection. The values here range as low as .44. The next column is the size of the cohort, in number of deaths. Particularly noteworthy are some of the large refinery populations showing remarkably small SMRs for all cancer and for NMRD, e.g., Nos. 13,15.

To control selection confounding, one ought to compare specific exposure subpopulations in relation to appropriate unexposed subpopulations in the same cohort. SMR analyses do not directly do this. Using SMRs to study cancer means that exposure effects will be underestimated on average because the unexposed cancer SMRs are generally less than 1.0. The next column, giving the reciprocal all-cancer SMR, is the factor by which a specific cancer on average would be underestimated in using SMRs in these cohorts. In the hypothesis testing of an observed SMR against the value 1.0, significant elevations will be missed in some cases. The next column, the all-cancer SMR divided by the all-causes SMR, is an approximation to the PMR. Using the PMR to estimate cancer risk usually would give an overestimate of the risk, as the PMR values for the unexposed population are generally greater than one. Notice, however, that the overestimation bias of the PMR is generally less than the underestimation bias of the SMR. Over the 17 studies, including some with attributable cancer, the average SMR bias was 1.11, while the average PMR bias was 1.06. When the studies with statistically significant exposure-associated cancers are omitted, the SMR and PMR biases become 1.16 and 1.03, respectively. Because some of the cohorts are considerably larger than others and thus have more precise estimates, the same calculations were done, limited to studies with 1,000 or more deaths. When studies with cancer-related exposures were excluded as well, the biases are 1.20 and 1.04 for SMRs and PMRs.

While a more comprehensive inventory of unexposed populations ought to be assembled on this issue, I believe these numbers strongly challenge the conventional wisdom regarding PMRs. Because actions rarely follow from single epidemiological studies, it is much more desirable to risk an occasional false positive finding arising from small PMR overestimations than to risk more frequent false negatives from the larger SMR underestimates.

Another problem which plagues both SMR and PMR studies is the loss of power from the stratification needed to simultaneously examine duration and latency effects. In some of our studies we have defined a latency-weighted duration measure for investigating mortality from long-latency disease. This measure assigns periods of employment or exposure a weight depending on how closely in time they preceded the death. The weighting function is shown in Table 2. The parameters defining the function were chosen based entirely on prior judgment. Consolidating latency and duration permits less stratification in ordering subpopulations at risk. Another use, however, is in modelling proportional mortality using logistic regression.

Together with Dr. William Butler at University of Michigan, we have developed a logistic regression model for proportional mortality that incorporates national mortality rates and gives simultaneous estimates of both selection and exposure effects.⁽⁶⁾ The model is as follows:

$$\ln(p/1-p) = a + \ln(r/1-r) + bX_1 + cX_2 + \dots$$

where $p/1-p$ is the odds of being a case (death due to a specific cause vs. all other causes);

$r/1-r$ is the expected odds for the same outcome, using national age-, year-, sex-, race-specific proportional mortality rates (r);

a is estimate of the (log) standardized mortality odds ratio (MOR) of the unexposed:

$$a = \ln(p/1-p)/(r/1-r) \text{ or } \exp(a) = \text{MOR given } X_i = 0;$$

b is estimate of effect of X_1 , etc.:

$$bX_1 = \ln(p/1-p) \Big|_{X_1 \neq 0} - \ln(p/1-p) \Big|_{X_1 = 0}$$

$$\text{or, } \exp(bX_1) = \text{MOR } (X_1 \neq 0 \text{ vs. } X_1 = 0)$$

The intercept, a , is an estimate of the overall selection effect on the particular disease odds relative to the reference population. Notice that the coefficient of the term $\ln(r/1-r)$ is fixed at 1.0. (This can be done in some statistical package procedures such as BMDP3R with a little effort.⁽⁷⁾)

In our second bearing plant study,⁽⁵⁾ again we found excess GI cancer proportional mortality. (Table 3) In particular, we found elevated stomach and pancreatic cancer in grinding jobs. In workers with 10 or more years' (latency-weighted duration) exposure, the PMRs for stomach and pancreatic cancer were 3.4 and 2.8, respectively. (Table 4) Applying the logistic regression model in this study, we estimated that the standardized MOR for stomach cancer associated with 25 years' work (latency-weighted duration) in grinding was 2.2 ($p=.02$). (Table 5) About 650 men had had some grinding experience. For members of the study population born in Canada (largely French Canadians), a separate statistically significant risk of stomach cancer was estimated, $\text{MOR}=2.5$. For those born in Central Europe a nonsignificant MOR of 1.8 was observed, and the estimate of the MOR for the unexposed was 1.16, a credible healthy worker elevation (corresponding to a PMR of about 1.13). This model without the birth in Central Europe term gave almost identical estimates except for the unexposed MOR, now 1.29. These findings illustrate the versatility of this method. Other findings in this study include cancer of the pancreas associated with machining in straight oil cutting fluids and lung cancer with forge and heat treat jobs. Comparison of the predicted and observed numbers of deaths in exposure strata indicates that the model fits the data quite well. (Table 6)

Another study concerned deaths of 200 men and 75 women who had worked in an electronics fabrication plant in Michigan, producing aircraft and missile instruments and control systems.⁽⁸⁾ This plant has existed since 1942, and seniority lists were obtained beginning in 1952. The exposures of concern were halogenated solvents, epoxy resins, and a variety of other polymerizing systems, cutting fluids, and soldering fumes. Over 200 job classifications and 100 departments were consolidated and profiled to produce gross rank-orderings on 5 general exposure categories: solvents, epoxy-like materials, cyanoacrylate-like materials, cutting fluids, and solder fume. PMR analyses identified excess

pancreatic cancer in men and women: 11 observed for 3.6 expected. In men the pancreatic cancer was associated with epoxy-like materials (PMR=5.2). For women there were excesses of colon and ovarian cancer as well. Exposure-stratified PMRs suggested associations but were ambiguous because multiple exposures had been experienced. Crude mortality odds ratios were uninformative for the cancer excesses in women, because most women were exposed and there were few or no unexposed cases. Logistic regression modelling disclosed a significant association between colon cancer and exposures to epoxy-like materials occurring in the 1950s. (Table 7) The goodness of fit is shown in Table 8. The ovarian cancer was significantly associated with assembly jobs using cyanoacrylate or similar agents and also with inspection jobs. Because of the near absence of unexposed cases, the intercept estimates for the MOR of the unexposed are very unstable and the exposure effect estimates are correspondingly imprecise. We believe these logistic regression-generated findings constitute important hypotheses for future testing, although small numbers and lacking prior hypotheses limit their significance.

In our study of auto repair workers in which latency-weighted durations of employment were used, the modelling could infer selection effects only by extrapolation to zero employment duration.⁽⁹⁾ This estimate, the intercept, would be expected to be quite sensitive to the shape of the weighting function and thus not reliable. Nevertheless, while the effect estimates resulting were imprecise, they suggested some important findings. For cancer of the esophagus, there was no dependence on age at death as one might expect if the cancer were related to diet or drinking behavior that differed from the reference population's. (Table 9) For nonmalignant respiratory disease, an important negative trend was observed with age at death along with the positive dependence on weighted duration of employment. (Table 10) When other measures of employment duration were used — unweighted duration or latency — the effect estimates for NMRD were not as good, suggesting that the latency-weighted measure intended for long-latency malignancies was also appropriate for NMRD, perhaps reflecting a progressive nature of the disease: continuing deterioration after exposure has stopped.

In conclusion, we believe that proportional mortality designs are legitimate and valuable means for investigating occupational disease mortality. The methods now available allow meaningful separation of selection and exposure effects in many study situations.

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Table 1

Estimates of Bias When Using SMR and PMR Analyses, from Selected Populations

| Population | SMR | All-CA
SMR | NMRD
SMR | No.
Deaths | SMR
Bias | PMR
Bias | NMRD |
|---------------------------------------|------|---------------|--------------|---------------|-----------------------------|---------------------|------|
| | | | | | All-CA ⁻¹
SMR | All-CA
PMR(est.) | |
| 1. Petrochemical ⁽¹⁰⁾ | .83 | .86 | .58 | 765 | 1.16 | 1.04 | .70 |
| 2. Plastics ⁽¹¹⁾ | .96 | 1.07* | 1.00 | 603 | .93 | 1.11 | 1.04 |
| 3. Nuclear Fuel ⁽¹²⁾ | .82 | .85 | 1.50(est.)** | 219 | 1.18 | 1.04 | 1.83 |
| 4. Refinery ⁽¹³⁾ | .76 | .91* | .44 | 640 | 1.10 | 1.20 | .58 |
| 5. Refin./Petrochem. ⁽¹⁴⁾ | .92 | .90 | .65 | 3147 | 1.11 | .98 | .71 |
| 6. Iron Ore Mining ⁽¹⁵⁾ | .93 | .97* | .79 | 4699 | 1.03 | 1.04 | .85 |
| 7. Refinery ⁽¹⁶⁾ | .87 | .97 | .69 | 3441 | 1.03 | 1.11 | .79 |
| 8. Mercury-Exposed ⁽¹⁷⁾ | .86 | .94 | .75 | 378 | 1.06 | 1.09 | .87 |
| 9. Undertakers ⁽¹⁸⁾ | .99 | .87 | .74 | 319 | 1.15 | .88 | .75 |
| 10. Paint-Coatings ⁽¹⁹⁾ | .86 | 1.00* | .81 | 2633 | 1.00 | 1.16 | .94 |
| 11. Aluminum Red. ⁽²⁰⁾ | .84 | .85 | .91 | 426 | 1.18 | 1.01 | 1.08 |
| 12. Aluminum Red. ⁽²¹⁾ | .76 | .83 | .66 | 598 | 1.21 | 1.09 | .87 |
| 13. Refin./Petrochem. ⁽²²⁾ | .75 | .75 | .52 | 4024 | 1.33 | 1.00 | .69 |
| 14. Railroad (Diesel) ⁽²³⁾ | .87 | .92 | .54 | 532 | 1.09 | 1.06 | .62 |
| 15. Refinery ⁽²⁴⁾ | .72 | .76 | .56 | 2292 | 1.32 | 1.05 | .78 |
| 16. Const. (Diesel) ⁽²⁵⁾ | .81 | .93* | .84** | 3345 | 1.08 | 1.15 | 1.04 |
| 17. Cement ⁽²⁶⁾ | .95 | 1.03* | .86 | 419 | .97 | 1.08 | .91 |
| Mean | .853 | .906 | .755 | | 1.113 | 1.064 | .885 |
| Mean
(exclude exposed*) | .839 | .864 | .700 | | 1.165 | 1.031 | .812 |
| Mean
(n > 1000) | .837 | .897 | .694 | | 1.128 | 1.070 | .730 |
| Mean
(n > 1000, exclude exposed*) | .815 | .845 | .670 | | 1.197 | 1.035 | .652 |

* Stat. signif. associations between cancers and exposures.

** Stat. signif. associations between nonmalignant resp. dis. (NMRD) and exposures.

Table 2

SELECTED VALUES OF WEIGHTING FUNCTION USED IN
CALCULATION OF CUMULATIVE EXPOSURES

| | | | | | | | | | |
|-------------------|------|------|------|------|------|------|------|------|------|
| TIME ¹ | 30 | 25 | 20 | 15 | 12.5 | 10 | 7.5 | 5 | 2.5 |
| W(T) ² | .999 | .993 | .953 | .731 | .500 | .269 | .119 | .049 | .018 |

1. TIME INTERVAL FROM MID-POINT OF EXPOSURE INTERVAL
UNTIL DEATH.

2. $W(T) = (1 + e^{.4(12.5 - T)})^{-1}$, A CUMULATIVE
LOGISTIC DISTRIBUTION.

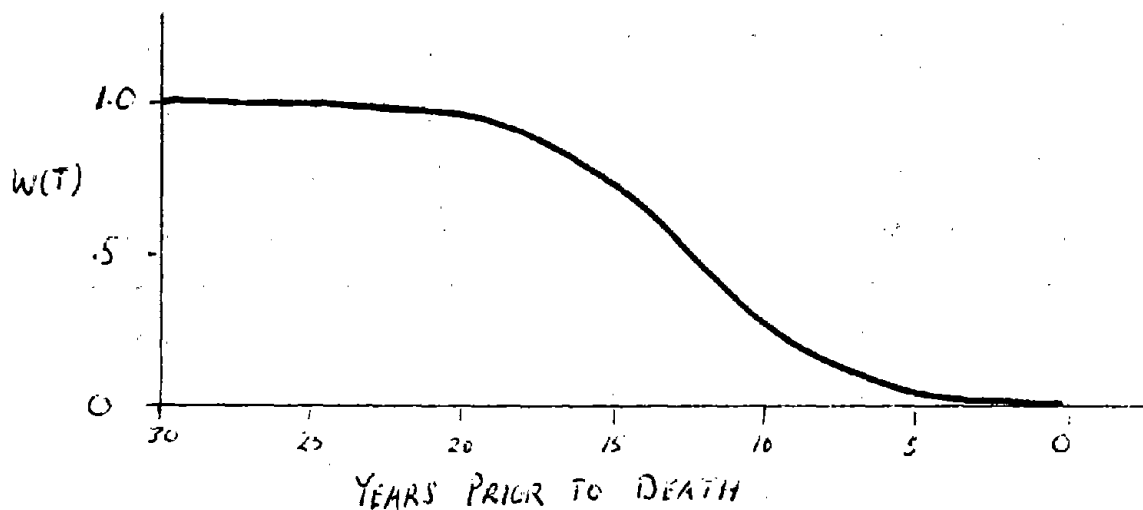


Table 3: Bearing Plant II

Standardized Proportional Mortality Ratios for Selected Causes of Death in All
White Men of Study Population (5 or More Years Duration of Employment¹)

| CAUSE OF DEATH | OBS | EXP | PMR | 95% CI | CHI SQ | P |
|---|------|--------|------|------------|--------|-------|
| TOTAL DEATHS | 1532 | | | | | |
| ALL MALIGNANT NEOPLASMS | 342 | 300.67 | 1.14 | 1.04, 1.25 | 7.16 | .01 |
| CANCER OF DIGESTIVE ORGANS AND PERITONEUM | 137 | 90.16 | 1.52 | 1.29, 1.79 | 25.90 | <.001 |
| CANCER OF ESOPHAGUS | 13 | 7.11 | 1.83 | 1.07, 3.12 | 4.91 | .03 |
| CANCER OF STOMACH | 35 | 17.74 | 1.97 | 1.43, 2.72 | 17.01 | <.001 |
| CANCER OF LARGE INTESTINE | 41 | 29.56 | 1.39 | 1.03, 1.88 | 4.52 | .04 |
| CANCER OF RECTUM | 14 | 10.28 | 1.36 | 0.81, 2.29 | 1.36 | |
| ALL CANCER OF LIVER - PRIMARY ONLY | 9 | 6.66 | 1.35 | 0.71, 2.59 | 0.82 | |
| CANCER OF PANCREAS | 24 | 16.79 | 1.43 | 0.96, 2.12 | 3.13 | .08 |
| ALL CANCER OF LUNG - PRIMARY AND SECONDARY | 83 | 90.47 | 0.92 | 0.75, 1.13 | 0.67 | |
| CANCER OF SKIN | 4 | 4.37 | 0.92 | 0.25, 2.34 | 0.0 | |
| CANCER OF PROSTATE | 29 | 29.03 | 1.00 | 0.70, 1.43 | 0.0 | |
| CANCER OF TESTIS | 0 | 0.82 | 0.00 | 0.00, 3.65 | 0.77 | |
| CANCER OF BLADDER | 14 | 11.09 | 1.26 | 0.75, 2.13 | 0.61 | |
| CANCER OF KIDNEY | 9 | 6.94 | 1.30 | 0.68, 2.48 | 0.0 | |
| CANCER OF BRAIN AND OTHER CENTRAL NERVOUS SYSTEM | 6 | 6.08 | 0.99 | 0.41, 2.19 | 0.03 | |
| ALL LYMPHOPOIETIC CANCER | 27 | 26.14 | 1.03 | 0.71, 1.50 | 0.06 | |
| LYMPHOSARCOMA AND RETICULOSARCOMA | 6 | 5.42 | 1.11 | 0.50, 2.46 | 0.11 | |
| LEUKEMIA | 12 | 10.89 | 1.10 | 0.63, 1.94 | 0.15 | |
| CANCER OF OTHER LYMPHATIC TISSUE | 6 | 7.01 | 0.86 | 0.39, 1.90 | 0.0 | |
| ALLERGIC, ENDOCRINE, METABOLIC, NUTRITIONAL DIS. | 26 | 26.13 | 1.00 | 0.68, 1.46 | 0.31 | |
| MENTAL, PSYCHONEUROTIC, AND PERSONALITY DISORDERS | 7 | 5.68 | 1.23 | 0.59, 2.58 | 1.21 | |
| ALL DISEASES OF NERVOUS SYSTEM AND SENSE ORGANS | 7 | 10.57 | 0.66 | 0.32, 1.38 | 0.02 | |
| ALL DISEASES OF CIRCULATORY SYSTEM | 881 | 883.86 | 1.00 | 0.96, 1.04 | 10.27 | .002 |
| ARTERIOSCLEROTIC HEART DISEASE, INCLUDING CHD | 650 | 589.09 | 1.10 | 1.04, 1.17 | 2.73 | .10 |
| ALL VASCULAR LESIONS OF CNS | 118 | 136.27 | 0.87 | 0.73, 1.03 | 0.98 | |
| ALL RESPIRATORY DISEASES | 94 | 103.73 | 0.91 | 0.75, 1.10 | 0.06 | |
| ALL PNEUMONIA | 37 | 38.52 | 0.96 | 0.70, 1.32 | 1.00 | |
| EMPHYSEMA | 20 | 24.94 | 0.80 | 0.52, 1.24 | 3.56 | .06 |
| ASTHMA | 3 | 3.33 | 0.90 | 0.19, 2.63 | 0.02 | |
| ALL DISEASES OF DIGESTIVE SYSTEM | 76 | 61.54 | 1.24 | 0.99, 1.54 | 3.05 | .10 |
| ALL GASTRIC AND DUODENAL ULCER | 10 | 10.42 | 0.96 | 0.52, 1.78 | 0.14 | |
| CIRRHOSIS OF LIVER | 35 | 26.19 | 1.34 | 0.97, 1.85 | 8.72 | .005 |
| CHRONIC NEPHRITIS | 7 | 6.07 | 1.15 | 0.55, 2.41 | 1.51 | |
| ALL ACCIDENTS | 30 | 50.44 | 0.59 | 0.42, 0.84 | | |
| SUICIDE | 13 | 18.19 | 0.71 | 0.42, 1.22 | | |

1. P-values and confidence intervals based on M-H chi-squared for expecteds > 5.0; otherwise based on Poisson; P-values are 2-tailed and not shown if > 0.1.

Table 4: Bearing Plant II

**Standardized Proportional Mortality Ratios for Selected Causes of
Death in White Men with Exposure to Cutting Fluids in Grinding Jobs¹**

| Underlying
Cause of
Death | Grinding
Comprehensive Definition | | Grinding
Restrictive Definition | |
|---------------------------------|--------------------------------------|-------------------|------------------------------------|------------------|
| | 40-119 Mo. | 120+ Mo. | 40-119 Mo. | 120+ |
| All Causes | 210 | 350 | 130 | 193 |
| | Obs/Exp PMR p ¹ | Obs/Exp PMR p | Obs/Exp PMR p | Obs/Exp PMR p |
| Malignant Diseases | | | | |
| All Cancer | 42/41. 1.03 — | 75/69. 1.09 — | 20/27. .74 — | 46/39. 1.19 — |
| Digestive | 11/12. .92 — | 40/20. 1.96 <.001 | 7/7.6 .92 — | 24/11. 2.11 .001 |
| Esophagus | 2/.95 2.1 — | 2/1.6 1.25 — | 0/.64 .00 — | 0/.89 .00 — |
| Stomach | 2/2.3 .86 — | 13/3.8 3.39 <.001 | 2/1.4 1.48 — | 8/2.1 3.76 .003 |
| Colon | 4/3.9 1.01 — | 13/6.9 1.89 .02 | 3/2.6 1.17 — | 7/3.8 1.83 — |
| Rectum | 1/1.4 .74 — | 3/2.3 1.30 — | 0/.83 .00 — | 3/1.3 2.33 — |
| Pancreas | 1/2.3 .44 — | 9/3.9 2.33 .04 | 1/1.5 .67 — | 6/2.2 2.78 .05 |
| Liver | 1/.88 1.13 — | 0/1.5 .00 — | 1/.55 1.83 — | 0/.84 .00 — |
| Lung | 12/12. .97 — | 13/21. .62 — | 8/8.8 .90 — | 7/12. .59 — |
| Lymphopoietic | 4/3.7 1.08 — | 5/6.0 .84 — | 0/2.4 .00 — | 4/3.4 1.19 — |
| Non-malignant | | | | |
| Digestive | 16/8.9 1.79 .02 | 18/13. 1.34 — | 12/5.8 2.07 .01 | 10/7.4 1.34 — |
| Cirrh. of Liver | 8/4.0 1.99 — | 8/5.5 1.46 — | 6/2.8 2.13 — | 6/3.1 1.96 — |
| Respiratory | 10/14. .71 — | 28/25. 1.12 — | 11/8.8 1.26 — | 14/14. 1.01 — |
| All Circ. Dis. | 111/119 .94 — | 200/203 .98 — | 70/71. .98 — | 105/112 .94 — |
| Arterioscl. HD | 82/80. 1.03 — | 143/136 1.05 — | 54/49. 1.10 — | 72/75. .96 — |
| All Dis. of Skin | 1/.16 6.34 — | 1/.26 3.84 — | 1/.10 10.4 — | 1/.14 7.00 — |
| Stroke | 13/18. .73 — | 34/32. 1.08 — | 10/9.7 1.03 — | 21/17. 1.23 — |
| Age at Death
(Mean) | 67.8 | 70.2 | 65.8 | 69.7 |
| Year of Death
(Mean) | 1969.6 | 1970.5 | 1971.2 | 1970.5 |

1. Cumulative exposure duration has been weighted according to interval prior to death; see Methods, page 10.
2. Based on 2-tailed test of significance (Mantel-Haenszel χ^2 used unless Exp. less than 5.0, then Poisson-P used).

Table 5: Bearing Plant II

**Standardized Mortality Odds Ratios from Logistic Regression Model
of Association between Stomach Cancer and Grinding Exposures in White Men¹**

| Predictors/
Risk Factors | n=1532 | df=3 | | | Adjusted Odds Ratio |
|--|--|--|------|-----|----------------------------|
| | Estimated
Effect
Coefficient, SE | Removal from Model ²
$\Delta(-2\ln L)$ | P1 | P2 | |
| (Constant) | .256, .240 | — | — | — | 1.29 |
| Expected Cause of
Death Odds ³
(age, year adjustment) | 1.00, — | — | — | — | — |
| Birth: Canada | .940, .424 | 4.72 | .015 | — | 2.56 |
| Grinding,
Comprehensive
(duration in months) | .00270, .00130 | 3.91 | .024 | — | 2.25
(25 yrs. exposure) |
| Tool Grinding
(duration in months) | .00406, .00327 | 1.65 | .10 | .20 | 3.38
(25 yrs. exposure) |

1. Final model of log (odds) of having died from stomach cancer:

$$\ln \left(\frac{p}{1-p} \right) = .256 + 1.00 \ln \left(\frac{r_i}{1-r_i} \right) + .940I_B + .00270X_G + .00406X_T$$

where:

r_i is age-, year-, sex-, race- specific proportional mortality rate for stomach cancer in U.S. population in 5-yr. intervals; $\ln \left(\frac{r_i}{1-r_i} \right)$ is log (odds);

I_B is indicator of Canadian birth (1 = Canada, 0 = not);

X_G is weighted duration of exposure in Grinding, Comprehensive in months (see Methods);

X_T is weighted duration of exposure in Tool Grinding in months.

2. Change in $-2\log(\text{likelihood})$ for removal of single risk factor from final model, one at a time, and associated P-value: 1-tailed (p_1) for hypothesized effects, 2-tailed (p_2) for others.
3. Effect estimate fixed at 1.0 in estimation procedure.

Table 6: Bearing Plant II

**Goodness of Fit of Logistic Regression Models
for Key Associations**

| Outcome | Exposure | Exposure Strata (Mo.) | | | | | Total |
|-------------------------------------|--------------------------------|-----------------------|------|--------|---------|------|-------|
| | | 0 | 1-50 | 51-150 | 151-350 | 351+ | |
| Stomach Cancer | Grinding, Comprehensive | | | | | | |
| Total No. in Stratum | | 883 | 121 | 232 | 243 | 53 | 1532 |
| No. of Cases Predicted ¹ | | 16.96 | 2.09 | 4.94 | 8.39 | 2.60 | 34.99 |
| No. of Cases Observed | | 17 | 3 | 5 | 8 | 2 | 35 |
| Cancer of Pancreas | Machining, Oil CF | | | | | | |
| Total No. in Stratum | | 1336 | 29 | 118 | 44 | 5 | 1532 |
| No. of Cases Predicted ¹ | | 18.7 | .54 | 2.52 | 1.91 | .74 | 24.41 |
| No. of Cases Observed | | 18 | 1 | 3 | 1 | 1 | 24 |
| Cancer of Pancreas | Grinding, Comprehensive | | | | | | |
| Total No. in Stratum | | 883 | 121 | 232 | 243 | 53 | 1532 |
| No. of Cases Predicted ¹ | | 12.42 | 1.72 | 2.81 | 4.37 | 3.10 | 24.42 |
| No. of Cases Observed | | 13 | 2 | 1 | 5 | 3 | 24 |
| Cancer of Lung | Forge, Heat Treat | | | | | | |
| Total No. in Stratum | | 1312 | 28 | 76 | 99 | 17 | 1532 |
| No. of Cases Predicted ¹ | | 65.7 | 1.91 | 5.34 | 7.82 | 2.20 | 82.99 |
| No. of Cases Observed | | 64 | 2 | 8 | 7 | 2 | 83 |

Table 7: Electronics Production

**Logistic Regression Analyses of Association Between
Death Due to Colon Cancer and Exposures in
Electronics Production in Women (n=75)**

| Model | Predictor ⁽¹⁾ | Effect Estimate, SE | Removal from Model ⁽²⁾ | | Adj. OR ⁽³⁾ | 95% CI |
|-------|---------------------------------|---------------------|-----------------------------------|------|------------------------|------------|
| | | | $\Delta(-2\ln L)$ | P | | |
| 1.(4) | Intercept | -.380, .896 | — | — | .68 | |
| | Epoxy | .00527, .00231 | 5.68 | .009 | 3.54 | 1.24, 10.0 |
| 2. | Intercept | -1.64, 1.46 | — | — | .19 | |
| | (Epoxy) 0.5 | .175, .080 | 5.89 | .008 | 15.1 | |
| 3. | Intercept | -1.70, 1.76 | — | — | .18 | |
| | Epoxy, truncated ⁽⁵⁾ | .017, .0096 | 3.81 | .03 | 30.0 | |
| 4. | Intercept | 1.290, .542 | — | — | 3.63 | |
| | Cyanoacrylate | -.00277, .00414 | .71 | — | .51 | |
| 5. | Intercept | .586, .519 | — | — | 1.80 | |
| | Solder/flux | .00333, .00251 | 3.64 | .03 | 2.22 | |
| 6. | Intercept | .296, .802 | — | — | 1.34 | |
| | Solvent | .00380, .00304 | 1.61 | .10 | 2.49 | |
| 7. | Intercept | -.578, 1.20 | — | — | .56 | |
| | Epoxy | .00489, .00291 | 3.98 | .02 | 3.23 | |
| | Solder/flux | .00267, .00467 | 1.94 | .08 | 1.90 | |
| 8. | Intercept | -.542, .996 | — | — | .58 | |
| | Epoxy | .00505, .00260 | 5.20 | .01 | 3.36 | |
| | Electronics Ass'y | .0148, .0185 | 1.68 | .10 | 2.43 | |
| 9. | Intercept | 1.10, .561 | — | — | 3.00 | |
| | Age at death | -.0110, .044 | .082 | — | .89 (10-yr.) | |

- Predictors are exposures (in exposure-months using latency-weighted durations and emphasizing pre-1960 exposures) or department durations (in months of latency-weighted duration, emphasizing the pre-1960 period) and age at death (years greater than age 55).
- Hypothesis testing consisted of removing a predictor from the model and assessing the change in $-2\ln(\text{likelihood})$, distributed as chi-squared; 1-tailed P-values were computed.
- The adj. OR for the intercept is the standardized mortality odds ratio estimated for the study population adjusting for the predictors in model, i.e., for the "unexposed." For exposure predictors, the adj. OR is the exposure odds ratio within the study population, standardized to the reference population and applied to 5 years (wt. duration) of exposure (and exposure level 4 where applicable). For age at death (model 9), the adj. OR is for a 10-yr. age increment. Confidence interval (95% CI) is based on chi-squared (test-based).

4. Final Model: $\ln\left(\frac{p}{1-p}\right) = -.38 + \ln\left(\frac{r}{1-r}\right) + .00527X$

where: p = probability that death was due to colon cancer;

r = age-, year-, race-, sex-specific proportional mortality rate for colon cancer in U.S.;

X = exposure to epoxy-like materials in exposure-months.

- Epoxy, truncated: all epoxy exposure values in excess of 200 exposure-months were set equal to 200.

Table 8: Electronics Production

Logistic Regression Analysis of Association Between Death Due to Cancer of the Colon and Exposure to Epoxy-like Materials in Women: Goodness of Fit

| Epoxy-like
Exposure Strata
(exposure-months) | n | Cases of Colon Cancer | | | | |
|--|----|-----------------------|----------|-------------|------------|-------------|
| | | Predicted(1) | Observed | Expected(2) | r, mean(2) | Pred. OR(3) |
| 0 | 8 | .171 | 0 | .248 | .0310 | 1.00 |
| 1-49 | 6 | .155 | 0 | .201 | .0335 | 1.14 |
| 50-99 | 5 | .153 | 1 | .146 | .0293 | 1.48 |
| 100-199 | 28 | 1.29 | 0 | .899 | .0321 | 2.21 |
| 200-399 | 15 | 1.27 | 2 | .499 | .0333 | 4.86 |
| 400+ | 13 | 2.96 | 3 | .323 | .0248 | 10.7 |
| Total | 75 | 5.99 | 6 | 2.32 | — | — |

1. Based on final model:

$$\text{Predicted} = \sum_S (1 + e^{-(.380 + \ln(\frac{r}{1-r}) + .00527 X)})^{-1};$$

S: members of stratum; X: exposure to epoxy-like materials (exposure-months, weighted for latency and emphasizing pre-1960 exposures).

2. Expected number of colon cancer deaths in stratum, based on national age-, year-, sex-, race-, cause-specific proportional mortality rates, r; Expected = $n(\text{mean } r) = \sum_S r$
3. Predicted odds ratio: $OR = e^{(.00527 X)}$; X chosen as stratum mid-point except for highest stratum where X = 450.

Table 9: Auto Repair Workers

Standardized Mortality Odds Ratios for Association
of Cancer of Esophagus With Employment Duration⁽¹⁾

| Model ⁽²⁾ | Risk Factor | Estimate (SD) | $\Delta(-2\ln L)^{(3)}$ | p ₁ | p ₂ | Adj. OR |
|----------------------|---------------------------|----------------|-------------------------|----------------|----------------|---------------|
| I | Empl. Duration (wt. mos.) | .0077 (.0070) | 3.02 | .04 | .08 | 2.5 (10 yrs.) |
| | (Intercept) | -.3395 (1.645) | — | — | — | 0.71 |
| II | Age at Death (yrs.-55) | .0235 (.0972) | 0.17 | — | — | 1.3 (10 yrs.) |
| | (Intercept) | .769 (1.074) | — | — | — | 2.2 |

1. In white men; n=290 (5 missing duration data).

2. General Model:

$$\ln\left(\frac{p}{1-p}\right) = a + \ln\left(\frac{r}{1-r}\right) + bx$$

where: p - probability of a death having been due to cancer of esophagus;

r - age-, year-, sex-, race-specific proportional mortality rate for cancer of esophagus in U.S. population;

a - intercept: estimate of SMOR for zero duration (Model I), or at age 55 at death (Model II);

b - effect estimate for risk factor x;

x - risk factor: employment duration (Model I) or age at death (Model II).

3. Statistical significance is estimated from change in $-2\ln(\text{Likelihood})$ when term is removed from model, assuming change is distributed as chi-squared variable under the null hypothesis (1df). P₁ and p₂ are one- and two-tailed P-values.

Table 10: Auto Repair Workers

Standardized Mortality Odds Ratios for Association of
Nonmalignant Respiratory Disease with Duration of Employment⁽¹⁾

| Model ⁽²⁾ | Risk Factor | Estimate | (SD) | $\Delta(-2\ln L)^{(3)}$ | p ₁ | p ₂ | Adj. OR |
|----------------------|---------------------------|----------|----------|-------------------------|----------------|----------------|---------------|
| I | Empl. Duration (wt. mos.) | .00456 | (.00260) | 3.40 | .03 | .07 | 1.7 (10 yrs.) |
| | Age at Death (yrs.-55) | -.0342 | (.0249) | 1.81 | .09 | .18 | .71 (10 yrs.) |
| | (Intercept) | -.342 | (.418) | — | — | — | .71 |
| II | Empl. Duration (wt. mos.) | .00294 | (.00196) | 1.86 | .09 | .17 | 1.4 (10 yrs.) |
| | (Intercept) | -.452 | (.413) | — | — | — | .63 |
| III | Latency (unwt. mos.) | .00399 | (.00226) | 3.12 | .04 | .08 | 1.6 (10 yrs.) |
| | Age at Death (yrs.-55) | -.038 | (.027) | 2.02 | .08 | .16 | .68 (10 yrs.) |
| | (Intercept) | -.790 | (.602) | — | — | — | .45 |
| IV | Duration (unwt. mos.) | .00251 | (.00230) | 1.16 | — | — | 1.4 (10 yrs.) |
| | Age at Death (yrs.-55) | -.018 | (.0208) | .584 | — | — | .83 (10 yrs.) |
| | (Intercept) | -.378 | (.556) | — | — | — | .68 |
| V | Age at Death (yrs.-55) | -.0112 | (.0188) | .271 | — | — | .89 (10 yrs.) |
| | (Intercept) | .136 | (.305) | — | — | — | 1.1 |

1. In white men; n=290 (5 missing duration data).

2. General Model:

$$\ln\left(\frac{p}{1-p}\right) = a + \ln\left(\frac{r}{1-r}\right) + b_1x_1 + b_2x_2$$

where: p - probability of a death having been due to nonmalignant respiratory disease;

r - age-, year-, sex-, race-specific proportional mortality rate for nonmalignant respiratory disease in U.S. population;

a - intercept: estimate of SMOR for zero duration (Model I-IV), or at age 55 at death (Model I, V);

b_i - effect estimate for risk factor x_i;

x_i - risk factor: employment duration, age at death, etc.

3. Statistical significance is estimated from change in -2ln(Likelihood) when term is removed from model, assuming change is distributed as chi-squared variable under the null hypothesis (ldf). P₁ and p₂ are one- and two-tailed P-values.

QUESTIONS AND DISCUSSION

DR. SHY: Question from the audience?

(No response)

Since there are no questions or comments for Mr. Park, let us move on to the next presentation. Carol Burnett, from the Institute for Occupational Safety and Health, will talk about research leads from occupational mortality data.

**RESEARCH LEADS FROM OCCUPATIONAL
MORTALITY DATA**

**Presented by Carol A. Burnett
Division of Surveillance, Hazard Evaluations and Field Studies
National Institute for Occupational Safety and Health**

Research Leads from Occupational Mortality Data

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Research Leads from Occupational Mortality Data

Background

One of the goals of the National Institute for Occupational Safety and Health (NIOSH) is the development of nationwide surveillance systems which identify disease and death related to occupation. The adaptation of existing health effects data bases is emphasized to conserve time and resources. A common deficiency in this approach is the limited availability of occupational exposure data linked to the health effects data. Consequently, surrogate exposure data is used to relate disease to the workplace. Such data may take the form of an employment history, frequently the usual or current occupation and industry.

Death certificate records are an example of an existing data base which has the potential for development into a national surveillance system. In addition to the cause of death and other pertinent demographic data, such as age, sex, race, and residence, information on the usual occupation and industry of the decedent is routinely collected by all states. However, as late as 1979, only six states coded both occupation and industry; five coded occupation only; and one coded industry only.¹

In 1979, the National Center for Health Statistics (NCHS) and the Bureau of the Census, in collaboration with NIOSH, investigated the feasibility of coding the occupation and industry entries on death certificates. The investigation found that the death certificate entries were sufficiently complete and detailed to be coded to the three-digit level of the Bureau of the Census classification scheme.

Activities Designed to Support Industry and Occupation Coding

In the early 1980's, NIOSH, NCHS, and the Bureau of Census continued their collaboration with the goal of fostering development of standardized routine coding of industry and occupation (I/O) entries from death certificates by state health departments. Training courses for coding occupation and industry according to the 1980 Bureau of Census classification system² were developed for both production and referral coders. Production coders perform routine coding, while referral coders resolve problem cases. Since 1981, 129 production coders from 32 states, New York City, the District of Columbia, and Puerto Rico have been trained, as have 38 referral coders from 22 states. The number of states coding I/O data using the Bureau of Census classification system has increased from nine in 1982 to 30, plus the District of Columbia, in 1986.

To enhance the quality of death certificate occupation and industry data, NCHS published two instructional manuals. "Guidelines for Reporting Occupation and Industry on Death Certificates"³ was published in 1983 as an aid to funeral directors, who are responsible for collecting this information. The aim of the manual is to upgrade the quality of the industry and occupation entries on the certificates, making them as specific and accurate as possible.

"Instruction Manual, Part 19: Industry and Occupation Coding for Death Certificates,"⁴ published in 1984, contains coding instruction procedures specific for industry and occupation entries on death certificates. These procedures were designed to permit states to code routinely these items and to be compatible with standard vital statistics coding practices carried out at the state and national levels.

In 1983, NCHS began performing quality control of occupation and industry coding. Quality control coding for the I/O items is done concurrently with the other demographic quality control coding, using a selected sample of records. The sample size, ranging from two to 10 percent, is based on the annual record volume for each state. Feedback to the states assists the coders to improve the quality of their data.

Eleven states submitted 1983 data to NCHS for quality control. These states permitted the use of their 1983 data by NCHS and NIOSH for evaluating the quality of the data and developing tabulation and analysis procedures for use with future data. The evaluation of the quality of the 1983 data indicated that the states were capable of producing I/O data from death certificates which would meet the NCHS and NIOSH standards of an error rate of 5% or less.

NIOSH has developed two computer software packages which support the coding and analysis of death certificate data. The first one, a program for editing industry and occupation codes, checks all records and prints out records with three types of possible errors: (1) invalid codes; (2) code combinations in which industry is not reported, but could be imputed from the reported occupation, or occupation is not reported, but could be imputed from the reported industry; and (3) industry and occupation code combinations which are impossible or highly unlikely. This editing provides an additional means of monitoring the quality of the data. The program is intended for use at the state coding level to detect and correct coding errors.

The second computer program developed by NIOSH, a proportionate mortality ratio (PMR) analysis program, permits the routine analysis of occupational mortality data. The results of this program--sex and race specific, age-standardized PMR's--are used for the purpose of hypothesis generation. The PMR compares the proportion of deaths from a specific cause within a specific occupation or industry group with the proportion of deaths from that cause in a standard population, adjusting for age. The standard population usually used in surveillance studies is the population of decedents in the study.

1985 Activities

The National Cancer Institute (NCI), through an interagency agreement with NIOSH, has allocated funds for reimbursement of selected states for coded I/O data. These data would then be available to NCI, NIOSH, and NCHS for analysis and dissemination purposes. In 1985, representatives of NIOSH, NCHS, and the Association for Vital Records and Health Statistics established in-scope costs and the level of reimbursement for the purchase of coded I/O data from states. Five states--Georgia, Kansas, Kentucky, Utah, and Wisconsin--were selected for reimbursement for 1985 data. These states also agreed to permit NIOSH, NCI, and NCHS the use of their 1984 data. In addition, Colorado, Maine, Missouri, Nebraska, Nevada, New Hampshire, Oklahoma, Rhode Island, and South Carolina, although not reimbursed, have agreed to the use of their 1984 and 1985 data for analysis and publication. Ohio and Tennessee have agreed to the use of their 1985 data.

The 1983 data submitted to NCHS for quality control were further evaluated for quality. Using the I/O Edit program, fewer than 1% of the records were printed out as possible errors. A measure of the quality of the data reported on the death certificates is the percent of entries which are not codable to a specified industry category or a specified occupation category. The uncodable industry entries for the 11 states ranged from 2% to 14%. The uncodable occupation entries ranged from 2% to 16%. These results include homemaker as a codable industry and occupation.

The first proposed publication using the occupational mortality data base will be a "Monthly Vital Statistics Report" (MVSR) supplement, to be published by NCHS. The proposed MVSR will present a short descriptive analysis of the 1984 data. Using the 1983 data to estimate frequencies in proposed industry or occupation by cause of death cells, three lists were developed: (1) a List of 42 Selected Industries; (2) a List of 46 Selected Occupations; and (3) a List of 52 Selected Causes of Death (see Tables 1-3). The industry and occupation lists consist of categories derived by collapsing the detailed 1980 Bureau of Census codes. The cause of death list includes causes known to be related or possibly related to occupation, as well as the 13 leading causes of death in the United States during 1979-1983, excluding Congenital anomalies and Certain conditions originating in the perinatal period.

The data will be analyzed both for quality and for cause-specific mortality patterns by industry and by occupation. The quality of industry entries will be assessed by determining the number of certificates with industry entries coded to the following categories: All industries; Specified industry;

Military; Never worked, unemployed, homemaking, student, etc.; Retired; and Unknown. The following categories will be used for assessing quality of occupation entries: All occupations; Specified occupation; Military; Homemaker; Volunteer; Student; Unemployed, never worked; Retired; and Unknown. The PMR program will be used to analyze the association of industry or occupation and cause of death. The analysis will be age and race adjusted, sex-specific, by state of residence, for all decedents aged 20 years and over. The Mantel-Haenszel chi-square test⁵ will be used to determine if the PMR's differ significantly from 100 at the 1- and 5-percent levels. Proposed table plans are shown in Figures 1-4.

Results from Two NIOSH Death Certificate-Based Surveillance Studies

Analyses of data obtained by NIOSH through cooperative agreements with Rhode Island and Utah⁶ are used to demonstrate the types of results which can be expected using a death certificate data base. The data were extracted from all death certificates of Rhode Island residents aged 16 years and older for the period 1968-1978, and Utah residents aged 16 years and older for the period 1959-1978. For the Rhode Island data, an age and race standardized, sex-specific proportionate mortality ratio analysis was used for measuring occupationally related mortality. Because of the small number of non-whites in Utah, only whites were included in an age standardized, race- and sex-specific analysis. Statistical significance was tested with the Mantel-Haenszel chi-square statistic.

One purpose of these surveillance analyses was the generation of leads for further research. A PMR analysis of large data bases such as these results in many observed statistically significant associations between specific occupations or industries and specific causes of death. Because of the large number of comparisons made, many of the associations could be due to chance. Associations were evaluated with respect to their biological plausibility, consistency with other epidemiological studies, and the likelihood that they could be explained by confounding or bias. Some associations have been recognized and extensively studied; therefore, they cannot be considered as leads. Some of the associations considered to be leads were:

1. Malignant melanoma in the printing industry.
2. Non-Hodgkin's lymphoma, leukemia, and aplastic anemia in female textile industry workers.
3. Non-malignant respiratory disease in male textile industry workers.
4. Suicide in social workers.
5. Acute myocardial infarction in police officers.

The Rhode Island analysis suggested an association of malignant melanoma with the printing industry. Of a total of 577 white males whose usual industry was printing, six died from malignant melanoma. Only 1.3 deaths were expected, resulting in a PMR of 460, significant at $p < .01$. An analysis of the

printing trade occupation categories, containing 290 white males, yielded a PMR of 570, $p < .01$, with four observed deaths and 0.70 expected. The usual industry of all four decedents was printing.

To evaluate the consistency of this association, a thorough review of the literature⁷ was made of all studies for which observed and expected values were reported or could be calculated for malignant melanoma in the printing industry or in printing trades. The results are presented in Table 4. While the results of each individual study taken separately are not particularly noteworthy because of the small numbers, taken together these results provide substantial support for the hypothesis of a malignant melanoma risk in the printing industry. In eight of these nine studies, the observed-to-expected ratio for malignant melanoma was reported to be greater than 100. Summing the observed and expected values across the nine studies produced an aggregate observed-to-expected ratio of 198 ($p < .01$),¹⁶ with a total of 23 observed cases.

Based on current evidence, ultraviolet light from the sun is thought to be the most important factor in the etiology of malignant melanoma.^{17,18} However, the relationship between solar radiation and malignant melanoma appears to be complex, and nonsolar factors, such as chemicals, may also be involved.^{19,20} Workers in the printing industry are exposed to ultraviolet radiation through its use in printing processes such as photoengraving, lithographic plate making, and the curing of ink. They are also exposed to a wide variety of chemicals.

Rhode Island was a center for the textile industry during the first half of this century. Approximately 10% of the death certificates of the 1968-1978 period listed textile as the usual industry. A PMR analysis of female blue collar textile industry workers, using all other female blue collar workers as the comparison population, resulted in statistically significant associations between blue collar textile workers and non-Hodgkin's lymphoma, leukemia, and aplastic anemia. Since these causes of death are known to be related to chemical exposures, and a wide variety of chemicals have been used in textile manufacturing, this appears to be a lead worth pursuing.

Males in the textile industry had statistically significantly elevated PMR's for non-malignant diseases of the respiratory system. Among the occupations within the textile industry, Carding, lapping, and combing operatives had a high, statistically significant, PMR (PMR = 199, $p < .01$, with 21 observed deaths). This occupational group also has the highest risk of dust exposure in the textile industry. While there have been frequent reports of increased respiratory morbidity in the U.S. textile industry, this is the first report of excess mortality.

Suicide in social workers was found to be elevated for both males and females in both the Rhode Island and the Utah analyses. Rhode Island had four observed deaths for both males and females, with respective PMR's of 373 and 552, both statistically significant. Utah had three observed deaths for males, with a PMR of 131, and two observed deaths for females, with a PMR of 194. While these results are based on small numbers, they would support a hypothesis that social work would be stressful, possibly leading to suicide.

Although cardiovascular disease is listed as one of the NIOSH top ten occupationally related diseases,²¹ few studies have examined this relationship. Stress, both physical and psychological, and automobile exhaust are postulated to be risk factors for acute myocardial infarction (AMI). If this is true, police officers, an occupation thought to have episodes involving considerable stress and also exposure to automobile exhaust, should have high rates of AMI. Both the Utah and Rhode Island analyses support this hypothesis. If the deaths from AMI are associated with occupational factors, they would be expected to occur in the 16-64 age range. In the Utah analysis, the PMR for the 16-64 age range was 182, $p < .01$, with 22 observed deaths. Rhode Island decedents in the same age range experienced a PMR of 169, $p < .01$, with 64 observed deaths. The 65+ age group in the Utah analysis had a nonsignificantly elevated PMR of 134, with 28 deaths. In the Rhode Island study, the PMR was 92 with 51 deaths. These results suggest the need for follow-up studies.

Conclusions

While the Rhode Island and Utah analyses show the potential in the use of death certificate data for occupational health surveillance, the occupational mortality data base resulting from the NIOSH/NCHS/NCI project should have even greater surveillance capacity. It will contain more records than either of these. This will increase the power to detect associations in certain groups of interest: (1) smaller industry and occupation groups; (2) occupations within specific industries; (3) Blacks and possibly other racial minorities; (4) females; (5) specific geographical areas; and (6) industries that are concentrated in the states being analyzed.

The industry and occupation items in the data base should be more accurate than those in previous studies, because of the emphasis on upgrading the reporting and coding of the data. The improved data, in turn, will increase the accuracy of the results of the PMR analysis.

Lastly, the potential of linking occupation to exposure is being developed at NIOSH through the use of a job-exposure matrix. The matrix describes the association of exposures with occupations within industries. This would provide the possibility of testing the association of exposure with cause of death, using a death certificate data base.

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Table 1

List of 42 Selected Industries for the
Proposed Monthly Vital Statistics Report

| <u>Number</u> | <u>Bureau of Census
Industry Codes</u> | <u>Industry</u> |
|---------------|--|---|
| | | All Industries |
| 1 | 010-031 | Agriculture, forestry, and fisheries |
| 2 | 040-050 | Mining |
| 3 | 060 | Construction |
| | 100-392 | Manufacturing |
| | 100-222 | Nondurable goods |
| 4 | 100-130 | Food and kindred products |
| 5 | 132-152 | Textile mill and finished products |
| 6 | 160-162 | Paper and allied products |
| 7 | 171-172 | Printing, publishing and allied products |
| 8 | 180-192 | Chemicals and allied products |
| 9 | 200-201 | Petroleum and coal products |
| 10 | 210-222 | Rubber, plastics and leather products |
| | 230-392 | Durable goods |
| 11 | 230-242 | Lumber and other wood products, and
furniture |
| 12 | 250-262 | Stone, clay, glass and concrete products |
| 13 | 270-280 | Primary metal industries |
| 14 | 281-301 | Fabricated metal industries |
| 15 | 310-332 | Machinery, except electrical |
| 16 | 340-350 | Electrical machinery, equipment, and
supplies |
| 17 | 351-370 | Transportation equipment |
| 18 | 371-392 | Miscellaneous manufacturing industries |
| | 400-472 | Transportation, communications, and other
public utilities |
| | 400-432 | Transportation |
| 19 | 400 | Railroads |
| 20 | 410-411 | Trucking and warehousing |
| 21 | 401-402, 412-432 | Other transportation |
| 22 | 440-442 | Communications |
| 23 | 460-472 | Utilities and sanitary services |
| 24 | 500-571 | Wholesale trade |
| | 580-691 | Retail trade |
| 25 | 601-611 | Food, bakery, and dairy stores |
| 26 | 612-620 | Auto dealers and supply stores |
| 27 | 641 | Eating and drinking places |
| 28 | 580-600, 621-640,
642-691 | Other retail trade |
| 29 | 700-712 | Finance, insurance, and real estate |
| | 721-760 | Business and repair services |
| 30 | 750-751 | Automotive services and repair |

Table 1 (Cont'd.)

List of 42 Selected Industries for the
Proposed Monthly Vital Statistics Report

| <u>Number</u> | <u>Bureau of Census
Industry Codes</u> | <u>Industry</u> |
|---------------|--|--|
| 31 | 721-742, 752-760 | Other business and repair services |
| | 761-791 | Personal services |
| 32 | 761 | Private households |
| 33 | 772-780 | Beauty and barber shops |
| 34 | 762-771, 781-791 | Other personal services |
| 35 | 800-802 | Entertainment and recreation services |
| | 812-892 | Professional and related services |
| 36 | 812-840 | Health services |
| 37 | 842-860 | Educational services |
| 38 | 861-881 | Social services |
| 39 | 841, 882-892 | Legal, engineering, and other services |
| 40 | 900-932 | Public administration |
| 41 | 942 | Military |
| 42 | 951-990 | Industry not reported |

Table 2

List of 46 Selected Occupations for the
Proposed Monthly Vital Statistics Report

| <u>Number</u> | <u>Bureau of Census
Occupation Codes</u> | <u>Occupation</u> |
|---------------|--|--|
| | | All Occupations |
| | 003-037 | Executive, administrative, and managerial occupations |
| 1 | 003-019 | Executive and administrative occupations |
| 2 | 023-037 | Management related occupations |
| | 043-199 | Professional specialty occupations |
| 3 | 043-083 | Architects, engineers, and scientists |
| 4 | 084-106 | Health diagnosis and treatment occupations |
| 5 | 113-163 | Teachers |
| 6 | 164-199 | Other professional specialty occupations |
| 7 | 203-235 | Technicians and related support |
| 8 | 243-285 | Sales occupations |
| | 303-389 | Administrative support occupations, including clerical |
| 9 | 313-315 | Secretaries, stenographers, and typists |
| 10 | 325-344 | Records processing occupations |
| 11 | 354-357 | Mail and message distributing occupations |
| 12 | 303-309, 316-323,
345-353, 359-389 | Other administrative support occupations |
| | 403-469 | Service occupations |
| 13 | 403-407 | Private household occupations |
| 14 | 413-427 | Protective service occupations |
| 15 | 433-444 | Food preparation and service occupations |
| 16 | 445-447 | Health service occupations |
| 17 | 448-455 | Cleaning and building service occupations |
| | 456-469 | Personal service occupations |
| 18 | 473-499 | Farming, forestry, fishing occupations |
| 19 | 473-489 | Farm and other agricultural occupations |
| 20 | 494-499 | Forestry, fishing, and hunting occupations |
| | 503-699 | Precision production, craft and repair occupations |
| | 503-549 | Mechanics and repairers |
| 21 | 505-517 | Vehicle and mobile equipment mechanics and repairers |
| 22 | 503, 518-549 | Other mechanics and repairers |
| | 553-599 | Construction trades |
| 23 | 567-569 | Carpenters and apprentices |
| 24 | 575-577 | Electricians, apprentices, and electrical power installers and repairers |
| 25 | 579 | Painters, construction and maintenance |
| 26 | 553-566, 573,
583-599 | Other construction trades |

Table 2 (Cont'd.)

List of 46 Selected Occupations for the
Proposed Monthly Vital Statistics Report

| <u>Number</u> | <u>Bureau of Census
Occupation Codes</u> | <u>Occupation</u> |
|---------------|--|--|
| 27 | 613-617 | Extractive occupations |
| | 633-699 | Precision production occupations |
| 28 | 633 | Supervisors, production occupations |
| 29 | 634-659 | Precision metal and wood working
occupations |
| 30 | 666-674 | Precision textile, apparel, and
furnishings machine workers |
| 31 | 686-688 | Precision food production occupations |
| 32 | 675-684, 689-699 | Other precision production occupations |
| | 703-799 | Machine operators, assemblers, and
inspectors |
| | 703-779 | Machine operators and tenders, except
precision |
| 33 | 703-733 | Metal, plastic, and woodworking machine
operators |
| 34 | 734-737 | Printing machine operators |
| 35 | 738-749 | Textile, apparel, and furnishing
machine operators |
| 36 | 753-779 | Machine operators, assorted materials |
| 37 | 783-795 | Fabricators, assemblers, and hand working
occupations |
| 38 | 796-799 | Production inspectors, testers, samplers,
and weighers |
| | 803-859 | Transportation and material moving
occupations |
| 39 | 803-814 | Motor vehicle operators |
| 40 | 823-859 | Other transportation occupations |
| | 863-889 | Handlers, equipment cleaners, helpers and
laborers |
| 41 | 869 | Construction laborers |
| 42 | 889 | Laborers, except construction |
| 43 | 863-867, 873-888 | Other handlers, cleaners, and laborers |
| 44 | 905 | Military |
| 45 | 914 | Homemaker |
| 46 | 913, 915-999 | Occupation not reported |

Table 3. LIST OF 52 SELECTED CAUSES OF DEATH

| Number | Cause of death |
|--------|--|
| | All causes..... |
| 1 | Tuberculosis, including late effects 010-018,137 |
| 2 | Septicemia.....038 |
| | Malignant neoplasms, including neoplasms of |
| | lymphatic and hematopoietic tissues.....140-208 |
| 3 | Malignant neoplasms of lip, oral cavity, and pharynx.....140-149 |
| 4 | Malignant neoplasm of esophagus.....150 |
| 5 | Malignant neoplasm of stomach.....151 |
| 6 | Malignant neoplasm of colon.....153 |
| 7 | Malignant neoplasms of rectum, rectosigmoid junction, |
| | and anus.....154 |
| 8 | Malignant neoplasms of liver and intrahepatic bile |
| | ducts.....155 |
| 9 | Malignant neoplasms of gallbladder and extrahepatic |
| | bile ducts.....156 |
| 10 | Malignant neoplasm of pancreas.....157 |
| 11 | Malignant neoplasm of larynx.....161 |
| 12 | Malignant neoplasms of trachea, bronchus, and lung.....162 |
| 13 | Malignant neoplasm of pleura.....163 |
| 14 | Malignant neoplasms of bone and articular cartilage.....170 |
| 15 | Malignant neoplasms of connective and other soft |
| | tissue.....171 |
| 16 | Malignant melanoma of skin.....172 |
| 17 | Malignant neoplasm of female breast.....174 |
| 18 | Malignant neoplasm of cervix uteri.....180 |
| 19 | Malignant neoplasm of body of uterus.....182 |
| 20 | Malignant neoplasms of ovary and other uterine adnexa.....183 |
| 21 | Malignant neoplasm of prostate.....185 |
| 22 | Malignant neoplasm of testis.....186 |
| 23 | Malignant neoplasm of bladder.....188 |
| 24 | Malignant neoplasms of kidney and other and unspecified |
| | urinary organs.....189 |
| 25 | Malignant neoplasms of brain and other and unspecified |
| | parts of nervous system.....191-192 |
| 26 | Hodgkin's disease.....201 |
| 27 | Malignant lymphoma other than Hodgkin's disease.....200,202 |
| 28 | Multiple myeloma and immunoproliferative neoplasms.....203 |
| 29 | Leukemia.....204-208 |
| 30 | All other malignant neoplasms, including neoplasms of |
| | lymphatic and hematopoietic tissues.....152,158-160,164-165, |
| | 173,175,179,181,184, |
| | 187,190,193-199 |
| 31 | Diabetes mellitus.....250 |
| 32 | Aplastic anemia.....204 |

Table 3. LIST OF 52 SELECTED CAUSES OF DEATH--Con.

| Number | Cause of death |
|--------|---|
| | Diseases of heart.....390-398,402,404-429 |
| 33 | Hypertensive heart disease.....402 |
| 34 | Hypertensive heart and renal disease.....404 |
| | Ischemic heart disease.....410-414 |
| 35 | Acute myocardial infarction.....410 |
| 36 | All other ischemic heart disease.....411-414 |
| 37 | All other diseases of heart.....390-398,415-429 |
| 38 | Hypertension with or without renal disease.....401,403 |
| 39 | Cerebrovascular diseases.....430-438 |
| 40 | Atherosclerosis.....440 |
| 41 | Pneumonia and influenza.....480-487 |
| 42 | Chronic obstructive pulmonary diseases and allied conditions.....490-496 |
| 43 | Pneumoconioses and pneumopathy due to inhalation of other dust.....500-505 |
| 44 | Ulcer of stomach and duodenum.....531-533 |
| 45 | Chronic liver disease and cirrhosis.....571 |
| 46 | Nephritis, nephrotic syndrome, and nephrosis.....580-589 |
| | Accidents and adverse effects.....E800-E949 |
| 47 | Motor vehicle accidents.....E810-E825 |
| 48 | Accidents mainly of industrial type.....E846,E881-E882,E916-E919,
E921,E923-E926 |
| 49 | Other accidents and adverse effects.....E800-E807,E826-E845,
E847-E880,E883-E915,
E920,E922,E927-E949 |
| 50 | Suicide.....E950-E959 |
| 51 | Homicide and legal intervention.....E960-E978 |
| 52 | All other causes.....Residual |

Table 4

Reports of Malignant Melanoma in the Printing Industry^a

| Study | Study Period | Mortality or Incidence Index ^b | | (Observed/Expected) X100 | |
|---|--------------|---|----------|--------------------------|----------|
| | | Observed | Expected | Observed | Expected |
| Utah ⁶ | 1959-78 | PMR | 3 | 1.30 | 231 |
| Massachusetts ⁸ | 1971-73 | MOR | 3 | 1.65 | 182 |
| Washington State ⁹ | 1950-79 | PMR | 4 | 2.67 | 150 |
| California ¹⁰ | 1959-61 | PMR | 2 | 1.85 | 108 |
| Roswell Park ¹¹ | 1956-65 | IOR | 2 | 0.13 | 1502* |
| Third National Cancer Survey ¹² | 1969-71 | IOR | 2 | 0.67 | 300 |
| U.S. Government Printing Office employees ¹³ | 1948-77 | PCMR | 5 | 2.81 | 178 |
| London newspaper workers ¹⁴ | 1954-66 | PMR ^c | 2 | 0.39 | 517 |
| Milan newspaper workers ¹⁵ | 1956-75 | PMR ^d | 0 | 0.14 | 0 |
| Total | | | 23 | 11.61 | 198** |

^aAll of these studies were of males.

^bPMR = proportionate mortality ratio; MOR = mortality odds ratio;
PCMR = proportionate cancer mortality ratio; IOR = incidence odds ratio.

^cIn this study, two of 670 deaths were reported to be from malignant melanoma. However, a PMR for malignant melanoma was not presented. The expected number of deaths and PMR were calculated for this table using data presented in the paper on the number of deaths by year and age group in conjunction with year-, sex-, age-, cause-specific mortality tables for England and Wales.

^dIn this study, the expected number of malignant melanoma deaths was not reported. It was calculated for this table using data presented in the paper on the number of deaths by age group in conjunction with year-, sex-, age-, cause-specific mortality tables for Italy.

* $p < .05$, Significance test according to Bailar and Ederer, 1964.¹⁶

** $p < .01$, Significance test according to Bailar and Ederer, 1964.¹⁶

Figure 1

Proposed Table Plans for MYSR Supplement

Figure 1. Deaths and percent distribution for selected occupation codes: 13 reporting States, 1984

| State | All occupations | Specified occupation | Military (905) | Homemaker (914) | Volunteer (916) | Student (915) | Unemployed, never worked (917) | Retired (913) | Unknown ^{1/} (999) |
|---------------------------|-----------------|----------------------|----------------|-----------------|-----------------|---------------|--------------------------------|---------------|-----------------------------|
| All Reporting States----- | | | | | | | | | |
| Colorado----- | | | | | | | | | |
| Georgia----- | | | | | | | | | |
| Kansas----- | | | | | | | | | |
| Kentucky----- | | | | | | | | | |
| Maine----- | | | | | | | | | |
| Missouri----- | | | | | | | | | |
| Nebraska----- | | | | | | | | | |
| Nevada----- | | | | | | | | | |
| New Hampshire----- | | | | | | | | | |
| Oklahoma----- | | | | | | | | | |
| Rhode Island----- | | | | | | | | | |
| South Carolina----- | | | | | | | | | |
| Wisconsin----- | | | | | | | | | |
| All Reporting States----- | 100.0 | | | | | | | | |
| Colorado----- | 100.0 | | | | | | | | |
| Georgia----- | 100.0 | | | | | | | | |
| etc. for reporting States | | | | | | | | | |

Programmer's Notes: Table includes only ages 20 years and over and requires 1 page for MYSR Supplement.

Percent distribution

^{1/}Includes deaths that occurred in non-reporting States.

Figure 2

Proposed Table Plans for MVSr Supplement - con.

Figure 2. Proportionate mortality ratios (PMR) for 46 selected occupations and 52 selected causes of death, by sex:
13 reporting States, 1984

(Numbers after causes of death are category numbers of the Ninth Revision International Classification of Diseases, 1975)

| Bureau
of the
Census
Occupation
Code | Occupation and sex | All
causes | Tuberculosis,
including late
effects (010-
018, 137) | Septi-
cemia
(038) | Malignant neoplasms,
including neoplasms
of lymphatic and
hematopoietic tissues
(140-208) | Malignant neoplasms
of lip, oral cavity
and pharynx
(140-149) | Malignant
neoplasm of
esophagus
(150) | etc. for
List of 52
selected
causes |
|--|--|---------------|---|--------------------------|---|--|--|--|
| | Male | 100 | 100 | 100 | 100 | 100 | 100 | etc. |
| 003-037 | All occupations-----
Executive, administrative,
and managerial occupations---- | | | | | | | |
| 003-019 | Executive and administra-
tive occupations----- | | | | | | | |
| 023-037 | Management related
occupations----- | | | | | | | |
| 043-199 | Professional specialty
occupations----- | | | | | | | |
| 043-083 | Architects, engineers, and
scientists----- | | | | | | | |
| 084-106 | Health diagnosis and treat-
ment occupations----- | | | | | | | |
| 113-163 | Teachers----- | | | | | | | |
| 164-199 | Other professional specialty
occupations----- | | | | | | | |
| 203-235 | Technicians and related
support occupations----- | | | | | | | |

Programmer's Notes:

1. Table includes only ages 20 years and over and requires 12 pages for MVSr Supplement.
2. Statistical tests will be used to determine if the PMR is significantly different from 100. Statistically significant PMRs will be indicated as follows | space to the right of the PMR:

+ - Significantly different at .05 level.

++ - Significantly different at .01 level.

etc. for List of 46 Selected
Occupations

Repeat for Female

Figure 3

Proposed Table Plans for MVSR Supplement - con.

Figure 3. : Deaths and percent distribution for selected industry codes: 13 reporting States, 1984

| State | All industries | Specified industry | Military
(942) | Never worked, unemployed, homemaking, student, etc.
(961) | Retired
(951) | Unknown ^{1/}
(990) |
|---------------------------|----------------|--------------------|-------------------|--|------------------|--------------------------------|
| Number | | | | | | |
| All Reporting States----- | | | | | | |
| Colorado----- | | | | | | |
| Georgia----- | | | | | | |
| Kansas----- | | | | | | |
| Kentucky----- | | | | | | |
| Maine----- | | | | | | |
| Missouri----- | | | | | | |
| Nebraska----- | | | | | | |
| Nevada----- | | | | | | |
| New Hampshire----- | | | | | | |
| Oklahoma----- | | | | | | |
| Rhode Island----- | | | | | | |
| South Carolina----- | | | | | | |
| Wisconsin----- | | | | | | |
| All Reporting States----- | 100.0 | | | | | |
| Colorado----- | 100.0 | | | | | |
| Georgia----- | 100.0 | | | | | |
| etc. for reporting States | | | | | | |

Programmer's Notes: Table includes only ages 20 years and over and requires 1 page for MVSR Supplement.

Percent distribution

^{1/}Includes deaths that occurred in non-reporting States.

Figure 4

Proposed Table Plans for MVSR Supplement - con.

Figure 4. Proportionate mortality ratios (PMR) for 42-selected industries and 52 selected causes of death, by sex: 13 reporting States, 1984

(Numbers after causes of death are category numbers of the Ninth Revision International Classification of Diseases, 1975)

| Bureau of the Census Industry Code | Industry and sex | All causes | Tuberculosis, including late effects (010-018, 137) | Septicemia (038) | Malignant neoplasms, including neoplasms of lymphatic and hematopoietic tissues (140-208) | Malignant neoplasms of lip, oral cavity, and pharynx (140-149) | Malignant neoplasms of esophagus (150) | etc. for List of 52 selected causes |
|------------------------------------|---|------------|---|------------------|---|--|--|-------------------------------------|
| | Male | 100 | 100 | 100 | 100 | 100 | 100 | etc. |
| 010-031 | All industries-----
Agriculture, forestry, and fisheries-----
Mining-----
040-050
060
Construction-----
100-392
Manufacturing-----
100-222
Nondurable goods-----
100-130
Food and kindred products
132-152
Textile mill and finished products-----
160-162
Paper and allied products
171-172
Printing, publishing, and allied products-----
180-192
Chemicals and allied products-----
200-201
Petroleum and coal products-----
210-222
Rubber, plastics, and leather products-----
230-392
Durable goods-----
230-242
Lumber, furniture, and other wood products-----
250-262
Stone, clay, glass and concrete products-----
270-280
Primary metal industries----- | 100 | 100 | 100 | 100 | 100 | 100 | etc. |
| | etc. for List of 42 Selected Industries | | | | | | | |
| | Repeat for Female | | | | | | | |

Programmer's Notes:

1. Table includes only ages 20 years and over and requires 12 pages for MVSR Supplement.
2. Statistical tests will be used to determine if the PMR is significantly different from 100. Statistically significant PMRs will be indicated as follows 1 space to the right of the PMR:
 + - Significantly different at .05 level.
 ++ - Significantly different at .01 level.

QUESTIONS AND DISCUSSION

DR. SHY: Any questions or comments on the presentation?

DR. VAUGHAN: Tom Vaughan from the Fred Hutchinson Cancer Research Center.

I was wondering if you would comment on the idea that's been advocated by Sam Milham from Washington State, and that's that the reference group in your PMR study should be not the entire population of the deceased but those of the same social class based on, say, occupation.

MRS. BURNETT: That's another way that we intend to analyze this data. And, in fact, the PMR program that we have is set up so that it can analyze by white collar and blue collar. And we do think that, in fact, some of these textile workers, we did analyze that way and got more significance than we would expect to.

So, yes I think that that is a better way than using the total population.

DR. VAUGHAN: Thank you.

DR. SHY: Could you comment on the issue of validity of the information on death certificates regarding occupation?

MRS. BURNETT: Well, there have been several studies done. They usually range in the area of 60 to 70 percent accuracy. But one comment that I would like to make is that usually the comparative data is not an occupational history that's been gotten from the decedent. But it's just another source of occupational data, and you don't know if it's any more correct than what's on the death certificate. It looks like it's about 70 percent accurate.

DR. BEAUBIER: Jeff Beaubier, Environmental Protection Agency. About the melanomas. Since the ocular melanoma rates are constant and the retina (which contains melanocytes) is protected from UV radiation, there must be some kind of intrinsic metabolic or chemical exposure that would cause melanoma to arise there.

Although I am not in this area any longer, I remember hydroquinone is a chemical that's very similar to one of the metabolic precursors in the bioproduction of melanin. And I was struck by your presentation because this is about the third time that I've heard papers discussing the fact that melanoma is high among the publishing and photographic trades where hydroquinone exposures are often found.

So I just throw that out. You might be interested in that.

MRS. BURNETT: That's interesting.

DR. STERN: Frank Stern, NIOSH. I was wondering if you would comment on the industry coding and occupational coding on the death certificate. It is the usual or the last? On the death certificates I've seen, it's the last job the person had rather than the usual job.

MRS. BURNETT: No. On death certificates, the question asked is usual industry and usual occupation.

DR. STERN: I know. But I think in a lot of cases it's the last. At least on some of the death certificates that I've noticed in my study, the person was not considered a tanner because his last job was something else and they coded it as something else.

And I think that's a definite problem.

MRS. BURNETT: Well, that is a problem. Although, in a way, if it were last, it would be more convenient because there are other databases that use last that could be used for denominator data. If it were consistently one way or another it would be best.

DR. CANTOR: Kenneth Cantor, National Cancer Institute. The use of three digits in occupational and industrial coding sometimes lacks adequate specificity. We have some recent examples where there were several industries or occupations that were of interest to us that were included in one of the three-digit categories.

I was wondering, in such a case, would it be possible to use the three-digit code as a screen so that the death certificates themselves could be examined as to the particular industry or occupation actually listed? For example, the census coding scheme combines dry cleaners with launderers. If one is interested in studying dry cleaners, the three-digit code encompasses too broad a range of occupations, and doesn't provide the needed specificity.

MRS. BURNETT: And what are you asking me, is it possible...?

DR. CANTOR: Is it possible, once the three-digit classification is known, to get back to the original death certificate so that further coding may be done to permit a study within that restricted group?

MRS. BURNETT: Not using this database.

DR. CANTOR: Okay.

MRS. BURNETT: I think that it is a possibility.

DR. SHY: Thank you, Mrs. Burnett.

The next two presentations will be given by Thomas Bloom from the National Institute for Occupational Safety and Health. Let us hold our questions for him until he has finished both presentations.

**EXPOSURE CHARACTERIZATION
STUDY OF WORKERS EXPOSED TO HYDRAZINES**

**Presented by Thomas F. Bloom
National Institute for Occupational Safety and Health**

Fourth NCI/EPA/NIOSH Collaborative Workshop:
Progress on Joint Environmental and Occupational Cancer Studies

Exposure Characterization Study of Workers Exposed to Hydrazines

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EXPOSURE CHARACTERIZATION STUDY OF WORKERS EXPOSED TO HYDRAZINES

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The National Institute for Occupational Safety and Health (NIOSH) contracted (Contract No. 200-82-2521) with Clayton Environmental Consultants, Inc. (CEC) to conduct an industrial hygiene assessment of the extent of exposure to hydrazine compounds. The study was initiated because a growing number of these compounds have been shown in laboratory research to be known animal carcinogens, and because there is a lack of workplace exposure data.

The study report discusses production and uses of hydrazine compounds, reviews and summarizes toxic effects of these compounds, summarizes relevant exposure standards, describes sampling and analytical methods relevant to exposure assessment, and summarizes observations made during surveys conducted at eight U.S. facilities where selected hydrazines were produced and/or used.

Observations reported include descriptions of operations and job titles of potentially exposed personnel, exposure control techniques in use, health and safety programs, and discussions of air sampling data reviewed or collected. In summary, the air sampling data showed that, with one exception, 8-hour, time-weighted average (TWA) personal exposures to the hydrazine compound(s) in use during normal operations ranged from below the limit of detection to 0.1 ppm--exposures that were below current OSHA permissible exposure limits of 0.5 to 5 parts per million (depending upon the specific compound).

Acknowledgement:

This study is supported by the National Cancer Institute, through the Interagency Agreement on Occupational Carcinogens (Y-01-CP-60505).

BACKGROUND

Under the terms of the authority and responsibility given to the National Institute for Occupational Safety and Health (NIOSH) to develop needed information regarding potentially toxic substances in industry, NIOSH contracted with Clayton Environmental Consultants, Inc. to conduct an industrial hygiene study of the extent of exposure to hydrazine compounds. Hydrazines were selected by NIOSH for study because a growing number of the compounds have been shown in laboratory research to be known animal carcinogens and therefore suspected human carcinogens⁽¹⁾ and because there is, at the time of this study, a lack of workplace exposure data.

Hydrazine and common hydrazine derivatives were discovered between 1887 and 1893 and first synthesized in 1906 by Raschig.⁽²⁾ The first major use of hydrazine was as a fuel during World War II for rocket-powered German fighter planes. Commercial production in the United States started in 1953.

NIOSH lists more than 300 hydrazine derivatives in the "Registry of Toxic Effects of Chemical Substances."⁽¹⁾ The primary hydrazines of interest include hydrazine, methylhydrazine, 1,1- and 1,2-dimethylhydrazine, and phenylhydrazine

PRODUCTION OF HYDRAZINES

Hydrazine and its Salts

Currently, three major producers in the United States have hydrazine production capacities of approximately 16,500 metric tons per year. (2,3,4,5) Small amounts may be manufactured by other producers as well.

One of the major producers reportedly has about 9,500 metric tons of capacity per year, and about 3,000 metric tons of this is dedicated to the U.S. government, mainly for fuels for aerospace applications. Hydrazine producers estimated the total demand in 1982 at about 9,000 metric tons. Industry sources estimate that annual growth in the next five years will average about 8 to 10%. One of the predicted major areas of growth is in the use of hydrazines as chemical blowing agents. The blowing agents are used in the manufacture of some types of vinyl flooring and in automotive foam cushioning. Hydrazine is also used as an oxygen scavenger to control corrosion in boilers.

SUMMARY OF TOXICOLOGY

Since little information is available from the study of human populations, animal studies provide the primary source of data on the toxicity associated with the production and application of hydrazines. (6,7) Major target

organs and systems include the skin and eyes, the central nervous system, the liver, the blood, and the kidneys.

Hydrazine compounds effect the skin causing dermatitis and they also have potential for skin absorption. The central nervous system is also affected by a number of these compounds, effects include restlessness, breathing difficulties, convulsions, vomiting, and lethargy.⁽⁸⁾ The inhalation of hydrazine by mice at levels of 30 ppm - hours/week cumulative exposure resulted in severe fatty degeneration in the liver.⁽⁹⁾

Oral administration or injection of hydrazine sulfate has produced multiple pulmonary tumors in CBA mice and liver, lung, and breast tumor in Cb rats.⁽⁶⁾ In 1981, MacEwen et al released the results of a four-species inhalation study, in which multiple concentrations of hydrazine were administered to rats, mice, hamsters, and dogs.⁽¹⁰⁾ The exposures were for 6 hours/day, 5 days/week for one year; rodents were observed 12 to 18 months following termination of exposure and the dogs were observed for 38 months. Tested concentrations included 0.05, 0.25, 1.0, and 5.0 ppm. At 5 ppm and 1 ppm, respectively, microscopic nasal carcinomas in rats, and benign tumors of the nasal epithelium were observed. Benign respiratory tumors were also observed in mice and hamsters. When the 21 dogs were sacrificed after 38 months of observation, two tumors were found in one animal exposed at 0.25 ppm; however, the authors interpreted these findings as incidental.

Animal testing has also shown teratogenic and other reproductive effects. Studies by subcutaneous injection of hydrazine in rats resulted in death or stillbirth of all off-spring.⁽¹¹⁾ Hydrazine demonstrated mutagenic activity in the mouse host-mediated assay, Ames test, Drosophila melanogaster, and Chinese hamster cells. Mutagenicity was not demonstrated in the dominant lethal assay.⁽⁷⁾

OCCUPATIONAL HEALTH STANDARDS AND RECOMMENDED LEVELS

FOR HYDRAZINE COMPOUNDS

Exposure criteria and standards for occupational exposures have been developed for hydrazine and three of its derivatives (1,1-dimethylhydrazine, methylhydrazine, and phenylhydrazine). The exposure limits established, adopted, and recommended by the Occupational Safety and Health Administration (OSHA), the American Conference of Governmental Industrial Hygienists (ACGIH), and NIOSH, respectively, are indicated in Table I.^(6,12,13)

TABLE 1
Exposure Limits or Recommended Standards for Hydrazine and its Derivatives

| Chemical | Exposure Limits or Recommended Standards (ppm) | | | | |
|-----------------------|--|-----------|--------|------------|----------------------------------|
| | OSHA
PEL | ACGIH TLV | | | NIOSH
Recommended
Standard |
| | | TWA* | STEL** | CEILING*** | |
| Hydrazine | 1 | 0.1 | -- | -- | C 0.03 |
| 1,1-Dimethylhydrazine | 0.5 | 0.5 | 1 | -- | C 0.06 |
| Methylhydrazine | -- | -- | -- | 0.2 | C 0.04 |
| Phenylhydrazine | 5 | 5 | 10 | -- | C 0.14 |

¹PEL Permissible Exposure Limit: 8-hr TWA

²TLV Threshold Limit Value:

* TWA - Time-weighted average for an 8-hour workshift of a 40-hour workweek

** STEL (short-term exposure limit): A 15-minute TWA exposure

*** Ceiling - concentration not to be exceeded at any time

C Denotes ceiling concentration - the maximal concentration which cannot be exceeded in any 2-hour period.

Note: All the exposure limits or recommended standards carry the "skin" notation, warning of the potential for percutaneous absorption; these specific concentration limits are based on the presumption that there is not concurrent exposure via the skin and oral ingestion routes.

ACGIH lists hydrazine, methylhydrazine, and 1,1-dimethyl hydrazine in its A2 category, i.e. industrial substances suspect of carcinogenic potential in humans.

In 1983, the Committee on Toxicology of the National Academy of Sciences evaluated short-term exposures to hydrazine and has prepared exposure guidelines, as indicated in Table 2.⁽⁷⁾ A concentration-time multiplier concept with a safety factor of 10 was used. Guidelines were issued for emergency exposure limits (EEL), short-term public limits (STPL), and short-term public emergency limits (SPEL) as follows:

TABLE 2

| Exposure Time | TWA Limits for Indicated Exposure Time | | |
|---------------|--|-----------|------------|
| | EEL (ppm) | STPL(ppm) | SPEL (ppm) |
| 10 min. | 3.6 | 1.8 | 3.6 |
| 30 min. | 1.2 | 0.6 | 1.2 |
| 60 min. | 0.6 | 0.3 | 0.6 |
| 2 hours | 0.3 | 0.15 | 0.3 |
| 4 hours | 0.15 | 0.075 | 0.15 |
| 8 hours | 0.075 | 0.0375 | 0.075 |
| 12 hours | 0.05 | 0.025 | 0.05 |
| 16 hours | 0.0375 | 0.018 | 0.0375 |
| 24 hours | 0.025 | 0.012 | 0.025 |

FACILITIES SURVEYED

Walkthrough surveys were conducted at seven facilities where hydrazines are used, handled, or tested. In addition, an eighth facility was visited and hydrazine exposure monitoring was conducted to determine exposures occurring in that manufacturing facility.

The facilities, designated as Sites A through H, are briefly described.

| <u>Site</u> | <u>Use of Hydrazines</u> |
|-------------|--|
| A | Hydrazines-fueled propulsion system and power unit testing |
| B | Hydrazine production |
| C | Air Force fighter base |
| D | Hydrazines-fueled propulsion system testing |
| E | Fighter assembly plant |
| F | Space craft launching |
| G | Formerly hydrazine producer, currently derivative supplier |
| H | Hydrazine production facility |

SITE A

Description of the Site

This site, a major government installation, provides facilities for a variety of operational, administrative, and testing activities related to the space program. Over 3,000 government employees and approximately 9,000 contractor personnel work here.

Use of hydrazines (hydrazine, monomethylhydrazine, and to some extent, 1,1-dimethylhydrazine) at the site is limited to the thermo-chemical test

area (TTA). In this area, engines, power units, and components of engines which utilize hydrazines as propellants are tested. The TTA is separated from the normal traffic areas, and is isolated in the northwest area of the complex. Access is strictly controlled. Such testing has been done periodically for about 20 years at the site.

Description of Operations

Space-craft components (currently, primarily shuttle components) are performance-tested in the TTA. This requires the operation of the propulsion and fuel systems. The fuel for the auxiliary power unit (APU) on the shuttle is monopropellant grade (99.5% pure) anhydrous hydrazine.

Hydrazines are received in 55-gallon drums and stored in a designated and posted area. The fuel is moved around the facility in small portable tanks; the loading and offloading of hydrazines is accomplished with nitrogen pressure. At each test cell, the hydrazine fuel to be used is loaded into test fuel cells (both underground tank and run tank, as necessary) and into the space vehicle component fuel cells.

It was estimated that, at most, 50 gallons of hydrazines are used each month.

Exposure Data

Results of hydrazine sampling at Site A were made available by the facility. Samples were collected using impingers and analyzed by

colorimetry (a modified version of NIOSH Method S 237)¹⁴. The method does not distinguish between hydrazine and methylhydrazine. Tests conducted 30 to 40 days after tests in which hydrazine propellants were used showed trace (0.01 to 0.02 mg/m³) background hydrazines levels.

Area air samples in the vicinity of the wastewater holding pond showed concentrations up to 0.11 ppm when aeration was occurring. Hydrazine concentrations in the holding pond water were 240 ppm as monomethyl hydrazine or 48 ppm as hydrazine.

Personal sampling has not been done at Site A. During each activity that entails potential hydrazine or methylhydrazine exposure, full body air-supplied suits are worn. The suits are PVC based and equipped with special gloves.

SITE B

Description of the Facility

Site B is a large chemical plant in the south central United States with diverse production and manufacturing units including production of hydrazine hydrate.

The plant was constructed beginning in 1971. The hydrazine unit was built in 1975. Currently, approximately 800 persons are employed at the plant. Operators are site employees; maintenance is by an outside contractor.

Description of Operations

Hydrazine hydrate is produced by the Bayer-Ketazine process. Chlorine is reacted with sodium hydroxide to form sodium hypochlorite and sodium chloride. The hypochlorite is reacted with ammonia and acetone forming a dilute hydrazine stream through hydrazine and ketazine intermediates. The hydrazine hydrate is either shipped in bulk or in 30- or 55-gallon drums.

The hydrazine production unit is not enclosed and is not mechanically ventilated. The drumming operation is semi-enclosed and provided with ventilation using a large excess of exhaust air to keep all air movement well away from the operator.

Exposure Data

Frequent monitoring has been done to determine TWA exposures to hydrazine and short-term exposures in operations which entail the greatest potential for release of hydrazine. The sample collection medium currently in use is acid-coated firebrick absorbent; analysis is by desorption with dilute sulfuric acid, reaction of p-dimethyl-aminobenzaldehyde to form a yellow complex, and measurement of color intensity at 455 nm. In the past, samples have been collected in midget impingers with an 8% sulfuric acid absorbing solution, with analysis by the same colorimetric technique.

Exposure was assessed by reviewing the company's air sampling records.

Typical TWA results showed levels less than 0.01 ppm. Short-term (5 to 10 minutes) samples during specific operations showed levels up to 0.1 ppm.

Area samples showed levels up to 0.1 to 0.2 ppm.

SITE C

Description of Facilities

The facility is an integrated Air Force base, housing, in addition to other military units, a squadron of approximately 70 F-16 fighter planes. The presence of the F-16s requires maintenance and support capabilities unique to this aircraft since it is a "fly-by-wire" designed aircraft (that is, all control systems are based on electrical and/or hydraulic systems). It is imperative that emergency power be provided in the event of engine failure. Among these support operations are those for the handling and refilling of the Emergency Power Unit (EPU) fuel cells (bottles) containing 6.8 gallons of H-70 (a 70% hydrazine, 30% water mixture which provides fuel for the EPU upon engine failure). The maintenance squadron oversees these activities, which are carried out in an engine maintenance building and an adjacent service building. The service building houses hydrazine fuel cell refill operations and hydrazine drums. An emergency response trailer houses response equipment such as respirators, protective clothing, oxidizing bleach, and test materials.

Description of Operations

The operations involving hydrazine at the site include the removal of the hydrazine bottle from the aircraft, refilling the bottle as necessary, and tending aircraft (cleaning potentially contaminated areas on the aircraft and flight line) which have fired the EPU. These activities occur at two locations: on the flight line--just before or after landing--or in the service building.

Activities on the flight line involve stabilizing the EPU system, removal of the fuel bottle, and control and cleanup of any spills. Employees wear gloves, aprons, boots, and face masks during routine bottle handling on the flight line. Spill containment and cleanup is done by employees fully protected with air-supplied respirators, suits, gloves, and boots. This equipment is contained in an emergency response trailer.

Bottle refilling occurs in the service building. Refilling procedures are detailed in written instructions. The refilling stand is similar to that seen at the F-16 production facility (Site E), and is apparently being standardized for F-16 operations. The only ventilation is an overhead fan which is only operational when the heating system is in use. No ventilation was provided in the summer and the building was closed, apparently to protect nearby aircraft in the event of a hydrazine leak.

Exposure Data

Monitoring is performed using firebrick sorbent collection and colorimetric analysis.

Routine, predictable exposures do not occur, so environmental data are limited. Sampling during a spill showed hydrazine levels of 0.04 mg/m^3 for a 38-minute sample and 0.05 mg/m^3 for a 43-minute sample. Monitoring at a temporary fuel tank test stand, in use before the present permanent facility was ready, indicated concentrations of 0.01 and 0.13 mg/m^3 (30-minute samples) and 0.03 and 0.02 mg/m^3 (15-minute samples). The detection limit was 0.013 mg/m^3 . The Air Force hydrazine standard (AFOSH Standard 161-13) is 0.13 mg/m^3 (0.1 ppm).

SITE D

Description of Facility

Site D, which began operations in 1963, is located in the southwestern United States and is a major government test facility. Approximately 300 persons perform a large number and range of tests primarily related to the nation's space program. Tests involving the use of hydrazines conducted at the site include testing of propulsion systems, personal protective equipment, materials corrosion, and chemical analytical procedures and instrumentation.

Hydrazines are used in a number of areas at Site D. These include the engine and propulsion system test stands, and the materials testing area. Support areas include the chemistry lab, safety office, and medical clinic (contractor operated).

Description of Operations

Hydrazine fuels (hydrazine and monomethylhydrazine) are used during propulsion systems testing. A variety of engines are or have been tested. Currently, the majority of the tests involving hydrazines are connected with the space shuttle program.

Propulsion tests occur as required and not as part of a "routine" schedule. Actual test runs may last only 1 to 2 days. Test set-up and follow-up analysis may take several weeks for each test.

Materials testing is also done. Test materials are exposed in test cells to the compound of interest to determine corrosion and compatibility.

Hydrazine currently is and has been used in such testing.

Hydrazine waste streams are vented through scrubbers and the scrubber water directed to a holding pond. The water is treated by chlorination prior to discharge offsite.

Exposure Data

Monitoring is regularly performed before and after each test at Site D. Testing is done using integrated, colorimetric procedures and direct-reading instruments. Integrated samples are collected with acidified firebrick, eluted with water, and analyzed colorimetrically. This method reportedly provides a lower limit of detection of 0.02 ppm of monomethylhydrazine in a 25-liter air sample. The results of the monitoring have indicated concentrations primarily below the limit of detection. However, the data do not represent employee exposure since employees are protected with SCAPE suits (self-contained atmospheric protective ensemble).

SITE E

Description of the Facility

Site E is a large aircraft manufacturing complex. The current aircraft under production is the F-16, a combat fighter. The F-16 is equipped with an emergency power unit (EPU) which is fueled by hydrazine (H-70: 70% hydrazine, 30% water).

F-16 manufacturing is housed primarily in a large main assembly building with over 16,500 employees.

Description of Operations

The EPU must be installed and tested during assembly of the F-16 aircraft. Accordingly, the EPUs are fired ("green run"), and the hydrazine fuel cells (bottles) refueled and reinstalled. Hydrazine bottles are also refilled as necessary for transient aircraft.

The EPU fuel bottle contains approximately 6.8 gallons or 56 pounds of hydrazine. Bottle refueling and servicing involves three primary functions:

- (a) Removal and installation of bottles from the aircraft
- (b) Refueling and servicing the bottles
- (c) Spill cleanup and response

Exposure Data

Site E routine testing of hydrazine exposure is through the sulfuric-acid/firebrick collection technique, elution by water, and colorimetric analysis.

Results of area sampling during "green" runs (EPU test runs) indicated concentrations ranging from below the detection limit on the "upwind" side of the aircraft to 0.53 ppm at a point about 5 feet below the EPU exhaust port, based on sampling periods normalized to 15 minutes. (Actual sampling periods on the four dates ranged from 2.5 to 37 minutes because of changes and/or delays in the test sequences, in which the EPU was operated for only a few seconds at a time in each test mode).

The detection limit was not reported; however, hydrazine concentrations (normalized to 15 minutes) as low as 0.01 ppm were reported (0.19 micrograms of hydrazine found in a 7.4 minute sample). The report concluded that, because of the brevity of potential exposures, in which the EPU is fired for only a few seconds at a time and the exhaust cloud dissipates quickly (in 2 minutes or less), there is little risk of TWA exposures above the OSHA standard (1 ppm) or Air Force standard (0.1 ppm).

SITE F

Description of Facility

Site F is located in the southeastern U.S. The primary mission at the site is the launching of space vehicles into Earth orbit using chemically fueled rockets. Currently, a major program at the site is the Space Transportation System (STS), popularly called the "Shuttle Program."

The site controls approximately 150,000 acres. Hydrazine usage is centered in several facilities including:

- (a) Fuel Farm and Incinerator
- (b) Spacecraft Operations/Industrial Area
- (c) Vehicle Assembly Area

Support operations necessary for hydrazine use include administration, medical, and SCAPE suit maintenance.

Description of Operations

Hydrazines are used at the site as hypergolic propellants, primarily for the space shuttle and space shuttle payloads. Both monomethyl- hydrazine and hydrazine are used as follows:

(a) Space Shuttle Orbiter

- o Orbital Maneuvering Subsystem (OMS) - monomethylhydrazine
- o Reaction Control Subsystem (RCS) - monomethylhydrazine
- o Hydraulic Power Unit - hydrazine

(b) Space Vehicles (payload operations) - hydrazine, 1,1-dimethyl- hydrazine

Hydrazines are received at a fuel farm, which has three 22,000-gallon tanks and the capability to store 100 55-gallon drums. Fuel is also maintained at the launch pad fuel farm.

Potential hydrazine exposure occurs through the loading, testing, and off-loading of fuel on the various vehicles. Currently, most larger hydrazine handling activities in the shuttle program are centered on the launch pad in order to consolidate potential exposures. Smaller amounts of hydrazine are processed onto spacecraft in the cargo operations areas of the site.

Exposure Data

Since personnel are usually protected by SCAPE suits during activities that might release hydrazines into the air, little integrated sampling has been done to characterize TWA or full-shift potential exposures. Extensive leak detection, clearance, and re-entry sampling has been done with Draeger detector tubes; however, results of these tests do not represent exposure. The site is supporting research into a direct-reading instrument which will allow the more rapid "clearance" of areas where hydrazine propellants have been used. Impediments are the presence of ammonia as a potential interference and the frequent need to test in the presence of high humidity. Testing to date has not provided an instrument that is free of ammonia and humidity interference problems.

SITE G

Description of Facility

A visit was made to the corporate headquarters of a former hydrazine producer and current hydrazine derivative producer. The actual sites where hydrazine was formerly produced or used in the production of blowing agents or agricultural chemicals were not available for a walkthrough survey. Access was limited to the corporate headquarters where industrial health and safety programs are managed.

Description of Operations

The manufacturer does not currently produce hydrazine at any of its locations. Production was discontinued approximately 2 years ago.

Hydrazine (30% hydrate form) was produced from 1964 to 1981 in one of its southern chemical plants. Approximately 3 to 4 million pounds per year of hydrazine hydrate were produced.

Hydrazine hydrate (roughly 6 to 8 million pounds per year, although exact figures were not available) is still used in the production of certain derivatives at other locations of this company (blowing agents, agricultural chemicals). These derivatives are produced primarily in the South with occasional use in the East. Hydrazine for these processes is received in the 54 or 85% hydrate form, primarily via tank truck.

Any waste hydrazine is treated with hypochlorite (chlorine), held in a holding tank, and disposed of by deep-well injection.

Exposure Data

Monitoring at Site G for hydrazine exposure has increased in the last 5 years. The current ACGIH and NIOSH-recommended standards (0.1 ppm) require the ability to reliably detect low concentrations. The current method is collection on sulfuric acid-coated glass beads followed by colorimetric analysis. The limit of detection is 0.010 ppm in a 40-liter air sample. Impingers were used for sample collection before glass beads were used.

Historical exposure data were provided as shown in Table 3. Most 8-hour, TWA exposures are well below 0.1 ppm. Occasional exposures above 0.1 ppm may occur for limited time periods or in limited areas. Employees in these areas are often protected by use of respiratory protective equipment.

SITE H

Description of Facility

The Site H facility produces a variety of industrial chemicals, including sodium nitrate, urea, ammonia, nitric acid, toluene diisocyanate, a flame retardant additive, and hydrazine. There are two hydrazine production units, only one of which (the ketazine process plant) was operating during the survey.

The ketazine process plant, in operation since 1979, produces aqueous hydrazine hydrate at a concentration of 65 to 66% by weight (as hydrazine). The stored product is diluted with demineralized water to specified concentrations before shipment in 55-gallon drums or tank trucks.

Description of Operations

In the ketazine process at Site H, ammonia is oxidized by chlorine in the presence of acetone. The ketazine which is formed is hydrolyzed and aqueous hydrazine (65-66% by weight) is pumped to product storage.

TABLE 3
Summary of Hydrazine Monitoring* Data - 1978 through 1981
Site G

| Job Category | No. of
Samples | % of Samples | | | |
|----------------------------|-------------------|--------------|-------------|-------------|---------------|
| | | ppm
<0.01 | ppm
<0.1 | ppm
<1.0 | ppm
<2.0** |
| Boardman | 68 | 80 | 100 | | |
| Outside Tech. | 71 | 70 | 90 | 99 | 100 |
| Tank Farm Tech. | 13 | 15 | 85 | 100 | |
| Day Centrifuge Tech. | 8 | | | 100 | |
| Total Personal Samples | 160 | 67 | 89 | 99 | 100 |
| Fixed Point (Area) Samples | 89 | -- | 33 | 91 | 99 |
| | | | | | 100 |

*Sorbent collection; colorimetric analysis.

**Values between 2.0 and 12.0 ppm are for area samples. They do not represent worker exposure levels and were non-recurring incidents.

Exposure Data

Monitoring over two consecutive day shifts was conducted at Site H.

Sulfuric acid-coated firebrick was used to collect the samples. Analysis was by the p-dimethylaminobenzaldehyde colorimetric technique.

Results are presented in Table 4. Time-weighted average hydrazine exposures ranged from below the limit of detection to 0.27 ppm. Short-term hydrazine exposures (during process stream sampling) of up to 0.91 ppm were also measured.

BOILER WATER TREATMENT

A site visit was not made to a large boiler operation (electric power utility), but information on hydrazine exposures was submitted by a southern utility. The information contained the results of air sampling at nine power stations during the addition of hydrazine to the boiler water system. Exposure data are summarized in Table 5. Personal sampling indicated short-term exposures during specific activities (level checking, drum changing, pouring from drums, and level adjustment) ranging from 0.002 to 0.23 ppm (mean value 0.087 for samples indicating detectable levels) during sampling periods ranging from 5 minutes to 50 minutes. Eight-hour, TWA exposures (based on assumed "zero" exposures for the unsampled periods) ranged from less than 0.0002 to 0.005 ppm (mean value 0.002).

TABLE 4
Results of Hydrazine Sampling - Site H
February 8 and 9, 1984

| Sample Number | Sample Date | Description | Sampling Period | | Sample Volume (Liters) | Hydrazine Concentration (ppm) |
|---------------|-------------|---|-----------------|------|------------------------|-------------------------------|
| | | | Start | Stop | | |
| HYD-4 | 2/8 | Board operator | 0842 | 1523 | 25 | <0.003 |
| HYD-22 | 2/9 | Board operator | 0738 | 1454 | 28 | <0.003 |
| HYD-3 | 2/8 | Outside operator | 0817 | 1459 | 74 | 0.041 |
| HYD-23 | 2/9 | Outside operator | 0743 | 1454 | 81 | 0.008 |
| HYD-1 | 2/8 | Sample operator | 0742 | 1512 | 57 | 0.27 |
| HYD-6 | 2/8 | Sample operator, collecting and analyzing samples ^(a) | 0857 | 0903 | 5.6 | 0.68 |
| HYD-11 | 2/8 | Sample operator, sampling and dumping old samples ^(b) | 0933 | 0943 | 9.4 | 0.02 |
| HYD-12 | 2/8 | Sample operator, sampling and analyzing samples ^(c) | 1121 | 1148 | 25 | 0.91 |
| HYD-24 | 2/9 | Sample operator | 0800 | 1510 | 56 | 0.041 |
| HYD-28 | 2/9 | Sample operator, sampling hydrolysis overheads and hydrolysis bottoms | 1330 | 1337 | 6.5 | 0.06 |

- (a) Sampling concentrator bottoms, dilute caustic, and hypochlorite; analyzing concentrator bottoms sample.
(b) Sampling ketazine overheads, ketazine bottoms, ketazine tray 58, ketazine feed, hydrolysis feed, and hydrolysis bottoms; dumping old samples into pot to recycle waste line.
(c) Sampling ketazine bottoms, ketazine tray 69, hydrolysis trays 72, 68, 62, and 58; analyzing those samples for percent organics; sampling ammonia stripper bottoms, north and south ketazine reactors, ketazine tray 58, hydrolysis overheads, hydrolysis feed, and concentrator bottoms; weighing those samples.

Analytical limit of detection: 0.1 microgram per sample.

TABLE 4 (continued)
Results of Hydrazine Sampling - Site H
February 8 and 9, 1984

| Sample Number | Sample Date | Description | Sampling Period | | Sample Volume (Liters) | Hydrazine Concentration (ppm) |
|---------------|-------------|--|-----------------|------|------------------------|-------------------------------|
| | | | Start | Stop | | |
| HYD-7 | 2/8 | Drumming 54% N ₂ H ₄ | 0946 | 1518 | 17 | 0.01 |
| HYD-29 | 2/9 | Drumming 35% N ₂ H ₄ | 1337 | 1456 | 7.0 | <0.01 |
| HYD-2 | 2/8 | Pipefitter | 0752 | 1513 | 30 | 0.025 |
| HYD-21 | 2/9 | Pipefitter | 0724 | 1445 | 30 | 0.02 |
| HYD-5 | 2/8 | Instrument man | 0847 | 1521 | 67 | 0.002 |
| HYD-25 | 2/9 | Instrument man | 0808 | 1519 | 77 | <0.001 |
| HYD-26 | 2/9 | Millwright | 0812 | 1507 | 38 | <0.002 |

TABLE 5

**Hydrazine Exposures in Boiler Water Treatment
at Nine Electric Power Plants***

| Location | Sample Type (Number) | Hydrazine Concentration
(ppm) |
|----------|--------------------------|--|
| I | Area (2) | 0.006 0.013 |
| II | Area (2) | 0.007 0.011 |
| III | Area (4)
Personal (1) | 0.0031-0.0065
0.071 (14 minutes) |
| IV | Area (2)
Personal (1) | 0.002 0.006
0.23 (7 minutes) |
| V | Area (6)
Personal (1) | < 0.0002-0.077
< 0.002 (36 minutes) |
| VI | Area (4)
Personal (2) | 0.0032-0.024
0.024 (28 minutes)
0.099 (5 minutes) |
| VII | Area (4)
Personal (1) | 0.016-0.033
0.162 (9 minutes) |
| VIII | Area (5)
Personal (2) | 0.0017-0.024
0.065 (37 minutes)
0.023 (10 minutes) |
| IX | Area (4)
Personal (1) | 0.002-0.09
0.02 (50 minutes) |

*Data submitted by a southern utility in a written communication.

Area samples taken in drum storage and hydrazine dispensing areas indicated concentrations ranging from less than 0.002 ppm to 0.09 ppm.

DISCUSSION

Four types of hydrazines workplaces were reviewed during the course of this study: sites where hydrazines are used as propellants, hydrazine production sites, sites where hydrazine is used as an aircraft emergency power unit fuel, and sites where hydrazine is used in boiler water treatment. Several observations regarding hydrazines can be made on the basis of information obtained during this study:

1. Awareness of the acute toxicity of hydrazine and the concern raised by the results of chronic exposure testing in animals have generated a respect for the importance of avoiding exposure in handling hydrazine compounds. At the facilities visited during this study, employers and employees were aware of the need to avoid exposure, and had developed handling systems, work practices, and personal protective equipment programs to control exposure to hydrazine.
2. Use of hydrazines as propellants involves potential exposures during: (1) loading/unloading of the spacecraft and related propellant transfer operations, and (2) testing of spacecraft components which utilize hydrazines. During hydrazine propellant handling at the sites visited, the worst-case exposure potential is assumed; accordingly, full-body supplied-air suits are worn whenever

hydrazine is loaded, unloaded, or moved in vessels or pipes. Areas are "cleared" prior to resumption of activities without this protection. Airborne hydrazine concentration measurements made in propellant transfer operations are essentially instantaneous and are not intended to reflect personal exposures.

3. Hydrazine's production activities generate TWA exposures generally well below 0.1 ppm, primarily because the production processes involve closed systems. However, certain operations (centrifuging, outdoor activities, routine process stream sampling, and maintenance work) appear to entail a somewhat greater risk of exposure. The use of personal protective equipment was noted during such activities at the producers visited. Process stream sampling appears to present the greatest recurring risk of exposure; TWA exposures were generally within established limits (0.04 to 0.27 ppm), but excursions up to 0.91 ppm were noted.
4. In recent years, the use of hydrazine as a fuel in military aircraft emergency power units (EPUs) has resulted in an increase in the handling of small amounts of hydrazine. Routinely, this handling should not result in TWA exposures approaching current limits and exposure recommendations. The use of proper safeguards during EPU fuel cell handling and refilling has minimized the potential exposure. Prior to improvements in the venting system of the fuel stand, potential TWA exposures up to 0.19 ppm were noted. In servicing of aircraft, the increased use of hydrazine as an EPU fuel may involve increased risk of exposure from contact with residues on aircraft whose EPUs have been fired or from spills that may occur in flight line servicing of EPUs.

5. Hydrazine is widely used as an oxygen scavenger in boiler systems. The quantities used during each addition are rather small, and exposure data submitted by a utility in the course of this study indicated relatively low exposure. Short-term exposures (5 to 37 minutes) did not exceed 0.23 ppm. Long-term area samples indicated concentrations below 0.1 ppm. However, the potential for exposure exists if spills occur during transfers and, therefore, use of protective equipment (gloves, mask, shield) is appropriate in handling the hydrazine formulations. Hydrazine manufacturers recommend automated hydrazine transfer procedures in larger boiler systems.

In summary, data obtained in the course of this study (data submitted by companies, obtained from companies during site visits, or collected as part of an in-depth industrial hygiene survey) have shown that time-weighted average exposures to hydrazines are relatively low (generally below 0.1 ppm). The number of exposed workers is also small; only a few workers are involved in hydrazines manufacture and the routine use of hydrazines as propellants and fuels. The current manufacturing methods are relatively new and large scale propellant and emergency power unit usage is also fairly recent. Because of these factors, the accumulated person-years of exposure are relatively low, and it is unlikely that suitable cohorts exist for retrospective exposure studies.

RECOMMENDATIONS

1. Exposures to hydrazine and hydrazine compounds may occur during spills or other non-routine situations. Generally, where the potential for such exposures is in any way predictable, employees have been well protected (air-supplied protective suits), and the potential exposure areas have not been re-entered until it has been determined that hydrazine concentrations have adequately abated. There is a need for direct-reading devices to rapidly characterize the potential for exposure in such situations, especially where hydrazines are used as propellants and fuels (loading and off-loading, engine testing, and the bottle refilling and aircraft decontamination activities associated with servicing aircraft emergency power units).

However, existing sampling and analytical methods for hydrazine generally are suitable only for reliably determining TWA exposures. The direct-reading instruments presently available lack specificity (i.e., the instruments are cross-sensitive to other materials) or provide indications that are affected by humidity. Colorimetric tubes are being widely used in the interim, but reliable direct-reading instruments are needed for rapid characterization of exposure potential in such applications as establishing safety zones around known or suspected spills or "clearing" an area for entry by unprotected workers. Research on such instruments is currently being done, primarily to satisfy government requirements.

2. The increased use of hydrazine as a military aircraft emergency power unit fuel will undoubtedly entail a greater frequency of spills and leaks, due to increased servicing and refueling requirements. Refueling should be done only in well-ventilated facilities. Temperature control may be necessary to allow the use of protective devices (gloves, mask, apron) without causing skin exposure as a result of concentration of hydrazine against the skin as vapors dissolve in perspiration.
3. Hydrazine and hydrazine derivatives are manufactured and processed in closed systems. In such systems, the greatest risk of routine exposure occurs when the pressurized system is accessed for process and quality control sampling. Control of exposure during sampling may be improved through the use of closed-loop sampling systems and the more cautious disposal of excess samples.

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**INDUSTRIAL HYGIENE STUDY: EXTENT OF EXPOSURES
TO ORGANOTINS**

**Presented by Thomas F. Bloom
National Institute for Occupational Safety and Health**

FOURTH NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROCESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

INDUSTRIAL HYGIENE STUDY:
EXTENT OF EXPOSURES TO ORGANOTINS

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The National Institute for Occupational Safety and Health (NIOSH) contracted with Clayton Environmental Consultants, Inc. (CEC) to conduct an industrial hygiene assessment of the extent of exposure to organotin compounds. The study was initiated primarily on the basis of a report of an animal study indicating that triphenyltin acetate had induced malignant tumors in mice. (A later review of this study indicated that data supporting this observation were not conclusive.)

The study report discusses production and uses of organotin compounds, reviews and summarizes toxic effects of these compounds, summarizes relevant exposure standards, describes sampling and analytical methods relevant to exposure assessment, and summarizes observations made during surveys conducted at four U.S. facilities where selected organotins were produced and/or used.

Observations reported include descriptions of operations and job titles of potentially exposed personnel, exposure control techniques in use, health and safety programs, and discussions of air sampling data reviewed or collected.

In summary, air sampling data indicated a wide range of full-shift, time-weighted average airborne organotin concentrations, varying from below the detection limit (which varied according to the sampling and analytical methods used) to 2.9 mg/m³ (as Sn), depending on the operations and activities monitored. The use of personal protective equipment (respirator or hood) was noted in most situations in which concentrations exceeded the 0.1 mg/m³ (8-hour, time weighted average) limit for organotins (as Sn) established as a permissible exposure limit by the Occupational Safety and Health Administration (OSHA) and currently recommended by NIOSH. Thus, "actual" inhalation exposures generally were likely to be lower than the air sampling results would suggest.

This study was supported by the National Cancer Institute, Division of Cancer Cause and Prevention, through the Interagency Agreement on Research on Occupational Carcinogens (Y-01-CP-60505).

I. Introduction

A. General

The National Institute for Occupational Safety and Health, under an Interagency Agreement with the National Cancer Institute, conducted an industrial hygiene investigation in connection with workers exposed to organotins. As part of this investigation, workplace inhalation exposures to organotin compounds were evaluated.

The impetus for this investigation came from a citation in the 1976 NIOSH Registry of Toxic Effects of Chemical Substances (RTECS) indicating that triphenyltin acetate (TFTA) had induced malignant tumors in mice. (A subsequent review of this citation by the RTECS editorial board led to a 1983 reclassification of the report as one suggesting evidence of a neoplastic response. Neoplastic --as defined by the RTECS board--refers to a substance that produces a benign tumor, or a tumor that cannot definitely be classified as carcinogenic, or tumors produced in a study where the results are equivocal because of poor study design.) In addition, TFTA along with three other organotin compounds --tributyltin acetate (TPTA), tricyclohexyltin hydroxide (TCHH), and triphenyltin hydroxide (TPTH)--were the subject of interest by the Environmental Protection Agency (EPA) as a result of these

compounds being candidates for pesticide registration. In this connection EPA was requesting oncological related information for these substances. While the most recent RTECS (1984-85) did not report citations related to carcinogenic responses from the aforementioned or other organotins, several reports of animal studies were uncovered which suggested possible adverse reproductive effects associated with several organotin compounds of the triorganotin type which are associated with biocidal uses. For this brief investigation, NIOSH and contractor researchers performed industrial hygiene evaluations at three facilities. Personal and area air samples were collected at potential exposure locations at each setting. Samples were analyzed for total organotins.

During the industrial hygiene surveys, we also obtained employment related information. This information, along with exposure related information, was given to NIOSH epidemiologists for assessment of the feasibility of conducting a study of potential reproductive effects related to organotin(s) present.

B. Scope of Investigation

The scope of our overall investigation included:

- a. Identification of organotin producers and users;

- b. A review of toxicologic literature;
- c. Industrial hygiene surveys of facilities involved with organotin compounds; and
- d. Collection of reproductive study feasibility information

C. Uses of Organotins

Organotin compound usage can be categorized into two major areas: biocidal usage and non-biocidal usage. Biocidal uses include pesticides and antifouling coatings; non-biocidal uses include stabilizers and catalysts. Minor uses include antioxidants, and as metal plating agents. Figure 1 shows the primary organotin compound groups associated with the major use areas. The percentages are based on 1981 consumption data--estimated at 11,400 metric tons; 90% of consumption consists of mono and dialkyltins used as nonbiocides, and the remaining 10% consists of triorganotins used as biocides.

D. Activities of Potentially Exposed Employees

Workers may be potentially exposed to organotin compounds during (1) production of the compounds themselves; (2) formulation of compounds into biocides, stabilizers, catalysts or other minor products and, (3) application and use of

products containing organotin compounds, of which include fungicide sprays, paint sprays, and polyvinyl chloride compounds.

E. Occupational Health Aspects

1. General Toxicology Statements

There are more than 200 organotin compounds in the NIOSH "Registry of Toxic Effects of Chemical Substances". While a detailed discussion concerning the toxicities of specific organotins is beyond the scope of this paper, some general statements can be made:

Organotins exhibit a broad spectrum of toxicities, depending on the number and length of alkyl or aryl groups. Of the various classes, i.e., mono, di, tri, and tetraorganotins, the triorganotins have been shown to be the most toxic. Within a particular class, the lower alkyl groups are the most toxic to mammal, with the methyl and ethyl groups significantly more toxic than the higher alkyl groups.

Many organotin compounds are skin irritants and may be absorbed via the skin.

2. Reproductive Effects

Several reports of animal studies suggested possible adverse reproductive effects associated with several organotin compounds. A majority of the specific compounds in question were of the triorganotin type used for biocidal purposes. Examples are triphenyltin acetate and triphenyltin hydroxide. Reproductive effects reported included reduced pregnancy rates among female rats (mated with unexposed males), decreases in weights of the uterus and testes among rats (both male and female rats exposed), and decreases in size of ovaries.

It should be emphasized that, with the exception of one of these studies, all studies were based upon oral administration of the organotin in question. Further, it should be stated that these were animal studies. Therefore, such studies may not be necessarily indicative of the hazard potential associated with inhalation of these compounds, nor may they be necessarily indicative of human reproductive responses to these compounds.

2. Environmental Effects

On January 8, 1986, the Environmental Protection Agency (EPA), published a notice in the Federal Register indicating their intention to undertake a Special Review of certain tributyltin pesticide compounds used as paint additives to inhibit growth of certain aquatic organisms after determining that these compounds may present unreasonable risks to organisms such as mussels, clams, oysters, and fish. (The Special Review process is a mechanism by which the EPA collects information on the risks and benefits associated with the use of pesticide to determine whether any use causes unreasonable adverse effects to the environment). Tributyltin (TBT) compounds under review are listed in Table 1.

The TBTs are contained in antifoulant paints that are applied to shipyard boat hulls to inhibit the growth of marine and aquatic organisms such as barnacles. EPA reported that laboratory studies showed that TBT compounds were highly toxic

TABLE 1

TRIBUTYLTIN CONTAINING PESTICIDE COMPOUNDS SUBJECT TO SPECIAL REVIEW

Bis(tributyltin)oxide

Bis(tributyltin)adepate

Bis(tributyltin)dodecenyl succinate

Bis(tributyltin)sulfide

Tributyltin acetate

Tributyltin acrylate

Tributyltin fluoride

Tributyltin methacrylate

Tributyltin resinate

and potentially lethal to marine and fresh water organisms at the part per trillion (by weight) level. EPA is also reviewing several other studies which suggest that TBT compounds cause chronic problems such as abnormalities to the anatomy, adverse growth effects, and bioaccumulation in marine life.

F. Exposure Regulation/Recommendations

1. Organotins as a Group

The present OSHA PEL for organotins is 0.1 mg/m^3 measured as elemental tin. This level is based on the Threshold Limit Value (TLV) established by the American Conference of Governmental Industrial Hygienists (ACGIH) in 1965. At that time, ACGIH based its recommendation for organotins by analogy with mercury, thallium, and selenium compounds. The ACGIH level was recommended to prevent systemic toxicity. The current (85/86) TLV for organotin compounds is 0.1 mg/m^3 for an 8-hour TWA. (The ACGIH has eliminated the previous short-term exposure limit-- 0.2 mg/m^3 --(STEL) beginning with the 1986 listing.) The TLVs for organotins contain a "skin" notation, a warning of the potential for percutaneous absorption. ACGIH stated that these TLVs--for

inhalation exposures--are based on the presumption that there is no concurrent exposure via the skin and oral ingestion routes.

In 1976, NIOSH recommended that employee exposure to organotin compounds be controlled so that an employee is not exposed to organotin compounds, measured as elemental tin, at greater than a level of 0.1 mg/m^3 as a TWA for up to a 10 hour workday, 40 hour workweek. NIOSH stated that, since human and animal toxicity data neither support nor negate the basis for the current federal standard, that the current standard be retained for all organotin compounds until more definitive information has been obtained.

It should be noted that aforementioned exposure limits and recommendations have been established for organotins as a group.

2. TLV for Tricyclohexyltin Hydroxide (TCHH)

The ACGIH has, however, adopted a separate 8-hour, TWA TLV of 5 mg/m^3 . (The ACGIH also eliminated the previous STEL for TCHH-- 10 mg/m^3 --beginning with the 1986 listing.) This TLV

does not contain a skin notation. The TLV for TCHH corresponds to about 1.5 mg/m^3 of elemental tin, which is substantially higher than the 0.1 mg/m^3 TLV for organotin compounds as a group. ACGIH stated that the 0.1 mg/m^3 TLV appeared to have been based on the more toxic members of the group. Except for the proposed deletion of the STEL, ACGIH recommends that the 8-hour TWA TLV for TCHH be retained for the present.

NIOSH, for its part, has not recommended any exposure levels for specific organotins.

II. Selection of Organotin Compounds and Facilities for Evaluation

A. Parameters

In the selection of organotin compounds for investigation, toxicity data, particularly that cited in connection with the reproductive studies, U.S. consumption data, trends regarding future usage, data from the National Occupational Hazard Survey, and the perceived likelihood of workplace exposures were considered.

B. Site Selection

1. Types of Facilities Selected

After consideration of the aforementioned factors, NIOSH researchers decided to focus project efforts on the triorganotin compounds used in biocidal applications. Biocidal uses were associated with several triorganotin organotin compounds mentioned in the reports of animal reproductive studies.

Accordingly, we initiated efforts to locate facilities that produced or used triorganotin compounds which had biocidal applications. Three facilities were located and arrangements made for on-site surveys. They were:

- a. A producer of triorganotin compounds with potential biocidal applications
- b. A formulator of a triorganotin containing miticide
- c. A user of a triorganotin containing antifouling paint

III. Description of Facilities Surveyed and Exposed Groups

Table 2 is a summary showing: Type of facility; number of potentially exposed employees; job activities associated with potentially exposed groups; personal protective equipment used.

Facility A was a producer of several organotin compounds, including tributyltin oxide and tributyltin chloride. Approximately 15 employees were involved in production which is conducted 24 hours per day. The production process is carried out in a "closed" system. Process operators, involved in monitoring the production process, collecting quality control samples, and transferring of finished product to containers, are the primary exposure group. Use of personal protective equipment, including clothing, safety glasses, hard hats, shoes (rubber boots), and rubber gloves, is required in the production area. A variety of respiratory protection is available for use as the need arises.

Facility B was a formulator of a miticide containing the organotin tricyclohexyltin hydroxide. Approximately 25 people were involved in the formulation of the miticide over three shifts. Exposure groups include the millman, bag filler, weigh operator, bag seamer, and packer/boxer. Personal protective equipment provided and required to be worn included respirators, protective clothing, safety glasses, gloves, bump caps, and steel-toed shoes.

Facility C was a naval shipyard. At the time of the evaluation, shipyard personnel were coating the underside of an aircraft carrier with an organotin containing antifouling paint. Organotin compounds present in the coating were reported to be tributyltin methacrylate and tributyltin oxide. Approximately 15 employees

were involved in application of the coating. Primary exposure groups were the coating mixer, a driver transporting the coating to the various painting crews situated along the length of the carrier, two paint crew members (of each crew) tending the painting equipment, and one paint crew member applying the coating with a spray wand. All workers wore disposable coveralls with head coverings, shoe covers, and impervious gloves. All paint sprayers wore full-facepiece, continuous-flow, air-supplied respirators. Paint mixers, paint drivers, and pump crews wore full-facepiece, air-purifying respirators canisters approved for use with pesticides.

IV. Field Evaluation

A. Description of Sampling Strategy

At each of the three facilities, personal breathing zone samples were collected from exposed job titles for measurement of potential exposure to organotins. Area samples were also collected.

B. Sampling Methods

The sampling train consisted of a cassette containing a glass fiber filter followed by an XAD-2 sorbent tube in series to collect both solid and vapor states of the organotin(s) present. These sample media were connected via tygon tubing to a sample pump operating at approximately 1.5 liters per minute. This sample train was employed at the producer of organotins, and at the applicator of the organotin containing antifouling compounds. At the formulator, measurements were made using only the glass fiber sample media since TCHH is a solid with a, reportedly, very low vapor pressure.

C. Analytical Methods

At the conclusion of the evaluation, the glass fiber filters and the XAD-2 sorbent tubes were returned to the NIOSH contractor's laboratory where they were analyzed for total organotins. Because of the different organotin containing chemical matrices encountered at the site visited, three different analytical methods were used. Two methods used were of the extraction type-where the organotin analyte was removed from the sample media with an appropriate solution. The other method was of the destructive type-where the media along with the organotin analyte

was digested in an acid solution. For the producer, samples were desorbed using acetic acid and toluene followed by analysis of the extract using graphite furnace atomic absorption spectrophotometry (AAS). This method was suggested to us by the producer. For the formulator, samples were desorbed using acetic acid in acetonitrile followed by analysis of the extract using flame AAS. For the applicator, the filter media was digested with hydrochloric acid, and the sample solution following a fusion procedure--which "dissolved" the insoluble extract portion, then analyzed using flame AAS. This procedure was necessitated by the complex organotin containing chemical being used by the applicator.

The method used for the producer and formulator samples is analagous to the present NIOSH Method, Number 5504. In brief, the NIOSH method involves desorption of organotin analyte(s) present with acetic acid in acetonitrile from each sampling media, followed by analysis (for elemental tin) using graphite furnace AAS. If more than one organotin is thought to be in the sample extract, the method provides a procedure whereby the extract can be first injected into a high performance liquid chromatograph (HPLC) for separation of specific compounds. This NIOSH method has been validated for analysis and separation of four compounds. One compound is TCHH. The method used for the applicator samples

is a variation of NIOSH Method 7300--a method used for metals analyses. For the formulator and applicator samples, it should be pointed out that the use of flame AAS does involve some sacrifice of sensitivity in relation to graphite furnace. For samples obtained from the formulator, however, all analytical amounts were well above the limit of detection for the analyte (TCHH) in question. For the applicator, while many of the non-detectable results determined might well have been detectable using a graphite furnace AAS, we were, nevertheless able to quantitate concentrations at 20% of the OSHA PEL, ACGIH, and NIOSH recommended limits. Detection limits were 10 ug/sample for the flame AAS analysis and 0.6 ug/sample for the graphite furnace AAS analysis.

V. Results and Discussion

A. Data Presentation

Tables 3 through 5 summarize the results of personal samples obtained at each of the three facilities visited. The results depict concentrations of total organotins, measured as elemental tin.

The concentrations reported are for the actual period of sampling; 8-hour time weighted averages were not calculated. However, inferences regarding the 8-hour TWA can be made with reasonable confidence in their validity.

B. Results/Discussion--Producer

1. Total Organotins

Table 3 summarizes results of four personal samples obtained from process operators of a producer. These operators were working in several different areas of the plant. These results reflect measurements made during the day shift. Sample collection time for all samples ranged from seven to seven and one-half hours. Concentrations were considered to be reflective of the 8-hour TWA. Further, since employees were observed to be not wearing respiratory protection, these concentrations are probably indicative of actual exposure.

From Table 3 it can be seen that inhalation exposure of all workers measured who were involved with production process related activities was below the present OSHA PEL, and TLV and NIOSH recommended limits of 0.1 mg/m^3 measured as elemental tin.

C. Results/Discussion - Miticide Formulator

1. Total Organotins

Table 4 shows the results of 14 personal samples obtained over seven different activities associated with formulation of an organotin containing miticide. These results reflect measurements made during three separate shifts during one survey. Sample collection time for the sample results reported here varied from four to seven and one-half hours due to atypical formulation schedules which, in turn affected the work time associated with several of the job activities. Therefore, some concentrations in the ranges of the values reported here may not be indicative of 8-hour TWAs. It should also be stated that all formulation employees were wearing respiratory protection. Therefore, actual employee exposure to organotin concentrations may be less than that suggested by the values reported here.

Table 4 indicates that employees in most job activities (except the foreman and forlift driver) were potentially to total organotin concentrations exceeding the OSHA PEL and NIOSH recommended exposure limit of 0.1 mg/m^3 (expressed as elemental tin). One sample result that requires further explanation is the 2.90 mg/m^3 , obtained for the millman/formulator. This sample appeared to represent a maximum, worse-case exposure, as a problem was encountered

securing the drawstring closure of a bag of TCHH. This problem resulted in generation of considerable amounts of airborne TCHH. It is highly probable that a significant contribution to the 2.90 mg/m^3 level occurred during the approximately ten minutes required to resolve this problem. Depending upon the frequency of such a problem, which was not determined, this level may or may not be typical of everyday exposure potential. The other concentration recorded for this job activity, 0.46 mg/m^3 , may be more representative of potential exposure since the type of problem just mentioned did not occur during the sample period.

2. Specific Organotins

Because only one organotin compound was in use at this facility, that being tricyclohexyltin hydroxide, total organotin range results were mathematically converted to expressions of TCHH concentrations so as to enable comparison with the TLV. Using the ACGIH TLV of 5 mg/m^3 as the (only) exposure criteria, it can be seen that only one sample indicated a concentration-- 9.4 mg/m^3 --that exceeded this criterion. This sample however, obtained from the millman/formulator, appeared to represent a maximum, worst

case concentration for the reason previously discussed. The 1.5 mg/m³ value, again for the reason previously alluded to, may be more representative of potential exposure.

D. Results/Discussion - Applicator of Antifouling Paint

1. Total Organotins

A summary of the results of 38 personal samples obtained over four activity categories associated with application of an organotin containing antifouling paint to the underside of an aircraft carrier is shown in Table 5. Measurements were made over a three day application of the coating. Sample collection times averaged approximately six hours. Since the remaining two hours were spent, for the most part, in moving to and from the job site and donning personal protective equipment, 8 hour TWAs would probably be less than that suggested by the reported values. All workers were extensively clad in protective clothing as well as respiratory equipment. Sprayers wore continuous-flow air supplied respirators; all other workers wore air purifying respirators. Therefore, actual employee exposure to airborne concentrations of total organotins is, in all probability, less than that suggested by the reported values.

Table 5 indicates that breathing zone concentrations ranged from less than the detection limit to 0.2 mg/m^3 . The higher concentrations were found among sprayers coating the flat underside of the hull--a relatively confined space. Overall, 27 of the 38 samples collected indicated concentrations below the limit of detection. Only one of the 38 samples indicated a TWA organic tin concentration exceeding the 0.1 mg/m^3 (as Sn) limit adopted by OSHA and recommended by ACGIH and NIOSH. Three of the 38 samples indicated concentrations approaching or equal to the 0.1 mg/m^3 limit (0.07, 0.09, and 0.1 mg/m^3).

VI. Conclusions

From the results of the three surveys and related analytical activities the following conclusions were drawn:

- A. Breathing zone concentrations of total organotins in excess of 0.1 mg/m^3 are likely to be found in employment activities involving transferring, blending, or packaging of raw organotin containing product, or when application of an organotin containing product involves aerosolizing the product.

- B. The use of respiratory protective equipment and personal protective clothing and the availability of such equipment observed during the surveys could indicate that, where organotin compounds are being worked with, users are aware of the need for protective mechanisms. In addition, use of this equipment would invariably mean lower actual exposure to organotin compounds than that inferred by the breathing zone measurements shown today.
- C. At all three facilities, workplace exposure populations were considered to be of inadequate size to conduct a reproductive study. Exposure populations ranged from approximately 15 at both the producer and applicator to approximately 25 at the formulator. NIOSH epidemiologists estimate that approximately 40 male employees, exposed to organotin compounds for a minimum of 6 months, would be needed for an adequate semen study. In addition, an equal number of unexposed males as a control population would be needed. Such population characteristics would enable a study to detect at least a 25% decrease in sperm motility and velocity, a 25% increase in abnormal sperm shapes, and an approximately 80% decrease in sperm concentrations.
- D. While it is clear that the facilities and organotin compounds selected for the on-site surveys did not represent all possible exposed workplaces and exposures to all organotins associated with biocidal uses, organotins exposure data obtained from these

surveys were considered to provide information about workplace exposure scenarios associated with a not insignificant category of organotins usage. Data obtained may also provide meaningful information concerning operations which may be representative of activities which may be involved non-biocidal applications, i.e. production, mixing/blending, and spray application.

VII. Recommendations

From observations and measurements made during these surveys the following recommendations pertaining to additional studies and analytical development are considered appropriate:

- A. Since the number of exposed workers and extent of potential exposure in these manufacturing, formulation, and antifouling paint application facilities are low, and thus are unsuitable for a reproductive study, attention should be focused on potential exposure of agricultural populations to these compounds.
- B. Efforts should be made to characterize and report organotin exposure by specific species. Such information will be useful should development of standards for individual organotin compounds be undertaken. If the NIOSH Method (5504) is used, this method will require revalidation for each specie if the specie of interest is different than that for which the present method is validated.

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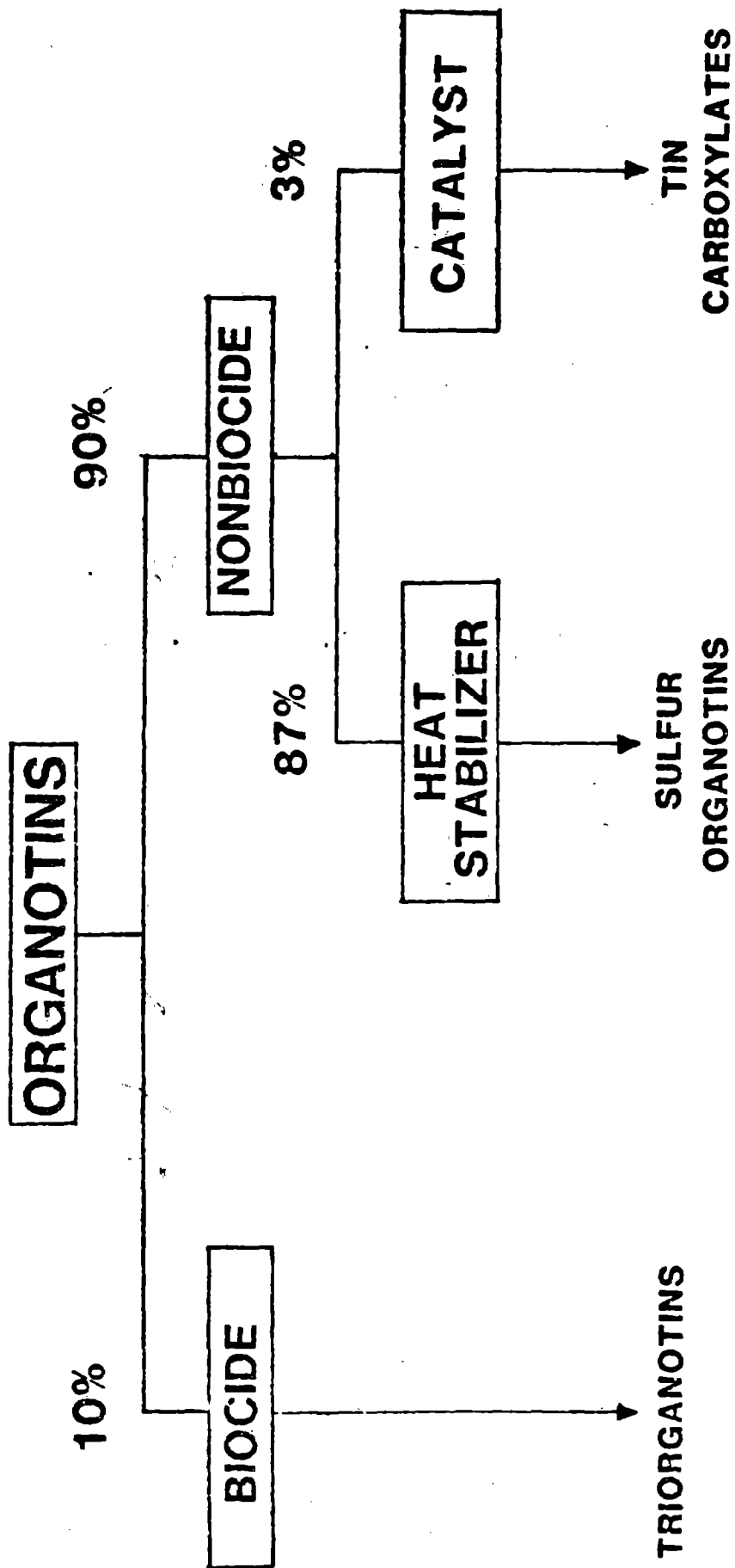


FIGURE 1: ORGANOTINS: MAJOR USES/ASSOCIATED COMPOUND GROUPS

TABLE 2

Facility Description Summary

| Type | Employees | Job Title(s) | PPE ¹ |
|------------|-----------|---|----------------------|
| Producer | 15 | Process Opr | RP, PC,
SG, G, SS |
| Formulator | 25 | Millman
Filler
Weigh Opr.
Seamer
Packer | RP, PC,
SG, G, SS |
| Applicator | 15 | Mixer
Driver
Applicator
Applicator Assts. | RP, PC,
G, SS |

¹ PERSONAL PROTECTIVE EQUIPMENT

RP--Respiratory Protection
 PC--Protective Clothing
 SG--Safety Glasses
 G--Gloves
 SS--Safety Shoes

TABLE 3

TOTAL ORGANOTINS BREATHING ZONE CONCENTRATION SUMMARY PRODUCER

| ACTIVITY | NO.
SAMPLES | CONCENTRATION
(MG/M³AS SN)* |
|--|------------------------|---|
| Collect QC
Samples/Monitor
Process | 1 | 0.05 |
| Collect QC
Samples/Monitor
Process/Drum
Raw Materials | 3 | 0.02-0.05 |

* LOD: 0.0012 MG/M³ (AS SN)

TABLE 4

ORGANOTINS

BREATHING ZONE CONCENTRATION SUMMARY

MITICIDE FORMULATOR

| ACTIVITY | NO. SAMPLES | CONCENTRATION**
(MG/M ³ AS SN) | CONCENTRATION
(MG/M ³ AS TCHH) |
|--------------------|-------------|--|--|
| Filler-Packaging | | | |
| Machine | 3 | 0.09* - 0.34* | 0.3* - 1.1* |
| Bag Seamer/Sealer | 2 | 0.31* ; 0.58 | 1.0* ; 1.9 |
| Final Weigh | | | |
| Operator | 3 | 0.07* - 0.29 | 0.2* - 0.9 |
| Packer/Boxer | 2 | 0.14 ; 0.21* | 0.5 ; 0.7* |
| Millman/Formulator | 2 | 0.46* ; 2.90† | 1.5* ; 9.4† |
| Forklift Driver | 1 | 0.01 | 0.03 |
| Foreman | 1 | 0.03 | 0.01 |

*May not be representative of 8-TWA

†Worse-case concentration

**LOD: 0.003 MG/M³ (as SN)

TABLE 5

TOTAL ORGANOTINS BREATHING ZONE CONCENTRATION SUMMARY APPLICATOR OF ANITFOULING PAINT

| ACTIVITY | NO. SAMPLES | CONCENTRATION RANGE
(MG/M ³ AS SN) | NO. SAMPLES
<LOD* |
|--|-------------|--|----------------------|
| Paint Mixing | 3 | ND | 3 |
| Transporting Paint | 3 | ND | 3 |
| Apply Paint | 12 | ND-0.2 | 4 |
| Fill Paint Reservoir
Tend Spray Pump/
Tend Hoses | 20 | ND-0.03 | 17 |

* LOD: 0.02 MG/M³ (AS SN)

QUESTIONS AND DISCUSSION

DR. SHY: Questions or comments?

DR. ANDERSON: Lucy Anderson, National Cancer Institute.

Do these folks know you're coming when you come to take these samplings?

The question is, do you find them adequately protected all the time or just when a visit is scheduled?

MR. BLOOM: In the facilities we visited for the organotins project, most employees were wearing respiratory protective equipment that we felt was appropriate to the task and the exposure potential.

DR. ANDERSON: Are they protected well because they know you're coming? Or are these spontaneous, unannounced visits?

MR. BLOOM: They are not unannounced visits. There is the chance that workers may not be protected when we are not there. The employer, and most of the time the employees, know when we're coming and, conceivably, the employees may have worn their respiratory protection only when we are there.

We did discuss that possibility with some of the workers, and indications are that while they probably aren't diligent in wearing their respiratory protection 100 percent of the time, for the most part they do wear their respiratory protection.

It is, in general, probably correct to say that employees working in some companies are better protected than employees working in other companies. This is due, in part, to health and safety surveillance services available to the companies. In the organotins project, for example, it occurred that the formulator of the tricyclohexyltin hydroxide-containing pesticide was under license to another company to produce this pesticide. In this case the formulator did have the benefit of the larger company's industrial hygiene services. Accordingly, one might assume that, in this case, employees would be more diligent in wearing respiratory protection and personal protective equipment in general due to the surveillance aspect. One might assume that such diligence applies when we are not there.

But as to whether, in fact, there were instances in which employees did not wear personal protective equipment, I can't preclude that possibility. Given the nature of the exposure scenario at the shipyard, however, I would say that those workers were probably wearing protective equipment when we were not there.

And for the producer, since the production involves for the most part a closed system, I would suspect that it probably wouldn't make a lot of difference if employees did or did not wear respiratory protection when we were not there. Exposure potential for most jobs was minimal and respiratory protection was not required except for emergency situations.

SESSION III—EXPERIMENTAL STUDIES

**Chairman: Dr. William Farland
U.S. Environmental Protection Agency**

Wednesday Morning, April 23, 1986

CHAIRMAN'S REMARKS/INTRODUCTION

**Dr. William Farland
U.S. Environmental Protection Agency**

CHAIRMAN'S REMARKS/INTRODUCTION

Dr. William Farland
U.S. Environmental Protection Agency

DR. FARLAND: Our session this morning will begin with two papers related to experimental studies, and then a report from a workshop that was funded through this collaborative program. This is our second session in experimental studies, and I think we have several very interesting papers to deal with this morning.

The first paper is going to be a discussion of Metabolism of 4,4'-Methylene-bis(2-Chloroaniline) in Explant Cultures of Human and Dog Bladder and Dog Liver Cell Cultures. Our presenter is the principal investigator on this project, Dr. Gary Stoner, from Medical College of Ohio. Following that presentation, we will have a discussion of the Characterization and Validation of Carcinogen-Induced Transformation of Human Cells In Vitro. Dr. George Milo of the Ohio State University will be the presenter--also the principal investigator there.

On your program, the third activity this morning is the report on the International Workshop on the Use of In Vitro Transformation of Established Cell Lines for the Prediction of Carcinogenic Chemicals--a workshop that was held in 1985. Dr. Steve Nesnow from the EPA Health Effects Research Lab was to present that report. Steve called and because of an unavoidable family problem will not be here, although we do have his comments and Tom Cameron will be presenting that paper for Steve.

**METABOLISM OF 4,4'-METHYLENE-BIS (2-CHLOROANILINE)
IN EXPLANT CULTURES OF HUMAN AND DOG BLADDER
AND DOG LIVER CELL CULTURES**

**Presented by Dr. Gary D. Stoner
Department of Pathology
Medical College of Ohio**

**METABOLISM OF 4,4'-METHYLENE-BIS (2-CHLOROANILINE)
IN EXPLANT CULTURES OF HUMAN AND DOG BLADDER
AND DOG LIVER CELL CULTURES**

**Dr. Gary D. Stoner
Medical College of Ohio**

In vitro models using cultured human tissues and cells have become a valuable bridge between clinical investigations and animal model studies of carcinogenesis. Normal tissues and cells from most of the major sites of human cancer can now be successfully cultured. Some of the early events considered to be important in tumor initiation are the metabolic activation of chemical carcinogens and the formation of carcinogen-DNA adducts.

During the past few years, with assistance from a cooperative agreement with the EPA, and in collaboration with Dr. Bernie Daniel of the EPA, we investigated the metabolism, DNA binding, and DNA adduct formation of benzo(a)pyrene, aflatoxin B₁, 2-acetylaminofluorene, 7,12-dimethylbenz(a)-anthracene, and 4-aminobiphenyl in cultured human lung and/or bladder tissues. The metabolism in human tissues was compared to that in the corresponding animal tissues in which the carcinogens were shown to induce tumors.

Our present cooperative agreement with the EPA, in which Dr. Marc Mass serves as Project Officer, is an extension of previous studies, and includes investigations of the metabolism, DNA binding, and DNA adduct formation of a commercially important compound, 4,4'-Methylene-bis(2-chloroaniline) (MOCA) in human and dog tissues.

MOCA is an arylamine used principally in industry as an intermediate in the synthesis of polyurethane elastomers. In 1977, approximately 3.9 million pounds of MOCA were consumed in the United States. Estimates of the total direct worker population at risk range from 1,000 to 2,400 employees. The maximum estimate of the indirect population at risk is approximately 25,000 employees. The two important routes of exposure to MOCA are by inhalation and skin exposure.

MOCA has been tested for mutagenicity and carcinogenicity. It is mutagenic in bacteria, i.e., in the Ames Salmonella microsome assay. With respect to carcinogenicity, MOCA has been shown to induce hepatomas and lung tumors (adenomas and adenocarcinomas) in rats. It also induces vascular tumors and hepatomas in mice, and urinary bladder tumors in the dog. When compared to other arylamines, MOCA is a stronger carcinogen for the urinary bladder of dogs than benzidine, but weaker than 2-acetylaminofluorene, 2-naphthylamine, and 4-aminobiphenyl. There is no direct evidence of its carcinogenic potential in the human bladder, but it is found in the urine of workers exposed to MOCA. Since humans are exposed to MOCA, and since MOCA is a carcinogen for the dog bladder, we decided to compare its metabolism, DNA binding, and DNA adduct formation in human and dog bladder tissues in vitro.

The specific objectives of our studies are:

1. To investigate the metabolism of MOCA in cultured human and dog bladder and liver tissues;
2. To compare the levels of DNA damage and DNA adduct formation in human and dog bladder tissues with that of another arylamine carcinogen, 2-acetylaminofluorene; and,
3. To determine the ability of MOCA to induce DNA strand breaks and sister chromatid exchanges in cultured human and animal bladder epithelial cells. The studies are being done in collaboration with Dr. Ih-Chang Hsu at the University of Maryland.

Before I go into a description of the data obtained to date, I'd like to tell you about our program for collection of human tissues at the Medical College of Ohio. During a period of two years, there are approximately 35-40 cases from which are obtained specimens of bronchus and bladder, and 20 cases for the esophagus. Specimens of colon, skin, liver, cervix, testes, and pituitary are also obtained either for our laboratory or for other laboratories. Although most tissues are collected from autopsies, some are obtained from surgeries. Tissues are collected from the Medical College Hospital as well as three other hospitals in the Toledo area.

The approximate period after death during which human tissues are collected in order to culture them in a viable state differs depending on the various organ sites. Specimens of trachea-bronchus should be collected within five to six hours; bladder, four to five hours; colon, two to three hours; esophagus, three hours; liver, one hour; skin, at least up-to-24 hours or longer; and oral cavity, five hours or longer. So it's possible to collect certain tissues, including the bladder, for periods of several hours after death and culture the tissues in a viable state.

The culture conditions we use for these tissues are as follows: the culture medium is PFMR-4, a modification of Ham's F12K supplemented with insulin, hydrocortisone, β -retinyl acetate, and antibiotics. The medium is serum-free. The atmosphere is a mixture of 50 percent oxygen, 47 percent nitrogen, 3 percent CO₂, and the temperature of incubation is 36.5° C.

Tissues are taken from the patient at autopsy or surgery, placed in a transport medium and transferred to the tissue culture laboratory. The tissue is then cut into pieces ranging from 0.3-0.5 cm². The pieces are then placed in tissue culture dishes (2-4 pieces/dish) and each dish receives 2.5 ml of medium. The dishes are then placed in a chamber and the chamber is gassed for a period of five to ten minutes before it is placed on a rocker platform.

The tissues are rocked back and forth in the chamber so that the epithelium is intermittently exposed to the atmosphere and to the culture medium. Utilizing this technique, it has been possible to maintain several human tissues for periods of weeks to months in vitro.

I will now discuss the typical morphology of a specimen of human bladder maintained for seven days in culture. During this period, the bladder epithelium thickens (due to hyperplasia). Generally, this morphology is maintained for a period of at least 21 days. No attempts have been made to culture the tissue for longer periods.

Four methods are used to assess the viability of human and animal tissues in culture. One is to examine the morphology of the tissue by high resolution light microscopy. Generally, if the tissue appears normal, it is viable. Secondly, a few fragments of tissue from each patient or animal are placed in standard cell culture to determine whether or not epithelial cells grow out onto the culture dish from the fragments of tissue. If the tissue is non-viable, you will not observe any outgrowths of epithelial cells. Thirdly, where appropriate, the tissues are examined for evidence of functional activity. For example, cultures of bronchial epithelium are examined for secretion of mucous and/or ciliary activity. Finally, biochemical studies are done to examine the incorporation of radiolabeled precursors into DNA or protein. Explants of dog bladder incorporated tritiated thymidine into DNA in a linear fashion for three days, after which time the incorporation was somewhat reduced through a period of seven days. These data correlated closely with the histological appearance of the tissue. We also examined the incorporation of tritiated leucine into protein of explants of dog bladder, and the leucine data correlated well with the thymidine data; i.e., consistent incorporation for three days after which it started to fall off.

Having established that explants of human and dog bladder could be maintained in a fully viable state for at least three days in culture, we exposed the tissue for 24 hours to radiolabeled MOCA at concentrations of 0.1, 1, and 10 μ M. DNA was then isolated from the tissues by hydroxylapatite chromatography, and the radioactivity bound to the DNA counted in a liquid scintillation counter. The DNA was then hydrolyzed and the MOCA-DNA adducts identified by high performance liquid chromatography and/or by ^{32} P post-labeling techniques. Studies are currently under way to identify the adducts.

The binding of MOCA to the DNA of dog bladder explants is comparable to that of 2-acetylaminofluorene (AAF). The binding of MOCA and AAF to dog bladder explants is dose related. In general, the binding levels of AAF exceed those of MOCA by a factor of two.

MOCA is also metabolized by explants of human bladder, and the binding levels of MOCA to human bladder are quite similar to that of the dog. Due to the high variability in metabolism from case to case, we prefer to analyze at least 20 cases before any final conclusions are made.

In a few cases, the binding of MOCA to DNA is very high relative to the other cases. These differences could be due to differences in the rate of acetylation of MOCA. This has also been observed with AAF in which groups of high binders are found along with groups of lower binders. More data from additional cases are needed to further evaluate the importance of binding levels to carcinogenic susceptibility in humans and animals.

For comparative purposes, we also examined the binding of MOCA and AAF to the DNA of cultured hepatocytes, since arylamine compounds are known to be metabolized principally in the liver. With both MOCA and AAF, the binding levels in the liver were higher than those in the bladder, but the differences were only two-fold. These data are preliminary and additional samples are being processed to further verify our results. Nevertheless, we were rather surprised to observe that the metabolism of MOCA in the liver exceeded that in the bladder only by a factor of two.

As indicated earlier, studies are under way to identify MOCA-DNA adducts in explant cultures of dog bladder, human bladder, and in dog hepatocytes. In collaboration with Dr. Ramesh Gupta of the University of Texas, ³²P post-labeling methods are being used to identify MOCA-DNA adducts in hydrolyzed DNA. At least four adducts appear to be formed between MOCA and DNA in dog bladder explants. This agrees with our HPLC data in which four peaks are routinely seen in chromatographs of DNA hydrolysates. At the present time, standards that will be used to identify these adducts are being prepared by chemical synthesis.

Another compound that we proposed to study in this cooperative agreement is benzotrichloride (BTC). There is some evidence that BTC is a human lung carcinogen, and we proposed to compare its metabolism with that of benzo(a)pyrene in human lung and rat trachea in vitro. Unfortunately, BTC is very unstable in culture medium, and we could not demonstrate any DNA binding with this compound. However, the ability of BTC to induce lung tumors in strain A mice was examined and compared with other carcinogens in this model.

BTC was injected i.p. 24 times during a period of eight weeks. Six months after the first injection, the animals were harvested, lungs removed, and the adenomas on the surface of the lung counted. BTC induced an average of 125, 40, and 17 tumors per lung at total doses of 7.37, 3.68, and 1.47 mmol/Kg, respectively. When compared to other compounds tested in this model, BTC was approximately as active as benzo(a)pyrene and seven times more active than urethan as an inducer of lung tumors.

In summary, the following observations have been made during the first year of this project.

- o The binding of MOCA to the DNA of dog bladder explants is dose related, and the binding levels are approximately one-half those of AAF.
- o ³²P post-labeling studies indicate that four adducts are formed between MOCA metabolites and dog bladder DNA. Studies are now being done with human bladder for comparative purposes.
- o The binding of MOCA to the DNA of human bladder explants is dose related, and the binding levels are approximately equal to those in dog bladder explants.
- o The binding of MOCA to the DNA of dog hepatocytes is higher than that to the DNA of dog and human bladder explants.
- o Benzotrichloride is a potent carcinogen in the strain A mouse lung tumor bioassay.

QUESTIONS AND DISCUSSION

DR. FARLAND: Thank you Gary. I guess I'd like to use the chairman's prerogative and ask the first question, and then I'll identify some additional questioners if there are any.

Gary, in the studies you described, when you do the binding studies with those pieces of tissue, have you looked, either by autoradiography or by some other method, at the labeling pattern in the squares of tissue to see whether you could enhance the recovery of the labeled DNA by somehow slicing the top layer, or are they uniformly labeled through the tissue?

DR. STONER: We have done the autoradiography with both MOCA and AAF, and there is a higher labeling in the epithelium of the tissue than in the underlying stroma. It is difficult to separate the epithelium from the stroma in the bladder, so we usually sonicate the tissues. After brief sonication, most of the connective tissue becomes separated from the epithelium, and it settles out rapidly. The supernatant contains predominantly epithelial cells, which are then poured off and centrifuged for subsequent isolation of DNA.

DR. BRESNICK: Edward Bresnick, University of Nebraska Medical Center. At least with some arylamines, the major metabolic pathway is through prostaglandin synthase H, and I wonder if you have done some studies with MOCA to see whether it is a P450-mediated activation or prostaglandin synthase H activation?

DR. STONER: We haven't done that as yet, but those experiments are planned.

DR. BRESNICK: You can do it very simply, though, I think, particularly with the explant--just add varying amounts of indomethacin or aspirin and see what it does to your DNA binding. I think that that might provide a first indication as to whether it's going to be prostaglandin synthase mediated or P450-mediated.

DR. STONER: Yes, that's a good idea.

DR. MILO: George Milo, Ohio State University. The reason that I wanted to say something is that this relationship with the arylamines is one of the things that Dr. Kadlebar and I were interested in a few years ago. We found that the pH was a very determining factor in getting the right metabolite, the right ionic species. We had to get nitraniumion. And the dog bladder pH is five. Human bladder pH ranges between, I believe, approximately 6.2 and 7.4, depending on the diet.

DR. STONER: Yes.

DR. MILO: Is it at all possible, knowing the structure of this in light of Dr. Bresnick's comments, that this could decidedly influence the spectrum of the metabolites? It may not produce the end result that you want. How would you adjust the human bladder pH environment so that it could reflect the natural or normal pH of the dog bladder's environment?

DR. STONER: You could do that by adding some HCl or pyruvate to the medium, but we haven't tried that. I think that if we dropped the pH of the medium significantly below what is normal for tissue culture, the tissue would not be well maintained.

DR. MILO: Yes, you can if you do it under an argon environment.

DR. STONER: We have not tried to do that, but it is worth trying.

DR. MILO: Yes.

DR. STONER: We do plan to look at the spectrum of metabolites that are produced in the medium and to compare the metabolites produced by dog and human tissues. At this point in time we do not have the standards for that, but are getting some of them chemically synthesized.

DR. MILO: Under those conditions, I think you might be surprised.

DR. STONER: Thank you. I appreciate that information.

DR. FARLAND: Dr. Bresnick, further comment?

DR. BRESNICK: Just one--it seems to me that you have a very nice system, particularly in regard to the human. If you can keep those bladder explants going for three weeks or so, that should be sufficient time for you to try to get a metaplasia-like experiment under way. Have you tried to transform these explants in culture?

DR. STONER: We haven't done any transformation studies with bladder tissue, but I think that you might have a better chance of transforming bladder tissue than other human tissue in culture.

DR. BRESNICK: Right.

DR. STONER: Bladder cells appear to have a higher rate of proliferation in culture than other human tissues.

DR. BRESNICK: If you do get around to it, you might be interested in seeing if MOCA could actually act as a promoter. I think that type of experiment should be done somewhere.

DR. STONER: You mean in terms of stimulating proliferation?

DR. BRESNICK: I'm thinking in terms of a two-stage or a multistage model. You can actually add some benzo(a)pyrene and then add MOCA afterwards; this should significantly increase hyperplasia.

DR. STONER: It's interesting that the dog bladder tissues do very well as explants in culture, and so do human bladder tissues, but when you try to isolate and develop cell cultures from human bladder, it's a real problem--significantly more difficult than cell cultures from human bronchus for example, and I don't really know the reason for that.

DR. BRESNICK: But part of the reason might be exactly what you told us before, that the adherence of the epithelial layer to the stroma layer in bladder is very tight.

DR. STONER: Right. That could be a reason. We haven't tried to culture bladder cells using a fibroblast cell feeder layer or on collagen-coated dishes.

DR. BRESNICK: Right.

DR. STONER: Basically, we have not tried to develop bladder epithelial cell cultures, although we recognize the importance of this.

DR. FARLAND: Any additional comments?

DR. CAMERON: Tom Cameron, National Cancer Institute. Gary, I know this isn't part of your original presentation, but would you take the opportunity to talk a little about the strain A system? I'm particularly interested in what you think about it as a screening tool, as a short-term in vivo assay.

DR. STONER: Yes, I'll be happy to do that, Tom. The strain A mouse lung tumor model is exquisitely sensitive to certain classes of carcinogens, such as the polycyclic hydrocarbons, carbamates, aziridines, nitrosamines, et cetera. However, it is not responsive to many of the liver and bladder carcinogens, such as certain aromatic amines, aminofluorines, et cetera. In fact, potent rat liver carcinogens, such as aflatoxin, are several-fold less active than is benzo(a)pyrene for the induction of lung tumors in strain A mice.

A few years ago, NCI, in collaboration with NTP, conducted a study to determine the ability of the strain A mouse lung tumor bioassay to detect carcinogens that were active in the standard NTP long-term carcinogenesis bioassays. Unfortunately, the compounds they picked for that comparison were predominantly aromatic amines and, of course, most of them were found to be negative in the strain A test. It was then concluded that the strain A test is not a valid test for carcinogenicity.

Of course, one could turn around and assay a series of polycyclic hydrocarbons and the test would do very well. So, the selection of compounds was not representative of the spectrum of chemicals to which the assay is sensitive or insensitive. Nevertheless, the inability of the lung tumor bioassay to detect a series of carcinogens that were active in long-term carcinogenesis bioassays is troublesome, and one can probably conclude that the assay is inappropriate as a routine screening test. Currently, we are developing a hybrid mouse model in which strain A mice (high lung tumor susceptibility) are being crossed with C3H mice (high liver tumor susceptibility) in order to develop a hybrid which might be susceptible to both "lung" and "liver" carcinogens. The preliminary data look very promising.

DR. BRESNICK: If there is time--could I add a flip side to that maybe? What's the possibility that the animal is just really an initiated animal and that the compounds in which you are demonstrating--the exception to that, of course, is urethane--the compounds in which you are demonstrating effectiveness in strain

A--polycyclics principally--are complete carcinogens, whereas aflatoxin is not a complete carcinogen, and some of the arylamines are not complete carcinogens? They are initiators, but they don't have much in the way of promoter activity.

DR. STONER: That could very well be the case. There are usually two questions here. Is the strain A mouse lung already initiated such that all chemicals are simply promoting an ongoing process? In this case, the stronger promoters would be the more active carcinogens. Or, as you suggest, are some chemicals more active in the strain A lung because they have both strong initiating and promoting effects?

I think the latter is the case, but I'm not certain how one can answer these questions without a clear understanding of the molecular aspects of lung tumor development in this model. Metabolism studies could shed light on the problem, as well as cell kinetic studies, but I think it's something more fundamental than that.

**CHARACTERIZATION AND VALIDATION
OF CARCINOGEN-INDUCED TRANSFORMATION
OF HUMAN CELLS IN VITRO**

**Presented by Dr. George E. Milo
The Ohio State University
Comprehensive Cancer Center**

CHARACTERIZATION AND VALIDATION OF CARCINOGEN-INDUCED
TRANSFORMATION OF HUMAN CELLS IN VITRO.

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INTRODUCTION

Anchorage independence (AI) determined as the acquisition of colony-forming ability in a semi-solid medium provides a useful system for studying a carcinogen induced change in both animal and human cells in vitro. A number of workers have demonstrated the existence of high correlation between the ability of animal cells transformed in culture by viruses or chemical carcinogens to form colonies in semisolid medium and their ability to form tumors in surrogate host (1-4). Normal diploid human fibroblasts have been shown to be transformed to AI phenotype in vitro when treated by both physical and chemical carcinogens (5-13). Most of the investigators have been able to obtain tumors following the injection of the progeny of these human cells into athymic mice, though some have failed in their attempts (6,10). However, only few of these tumors have been found to be invasive progressively growing tumors. The early conversion to an AI phenotype appears to be the best marker for quantitative assay systems for a carcinogen-induced change in human fibroblasts because the carcinogen-treated human fibroblasts do not systematically exhibit other phenotypic characteristics of transformation usually found in rodent systems, such as the formation of morphologically altered foci in monolayer culture, the capacity to proliferate under reduced serum or calcium concentrations, or the ability to grow on irradiated 3T3

monolayers. Because of the importance of understanding the process of malignant transformation of diploid human cells and the significance of possible connection in human cells between AI growth and tumorigenicity, it is highly desirable to carry out a detailed study of the AI growth of human fibroblasts treated with a variety of carcinogens and investigate its correlation with 'cellular tumorigenicity'.

METHODS

Chemicals

The chemicals selected for this study are: aflatoxin B1, aflatoxin B2, 1-naphthylamine, 2-naphthylamine, bis(chloromethyl)ether, N,N-bis(2-chloroethyl)2-naphthylamine (chloronaphazine) and cyclophosphamide. Two more compounds will be chosen at a later date.

Cell Culture

Human neonatal foreskin (HNF) fibroblast cell populations were established by previously described procedures (11). Briefly, HNF tissue was rinsed with complete medium, minced into small sections and digested with 0.25% collagenase in CM and 20% fetal bovine serum (FBS) at 37° C. Complete medium (CM) is composed of Eagle's minimum essential medium-Hank's balanced salt solution containing 25 mM HEPES buffered at pH 7.2, supplemented with 1.0 mM sodium pyruvate, 2.0 mM glutamine, 0.1mM nonessential amino acids, 0.2% sodium bicarbonate and 50 ug/ml of gentamicin.

After 4 - 6 hours incubation, the cell suspension was centrifuged at 650xg for 10 minutes and the pellet rinsed, suspended in CM + 20% FBS and seeded in T-75 flask. The next day, the culture was rinsed and refed with CM + 20% FBS. Three to four days later, when the culture was almost confluent, the foreskin fibroblasts were selectively removed by 2-3 minutes trypsinization

at 37° C with 0.1% trypsin. The trypsinization was stopped with 10 ml of CM + 10% FBS (Growth medium), the fibroblasts dispersed by up and down pipetting, and then reseeded at 10,000 cells per cm². The trypsinization and passaging were repeated when the cultures became confluent, to obtain cells at desired PDL. Contamination by yeast, bacteria, fungi or mycoplasma was routinely checked.

Cytotoxicity

Cytotoxicity was measured by determining the effect of the compounds on the relative cloning efficiency. Cells at PDL 4-6 were pooled from different cultures and suspended in cloning medium (CM + 1X vitamins + 0.5X essential amino acids + 20% FBS) and seeded at 500 cells in 25 cm² flasks. Four flasks were used per experimental point. Twenty-four hours later they were treated with varying concentrations of the compound in cloning medium. Following a 24 hour period, the treatment medium was removed and the cells incubated in cloning medium at 37° C in 4% CO₂ for 10-14 days. This medium was then removed, the cells fixed in 3% neutral formalin and stained with hematoxylin. Colonies of > 100 cells were counted in order to calculate the cloning efficiency as a percentage of the control.

Carcinogen Treatment and Transformation

Barely confluent (preconfluent or confluent for no more that 24 hours) HNF cells at PDL 4-6 were blocked at G1 phase of the cell cycle by seeding the cells in Dulbecco's modified Eagle's minimum essential medium, deficient in arginine and glutamine, supplemented with 1.0 mM sodium pyruvate, 50 ug/ml gentamicin and 10% dialyzed FBS (dialyzed against Dulbecco's Eagle MEM lacking arginine and glutamine), for 24 hours. The cells then were released from the block by feeding with growth medium supplemented with 0.5 units/ml insulin.

Ten hours after release, when cells were in early S phase, the carcinogen dissolved in a suitable solvent was added with or without human liver S-9 mixture (14) and the cultures incubated for 14-16 hours. Cell cultures to which the same concentration of solvent was added served as controls. At the end of the treatment period, control and treated cell cultures were rinsed with CM and subpassaged at a 1:2 split ratio in selection medium (CM containing 0.8mM nonessential amino acids, 8X nonessential amino acids, 2X vitamins, and 20% FBS). When the cell populations were 75% confluent, they were serially passaged at a 1:10 split ratio using the selection medium till PDL 16-20.

Characterization of Transformed Cells

a. Anchorage Independent Growth in Soft Agar (23,36)

Carcinogen-treated and control fibroblast populations at PDL 16-20 were seeded at 50,000 cells in 60 mm tissue culture plates in 2 ml of 0.33% prewashed agar prepared in Dulbecco's LoCal medium (Biolabs) supplemented with 1mM sodium pyruvate, 1X nonessential amino acids, 1X essential amino acids, 2.0 mM glutamine, 1X vitamins, 0.2% sodium bicarbonate, 50 ug/ml gentamicin, and 20% FBS (11). These cells were layered over 5 ml of a 2% agar base prepared in RPMI 1629 medium (GIBCO) supplemented with 1X sodium pyruvate, 1X nonessential amino acids, 1X essential amino acids, 2.0 mM glutamine, 1X vitamins, 0.2% sodium bicarbonate, 50 ug/ml gentamicin, and 20% FBS (1X Local medium). The cultures were kept in a high humidity incubator in a 4% CO₂ atmosphere and 0.5 ml of 1X LoCal medium was added once a week. The cultures were examined for colony (bolus) growth weekly and they were considered negative when no growth was seen in 4 weeks. Cultures were scored as positive when colonies of ca. 25 cells were observed. The number of colonies per plate were counted.

b. Tumorigenicity in Nude Mice

Monolayer cultures were reestablished by removing the colonies from the agar with a tuberculin syringe (20 gauge needle) and seeding them into 8X medium. After attachment and sufficient growth, the cells were removed by trypsinization and reseeded to distribute evenly the cells from the monolayer colonies that had developed. These cultures were then passaged until cell densities were reached sufficient to produce an inoculum of 10×10^6 cells per mouse. Four to six weeks old NIH Swiss nude mice were splenectomized and further immunized with 0.2 ml of antilymphocyte serum (ALS) prior to subcutaneous injection of AI cells suspended in CM. During the tumor development period, the animals were given biweekly injections of ALS to further immunosuppress them.

RESULTS AND DISCUSSION

To date, six environmental xenobiotics representative of different classes of chemicals have been so far evaluated for their cytotoxicity on low passage human neonatal foreskin fibroblasts in vitro. Cytotoxicity of these compounds were measured as the percentage inhibition of the cloning efficiency of the cells at different concentrations of each compound. The data are graphically represented in figures 1-3. Each data point is an average of at least four replicates. As is evident from the figures, a wide variation in toxicity to HNF cells was observed among these compounds. 1-naphthylamine and 2-naphthylamine produced less than 25% inhibition of the cloning efficiency at 100 ug/ml treatment medium, whereas 50 ug/ml of aflatoxin B1 and aflatoxin B2

exhibited ca. 95% and 30% inhibition respectively. The reason for such wide variation in toxicity is not clear. Since these compounds require metabolic activation, the variation in cytotoxicities may probably be due to the varying ability of the cells to metabolize each compound. The two direct acting carcinogens, bis(chloromethyl)ether and chloronaphazine exhibited almost 100% inhibition of the cloning efficiency at 10 ug/ml.

Aflatoxin B₁, 1-naphthylamine and 2-naphthylamine were tested, at five different concentrations with and without uninduced human S-9 liver mix, for their ability to induce anchorage independent (AI) phenotype, measured by the number of AI colonies in soft agar. Cultures treated with the solvent alone served as controls. The data are shown in figures 4-6. In the case of aflatoxin B₁ and 1-naphthylamine treatment, a dose dependent increase in the number of colonies was observed at lower concentrations, but a decrease at high concentrations with a further increase at the highest concentrations (50 ug/ml) used. 2-Naphthylamine treated cells exhibited a decrease in the number of AI colonies with the increased dosage, and the response was linear. The response of the cells to high concentrations of the carcinogens is complex. A plausible explanation is that the cells in early S phase, when treated with high concentrations of the carcinogens, exhibit a selective decrease in the number of responsive cells participating in DNA replication. Since preferential modification of the replicating DNA is presumably responsible for the increased neoplastic potential in early S phase, the inhibition of replication at high concentrations will cause a selective decrease in the expression of transformation. Further studies with the other compounds may provide us a more comprehensive understanding of the observed results and of the usefulness of the system to evaluate human environmental carcinogens.

Addition of human uninduced S-9 liver mix to the treatment medium produced an increase in the number of AI colonies at all concentrations of 2-

naphthylamine and at three of the five concentrations of aflatoxin B₁; but with 1-naphthylamine, an increase in AI colonies was observed only at 0.1 ug/ml (figs. 4-6). However the effect was not statistically significant. Tumorigenicity studies in nude mice, using the cells repopulated from the AI colonies, are in progress and will be published at a later date.

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FIGURE 1. CYTOTOXICITY CURVES FOR HUMAN SKIN FIBROBLASTS EXPOSED TO A) AFLATOXIN B1 AND B) AFLATOXIN B2. ERROR BARS REPRESENT STANDARD DEVIATION.

FIGURE 1

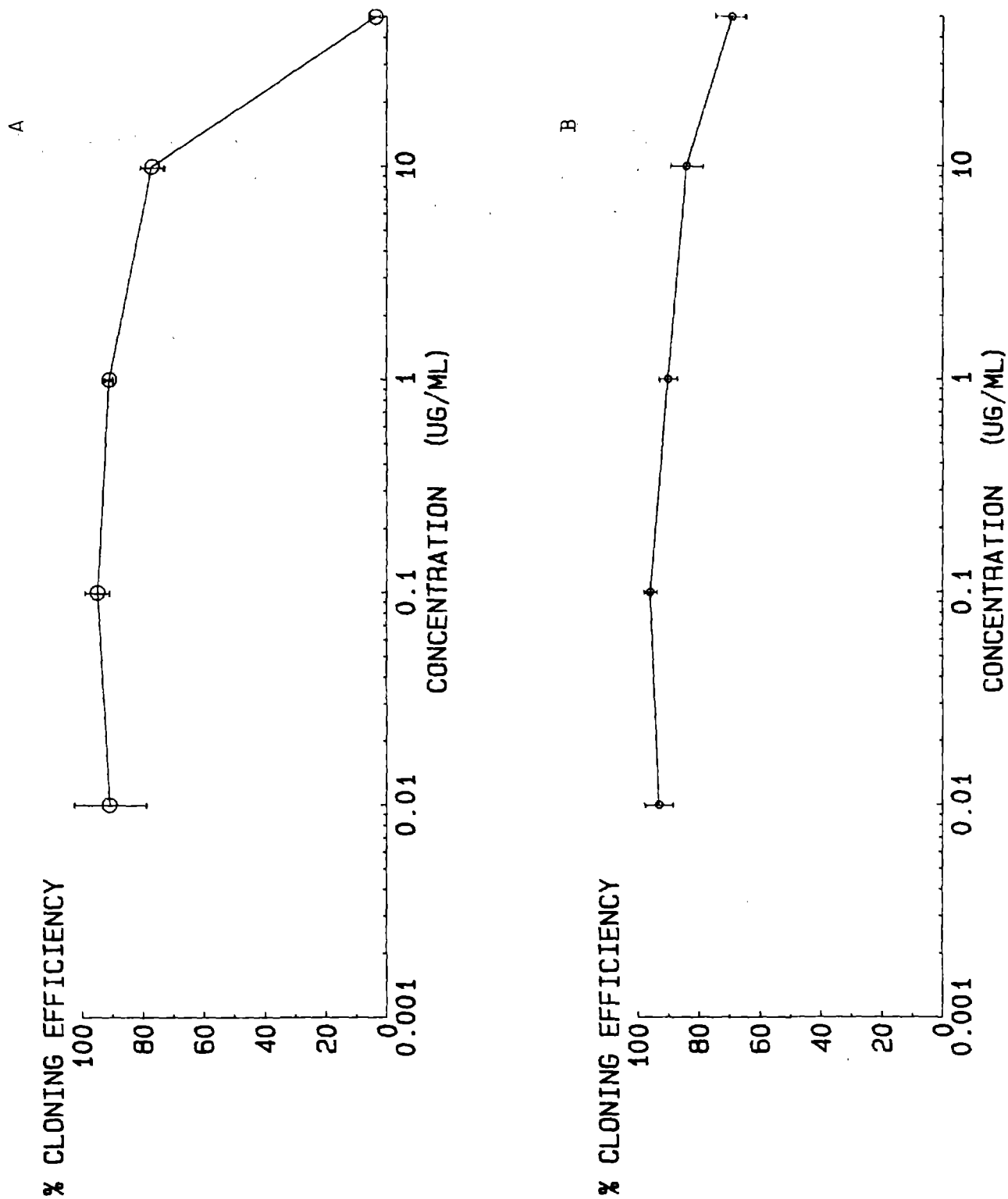


FIGURE 2. CYTOTOXICITY CURVES FOR HUMAN SKIN FIBROBLASTS EXPOSED TO A) 1-NAPHTHYLAMINE AND B) 2-NAPHTHYLAMINE. ERROR BARS REPRESENT STANDARD DEVIATION.

FIGURE 2

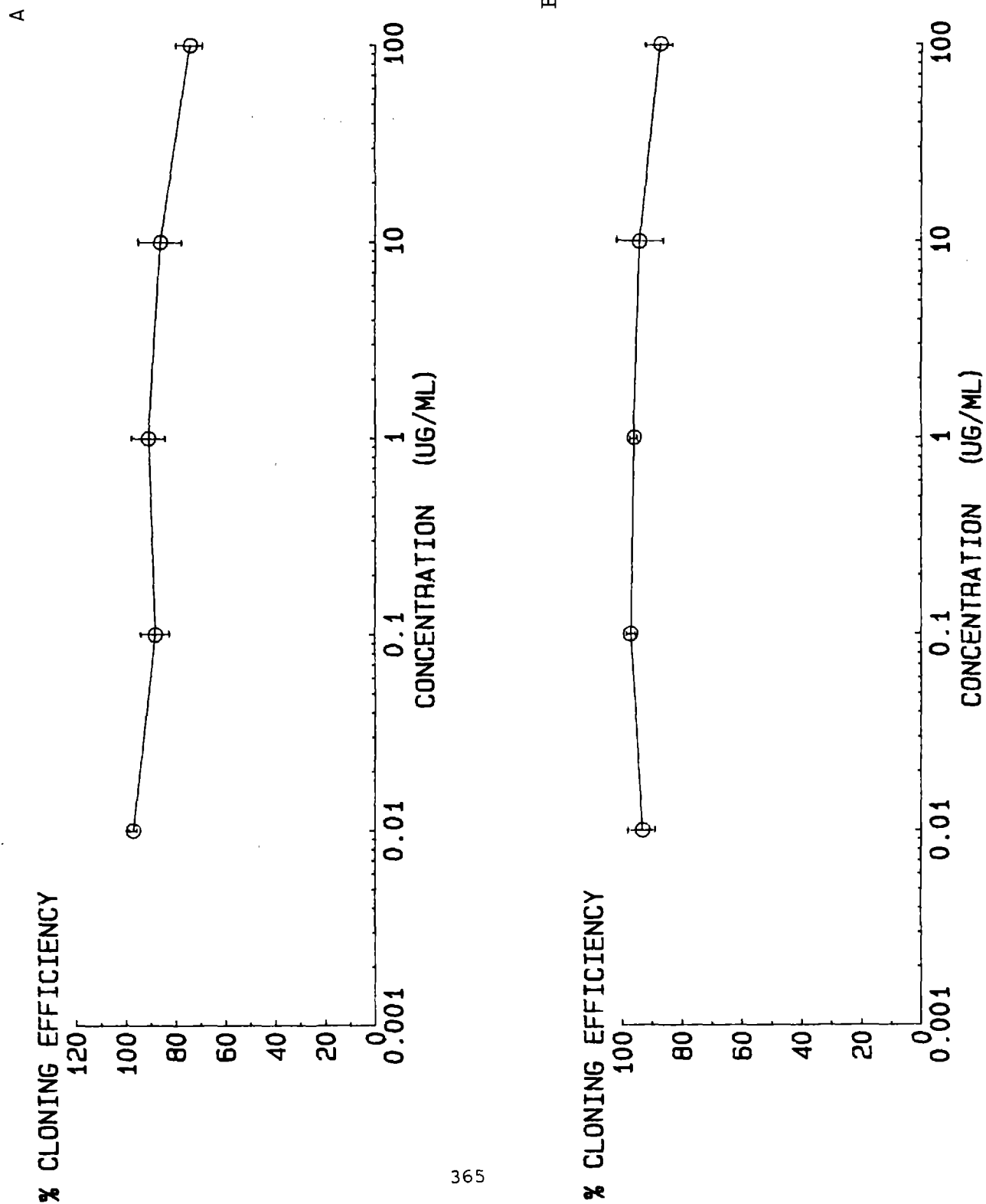
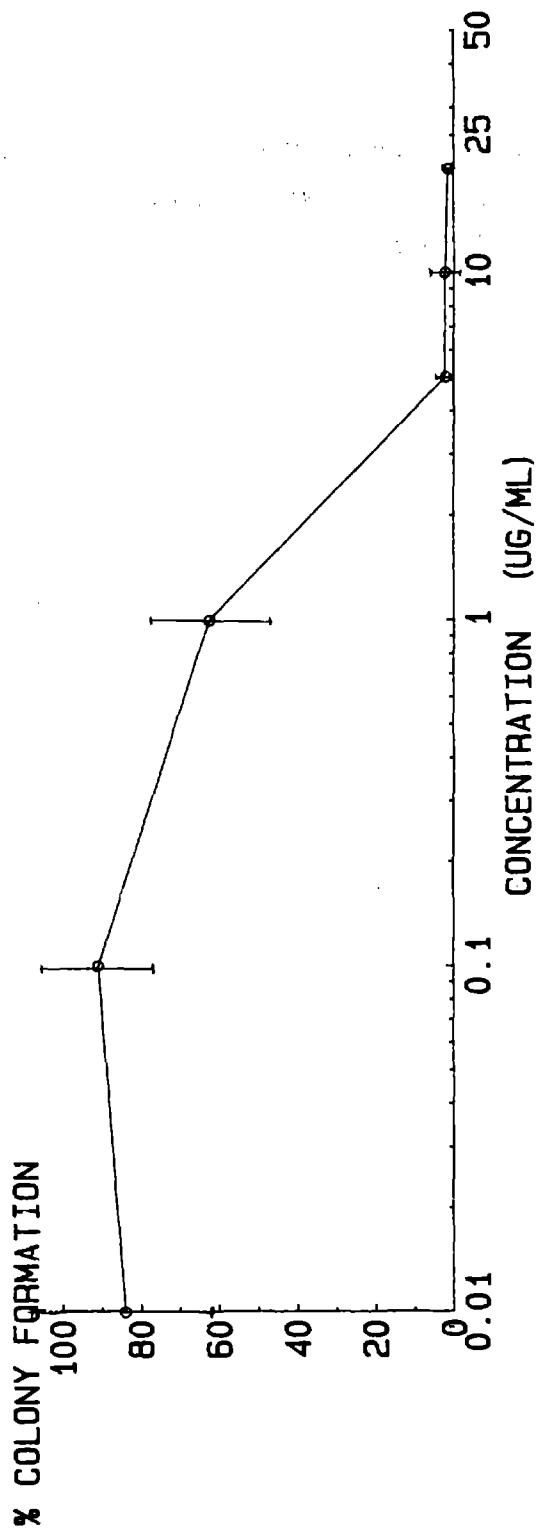


FIGURE 3. CYTOTOXICITY CURVES FOR HUMAN SKIN FIBROBLASTS EXPOSED TO A) BIS(CHLOROMETHYL)ETHER AND B) CHLORONAPHAZINE. ERROR BARS REPRESENT STANDARD DEVIATION.

FIGURE 3

A



B

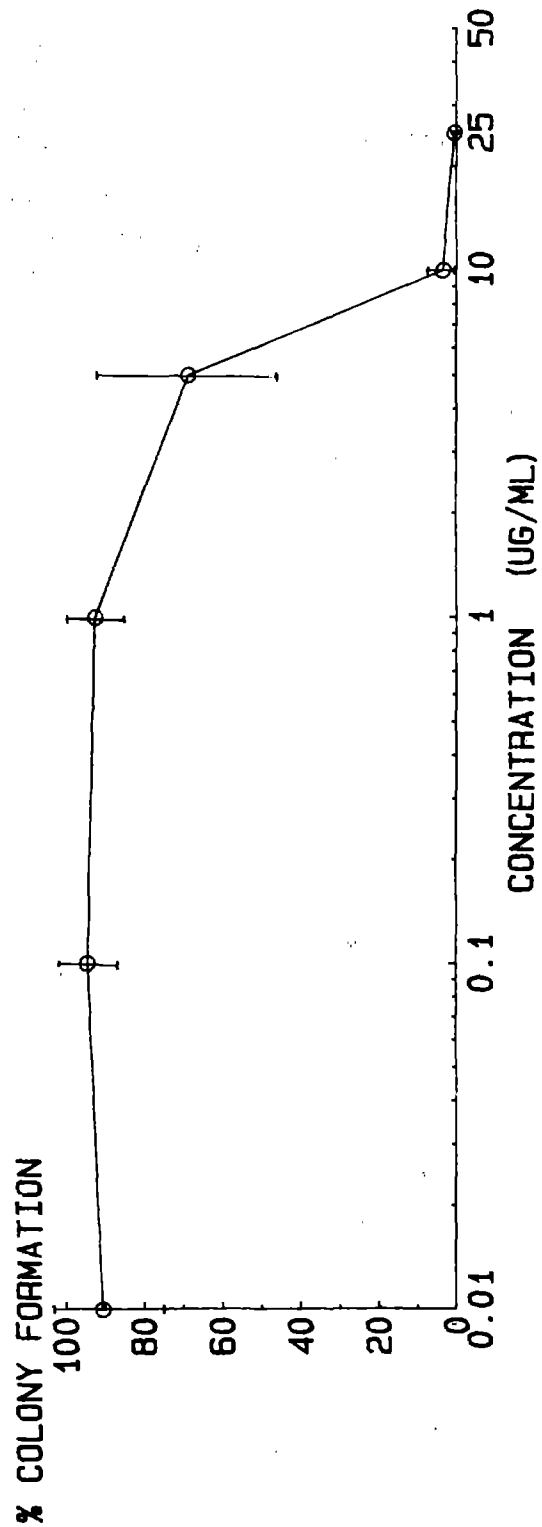


FIGURE 4. FREQUENCY OF A1 COLONIES IN HUMAN SKIN FIBROBLASTS TREATED WITH AFLATOXIN B1. EACH DATA POINT IS AN AVERAGE OF THE NUMBER OF COLONIES IN SIX PLATES.

FIGURE 4

ANCHORAGE INDEPENDENT GROWTH OF AFLATOXIN B1 TREATED CELLS

NO. COLONIES PER 50,000 CELLS

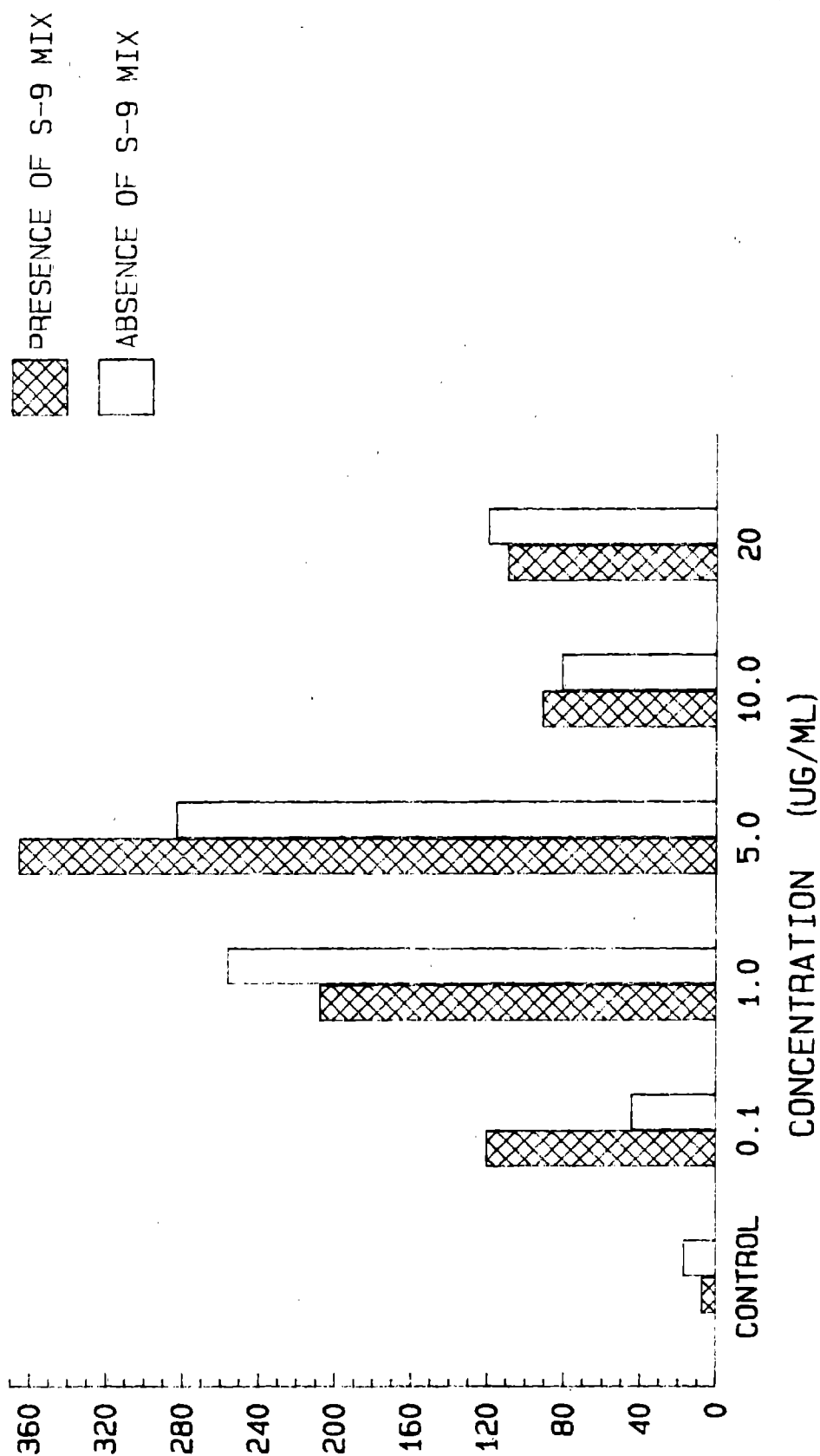


FIGURE 5. FREQUENCY OF AI COLONIES IN HUMAN SKIN FIBROBLASTS TREATED WITH 1-NAPHTHYLAMINE. EACH DATA POINT IS AN AVERAGE OF THE NUMBER OF COLONIES IN SIX PLATES.

FIGURE 5

ANCHORAGE INDEPENDENT GROWTH OF 1-NAPHTHYLAMINE TREATED CELLS

NO. COLONIES PER 50,000 CELLS

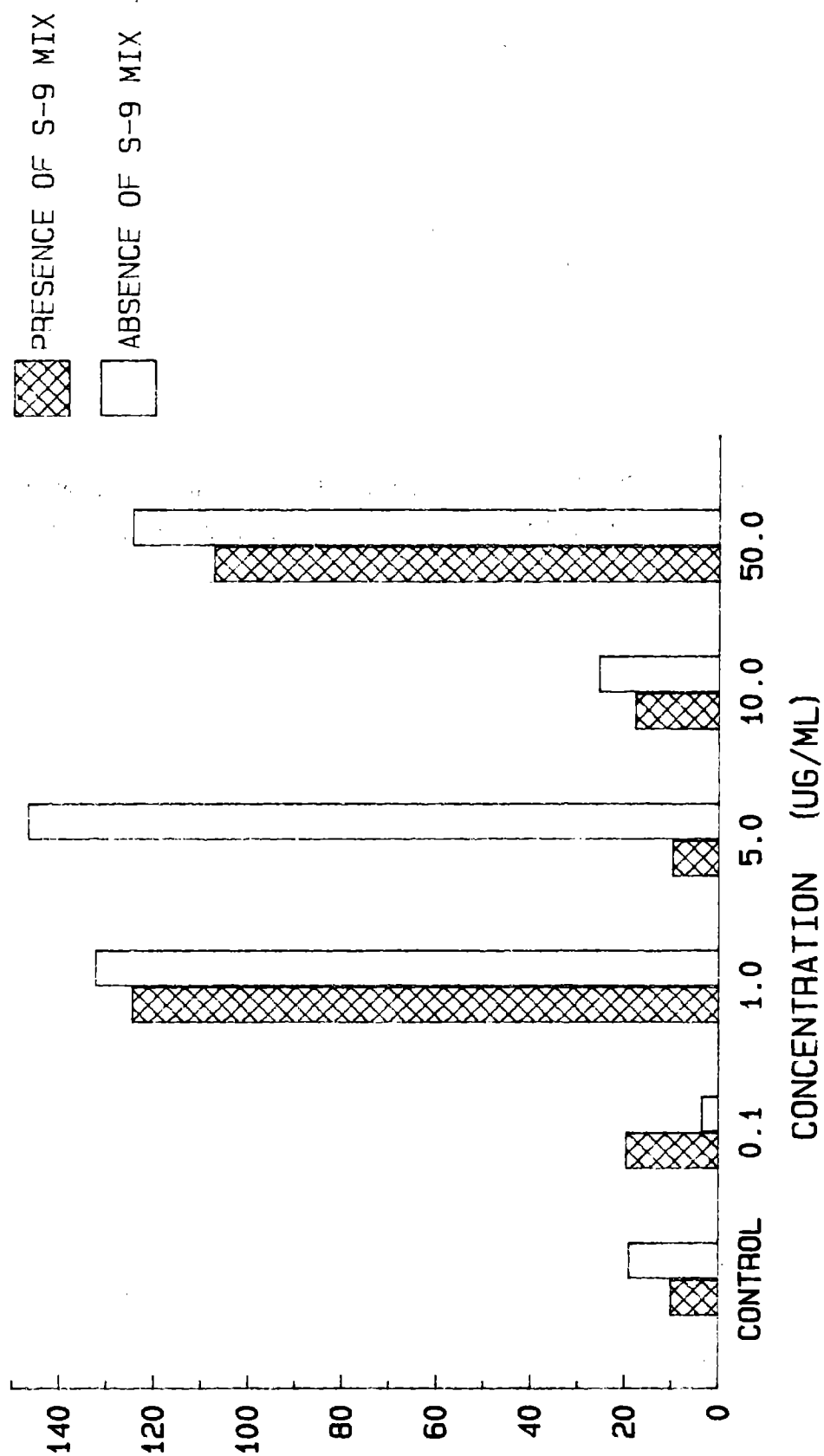
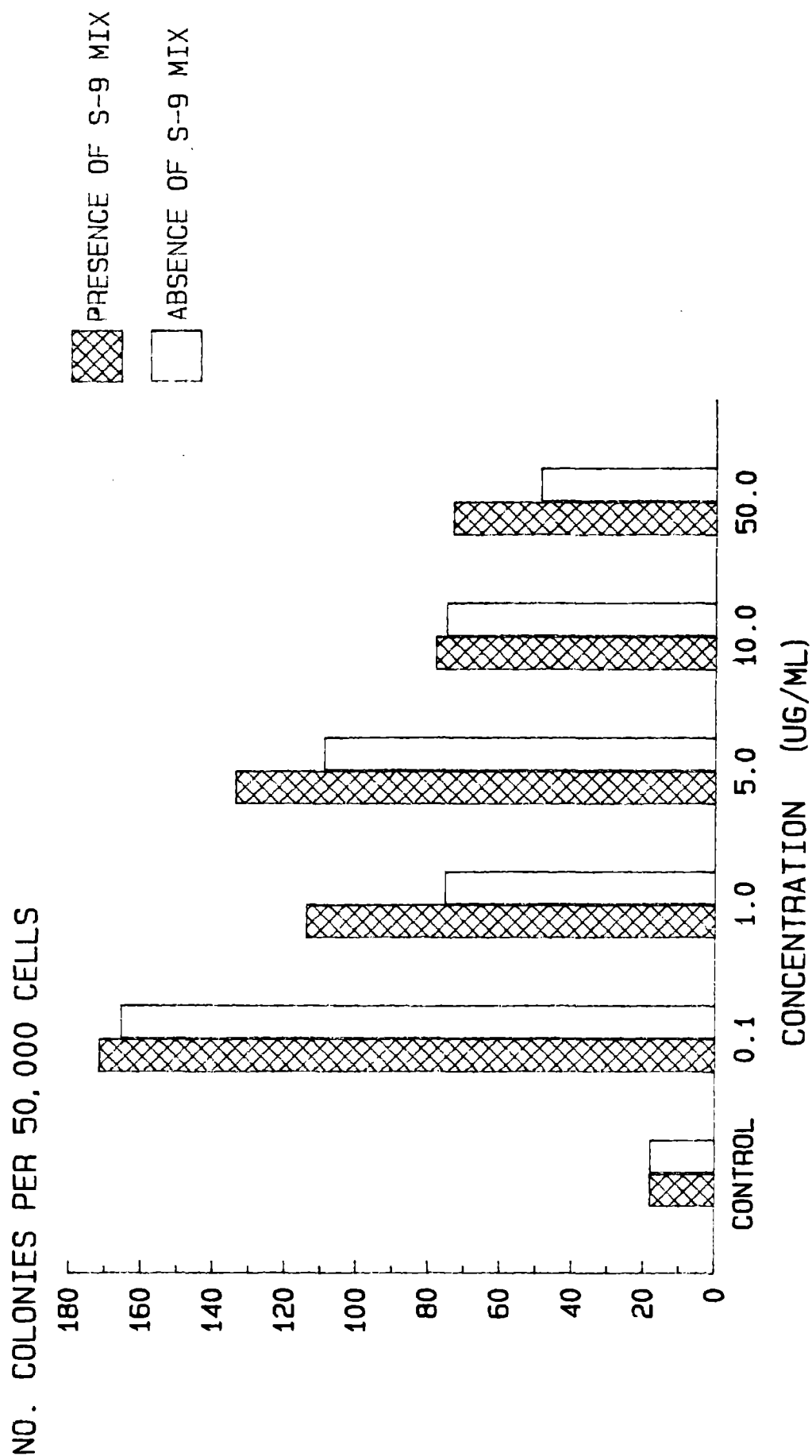


FIGURE 6. FREQUENCY OF AI COLONIES IN HUMAN SKIN FIBROBLASTS TREATED WITH 2-NAPHTHYLAMINE. EACH DATA POINT IS AN AVERAGE OF THE NUMBER OF COLONIES IN SIX PLATES.

FIGURE 6

ANCHORAGE INDEPENDENT GROWTH
OF 2-NAPHTHYLAMINE TREATED CELLS



QUESTIONS AND DISCUSSION

DR. FARLAND: Okay, again, I guess I'd like to start off with a question and then open it up. George, in your early comments you talked about some of the transfection experiments and indicated that you could get morphological transformation with the transfection. Can you go on to that anchorage independence also with that transfection?

DR. MILO: Yes, we can. It's interesting, but the linearity of response is essentially the same as a function of time, and the thing that is diminished in time--time being the X ordinate--is the association between the time of transfection and expression of anchorage independent growth is reduced from 15 population doubling down to 5.

DR. FARLAND: And how about the efficiency of that sort of a situation, it's--looking at your slides, going from morphological transformation to AI--not a very efficient process. You get a limited number of anchorage independent colonies out of your 50,000 cells. How about the efficiency with the transfection?

DR. MILO: At the present time, the efficiency is better than the chemically induced transformation. There was a comment made to me recently at a meeting that I attended that there is a machine out that apparently uses an electrical impulse to alter the poration of the surface of cells. We thought we might try it. Apparently they are able to introduce plasmids by this electroporation methodology so that you get up to a level of transformation that you would see with some chemicals alone or even better than you would expect with the more highly toxic chemicals.

DR. FARLAND: Thank you. Those would be very interesting studies to develop.

DR. STONER: I was wondering how much variation you find in ability to transform fibroblasts obtained from different individuals. Do they differ in their transformability, or is a fibroblast a fibroblast?

DR. MILO: I'm going to respond to that in a rather analogous manner. I think if everybody stood up in this room, you would find that there are all sizes, shapes, and forms. The same thing is also true of the human foreskin fibroblast. In other words, if I compare lung fibroblasts with skin fibroblasts, there are definitely some similarities. There's absolutely no question of that, but there are variations.

Now between people, for example, on handling benzo(a)pyrene, we have found human foreskin fibroblasts where there is no lipoprotein in the cytoplasm to carry the benzo(a)pyrene into a place where it can be metabolically activated and then produce the specific adduct. We have, therefore, people that are totally refractory to any carcinogenic insult from the component of benzo(a)pyrene by virtue of the fact that they don't have the cytoplasmic protein in their fibroblasts.

DR. BRESNICK: Edward Bresnick, University of Nebraska Medical Center. I have a couple of questions; one is sort of a comment. In terms of transfection, it seems to me that most people have found that there isn't any panacea. I mean one has to run through the battery of transfection procedures, including the last one--electroporation--and then some work really well, and some don't. In our hands, protoplast fusion works kind of the best, with the cells that we're interested in transfecting.

The question I have is--again, it's sort of by way of comment. I guess I'm one of the mavericks who hasn't come around to the feeling that human tissues are the best things to use for all experiments. It depends upon the question that's asked. In that regard, I would wonder about the choice of human S-9. The range of activity in terms of mixed function oxidase activity in the human is over 10,000. That's quite a range. If one's only interested in obtaining an activating system to then see if that activated metabolite is capable of interacting with the foreskin fibroblast DNA, then it strikes me the best way to do that is to take a uniform system. In that regard, to take a system such as the arochlor treated rat liver and use that as S-9, so that you know that from one experiment to the next you have a comparable level of activation, rather than taking the human liver at autopsy or whenever and then mixing it up and determining it has an S-9 level of 1 this time, and the next time you get a human liver sample, it has an S-9 level of 10,000--those experiments become difficult to interpret.

So, I would wonder why not use the arochlor system for what you want to do? The second part to that question is, why not also use as a comparable control, ram seminal vesicle preparation to take a look at what prostaglandin synthase II-mediated activity would do?

DR. MILO: I don't think I've got time to adequately respond, but I'll try and address part of it. I don't have any problem with any of the recommendations that you've made. I do have a comment on your comment. As far as the human liver is concerned, and other human cells, many of the compounds used to induce the P450 isozyme complexes are not active in the human system. They behave entirely different. So the subspecies of these P450 isozymes have to be induced by a different procedure.

As far as the arochlor is concerned, I don't have any problem with that at all. It just started out this way, and I wouldn't have any reservations about changing it to this other system.

DR. SCHECHTMAN: Leonard Schechtman, FDA. It is one thing to examine these various activation systems for the purposes of research; it is another thing to make use of them for screening purposes.

In this regard, one of the fundamental issues which continues to be addressed is the identification of an optimum, albeit unlikely an ideal, supplemental metabolic activation system. Limiting our discussion to just post-mitochondrial supernatant preparations, it is apparent that numerous permutations and combinations are feasible with respect to species, sex, tissue source, metabolic inducer(s), etc., in the generation of a suitable S-9 capable of supplying the required metabolic activity. Obviously, such a research effort is necessary and constitutes a part of our basic comprehension of the control of metabolism of xenobiotics.

In contrast, the screening process for regulatory purposes in the identification of potential genotoxins will likely be unable to accommodate the use of such a wide spectrum of S-9's. Time, resources (labor and monetary), and other limitations simply do not allow for this luxury. Expense alone would be self-limiting, having dramatically inflated the cost of an initially relatively inexpensive cell culture assay. Nevertheless, the pursuit of an ideal single metabolic activation system no doubt has its place in the research effort, and should be encouraged as we attempt to more closely approximate human metabolism of xenobiotics in vitro. However, for all practical purposes, screening programs are, by their very nature, considerably more limited.

DR. MILO: I don't have any problem with that.

DR. FARLAND: Comment from Dr. Bresnick?

DR. BRESNICK: Forgive me, I've always been provocative, and I don't have very much in the way of tact.--I think my response to that is "nonsense." I don't see anything wrong in having good basic research in a screening procedure. If you're going to look at activation by human S-9, then you don't superimpose on the back of that monkey a very, very expensive cell culture procedure, simply to look at quantitative aspects of S-9 activation, very simply. You don't need cell culture for that.

If you're going to look at what happens to the activated metabolite or activated substances in terms of the biology, and that's what George is doing, and doing very, very nicely, then you don't have to look at the activation mechanisms, because now you're superimposing on that monkey's back a tremendous variable which is going to compromise, to my way of thinking, what's going to come out on the other end. Not only do you have the variability of what happens to the active metabolite in terms of DNA and in terms of the subsequent alteration of the biology of the cell culture system, such as an anchorage independence, but you also impose the tremendous variable of the extent of activation by the S-9.

You can't homogenize people--S-9 preparations. It simply doesn't work. It's going to vary from one to 10,000. That's as given as some hair is black and some hair is red. So why take that variability if that's not really what you're trying to measure?

If you are trying to measure it, then why do the cell culture business? That's the very, very expensive aspect of these studies.

DR. MILO: I didn't realize that this would take this bent, but just let me add a little more fuel to this fire, in that now the match has been lit, I guess I can throw on a log. So I'll exercise the prerogative of the speaker and do so.

We have been very interested in this--as a side issue within the laboratory, so that, I try to develop these projects for the students to get their Ph.D's. So we've looked at this, which is a part of this contract. Looking at the S-9 action on the benzo(a)pyrene and looking at the profile of metabolites by HPLC, we've also taken part of the tissue and seeded it down in culture, and then looked at the internal and external distribution of the metabolites under those conditions.

The profile, as far as the qualitative aspects of the metabolites are concerned, is quite different from the animal system. I'm not giving you any answers, but Dr. Bresnick is correct. There is a tremendous amount of variation with humans as far as metabolism is concerned and the history of the individual is very important.

And so, I guess, the question that is being begged--is that of paramount importance or is the fact of standardizing the situation, such as what Dr. Bresnick has identified--the treatment of say, the rodent liver, with arochlor and looking at that spectrum of metabolites to see if these are the systems that we want to work into the activation scheme.

My bent in the lab is primarily basic research and I've adapted this system to doing this. It really can respond to either of the comments that have been made by either Dr. Schechtman or by Dr. Bresnick.

DR. FARLAND: Thank you. That's a very interesting system, there are going to be a lot of questions that will need to be asked and the questions that we were discussing with regard to activation are very basic questions that apply not only to this system, but to a number of screening systems that we might use in terms of identifying potential human carcinogens with screening assays.

**WORKSHOP ON "TRANSFORMATION ASSAYS OF
ESTABLISHED CELL LINES"**

**Presented by Dr. Thomas P. Cameron
National Cancer Institute**

WORKSHOP ON TRANSFORMATION ASSAYS OF ESTABLISHED CELL LINES

STEPHEN NESNOW, U.S. EPA

In February 1984, a workshop was held at the International Agency for Research on Cancer, Lyon, France, on the subject "Transformation Assays of Established Cell Lines: Mechanisms and Application". This workshop was conceived and directed by Dr. T. Kakunaga of the National Cancer Institute and Dr. H. Yamasaki of IARC, and was sponsored by the World Health Organization, the National Cancer Institute, and the U.S. Environmental Protection Agency, through the Collaborative Program on Joint Environmental and Occupational Cancer Studies.

The goals of this workshop were to discuss similarities between in vitro cell transformation and in vivo carcinogenesis, and the feasibility of screening environmental carcinogens using in vitro transformation assays in established cell lines and focused on the Balb/c 3T3 and C3H10T1/2 cell transformation models.

The participants of the workshop included an international group of scientists experienced in the use and application of established cell lines in cell transformation.

The workshop began with detailed discussions on cellular and molecular mechanism of cell transformation: cell density dependence on transformation, multi-stage models of transformation, the relationship between mutagenesis and cell transformation, effects of modifying factors, and the role of oncogene activation in the cell transformation process.

In vitro cell transformation and in vivo carcinogenesis were discussed with regard to structure-activity relationships, morphological and biochemical properties of transformed cells, and factors which modify the carcinogenic response in vitro and in vivo. Also discussed were the ability of the

Balb/c 3T3 and C3H10T1/2 cell transformation models to predict the carcinogenic activity of chemicals.

The issue of metabolic activation models for improving the response of cell transformation systems to a wide variety of classes of chemical carcinogens was discussed including the use of liver S9 fractions and rodent hepatocytes. Examples of difficult to test chemicals were used to describe the ability of these methods to activate chemicals to forms which morphologically transform cells.

The remainder of the workshop dealt with the development of experimental protocols for performance of the Balb/c 3T3 and C3H10T1/2 cell transformation bioassay models. The issues addressed were: performance problems and improvements to protocols, recommendations for standardized protocols, and established criteria for scoring morphologically transformed foci.

The results of this workshop have been reported as a meeting summary published in Cancer Research Volume 45 in 1985, and have been reported in detail as IARC Scientific Publication No. 67 published in 1985. The IARC volume is particularly useful for investigators interested in performing the Balb/c 3T3 and C3H10T1/2 cell transformation bioassays as it contains detailed protocols for the performance of these bioassays as well as photomicrographs of the different types of morphologically transformed foci observed in these systems. Also included in the IARC volume is a thorough review of chemicals tested in these two cell transformation bioassays.

In conclusion, this workshop funded and directed under this Collaborative Research Program has yielded an important and useful reference volume on cell transformation models using established cell lines and provides a basis for the continued development and use of these bioassay systems.

SESSION IV—EPIDEMIOLOGY

**Chairman: Dr. Joseph F. Fraumeni, Jr.
National Cancer Institute**

Wednesday Afternoon, April 23, 1986

CHAIRMAN'S REMARKS/INTRODUCTION

**Dr. Joseph F. Fraumeni, Jr.
National Cancer Institute**

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CHAIRMAN'S REMARKS/INTRODUCTION

**Dr. Joseph F. Fraumeni, Jr.
National Cancer Institute**

The first presentation is entitled "Time Trends and Risk Factors for Mesothelioma" to be presented by Dr. Robert Spirtas of the National Cancer Institute.

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TIME TRENDS AND RISK FACTORS FOR MESOTHELIOMA

**Presented by Dr. Robert Spirtas
National Cancer Institute**

TIME TRENDS AND RISK FACTORS FOR MESOTHELIOMA

Dr. Robert Spirtas
National Cancer Institute

This presentation has two parts. The first part deals with time trends in mesothelioma incidence and survival as well as information on mortality. Much of this material is included in a manuscript entitled "Recent Trends in Mesothelioma Incidence in the United States," which will be published as a report in an upcoming issue of the American Journal of Industrial Medicine.

The second part of today's presentation deals with etiology. Based on responses from a telephone interview with next-of-kin of cases and matched controls, odds ratios were calculated for nine occupational/avocational groups thought to have potential exposure to asbestos. This material was presented at the Society for Epidemiologic Research meeting in Chapel Hill, North Carolina. Collaborators affiliated with this study were the University of Southern California, New York State Health Department, Medical Follow-up Agency of the National Research Council, the Armed Forces Institute of Pathology, and the British Medical Research Council.

Mesothelioma is commonly described as occurring at two sites, the pleura and the peritoneum, as well as having three common histologic patterns--epithelial, fibrosarcomatous, and mixed or biphasic. The first series of slides, borrowed from the Armed Forces Institute of Pathology, describes these topographic and histologic properties.

This first slide is a photograph of the gross pathology of a case of mesothelioma of the pleura. Here the mesothelioma is the white material covering the lung. The tumor has attained massive proportions, particularly in the lower half, and it not only greatly compresses but extensively invades and destroys the lung.

The second slide presents the gross pathology for a case of peritoneal mesothelioma. Most of the peritoneal surface is studded with tumor nodules, which are most prominent in the cirrhosis of the small intestine, where they tend to coalesce. Small nodules are seen over the hepatic surfaces and the parietal peritoneum. In the left upper quadrant is a large tumor, especially massive in the omentum, that conceals the spleen and obscures the colon.

Here we see a microscopic slide of an example of the histologic type called epithelial. This is an example of the mesenchymal histologic type. It resembles a fibrosarcoma. The next case was characterized by a combination of epithelial and mesenchymal patterns. This type of mesothelioma is called mixed or biphasic.

The next series of slides describes incidence, mortality, and survival. This is the earliest information I could get on incidence in the United States. It comes from the Connecticut tumor registry. In the 1940s there wasn't a great deal of difference in incidence between males and females. For both, the age-adjusted incidence rate was below one case per million persons per year. This incidence pattern increased over time, and the differential in incidence between males and females began to increase in approximately the middle of the 1960s.

I have a map showing the SEER areas in the United States. For those who are unfamiliar with the SEER program, it's a program sponsored by the National Cancer Institute that pays several population-based tumor registries to collect information on cancer incidence, treatment, and survival, and pools it in a common format with common designations to create a large database.

The information that I will present on mesothelioma was taken from seven or eight of these registries and accumulated over a period of time. Included are the states of Connecticut (the same area that provided the data for the previous slide), Iowa, New Mexico, and Utah, as well as the urban areas of the San Francisco Bay area, Seattle, and I believe, Detroit. The seven areas covered represent approximately 10 percent of the population of the United States.

This is a slide showing you mesothelioma incidence by site, race, and sex from the SEER program for the period 1973 to 1980. As you can see now the rate is not only higher for pleural mesothelioma, but there's a differential between males and females, and a differential between white and black males for pleural mesothelioma. For peritoneal mesothelioma there is not as great an absolute difference among race and sex groups, although there is a relative difference.

Now we begin to see in white male pleural cases, an incidence of approximately 10 per million per year, or one per 100,000, moving the disease out of the very rare into the not-so-rare category of cancers.

Here we see incidence per year for white males from the period 1973 to 1980 for seven of the SEER registries, and as you can see there's an increase in trends at all these registries. Particularly interesting, although not easily explainable, is this big jump in the trend in Utah, from the lowest to the third highest. The areas that are highest, Seattle and the Bay area, were areas with shipyards.

This slide shows incidence by year for whites, by site and by sex. Here we see the upward trend occurring primarily in white male pleura. This is the same sort of information that you could glean from the previous slides; however, this shows it more directly. For white male pleural mesothelioma, incidence increased from approximately five or six cases per million persons per year in 1973 to above 10 cases per million persons per year in 1982, with no trend for females or peritoneal mesothelioma.

This slide shows incidence rates for each age-specific group for white male and female pleural cases. Here we see again that the big jump is in the older white males. The large excess in older white males appears to be related to time; that is, not only is the excess in older white males, but the excess appears to increase over time in older white males. Again, this is reflective of a disease that is etiologically related to an occupational exposure.

We tested the question of whether or not the increase in trend was related to diagnostic changes, that is, whether in recent times pathologists have been more likely to make the diagnosis of mesothelioma. From what we could gather, we didn't detect an effect due to changes in recognition among pathologists as a factor that could explain the increase in trend in mesothelioma.

This requires some explanation. I've presented this slide a few times before and it has not come across well. We were interested in whether we could use death certificates to study mesothelioma. There's a problem in classification with death certificates, especially for concerns in the peritoneum, but most pleural mesothelioma should be classified as malignant neoplasm of the pleura, which has the ICDA-3 code 163.0.

We considered all 558 cases of pleural mesothelioma diagnosed by SEER in this eight-year period, and looked at the death certificate for underlying cause of death to see if it was properly coded as 163.0. We found that only 23 percent of the death certificates were properly coded to the correct tumor classification, assuming that the classification was done correctly by the SEER registry. It was even worse for peritoneal mesotheliomas.

What this says is that if you believe that the tumor registries are correctly classifying mesotheliomas, then death certificates are undercounting the mortality rate by fivefold. That was the bad news.

The good news is that we thought even with this tremendous undercount that there should be no reason for the undercount to be differentially distributed geographically. So we did one of the famous NCI cancer maps for malignant neoplasm of the pleura, ICDA-8, code 163.0, for the entire time period of ICDA-8, 1968-78. We found about what we expected to see. For the areas where there were either large shipyards or asbestos manufacturing, there were the greatest excesses. These occurred in several areas in New Jersey, in Philadelphia, Norfolk, the Bay area of California, Seattle and Bremerton, Washington, and also in Chicago. For females, the excesses were primarily in Chicago and New York City.

This analysis was done on the basis of state economic areas, so that we had to put together fairly large geographic areas in order to get enough numbers to make this map hold together. The thing that was interesting to us was that Chicago popped up, especially for females, when we weren't aware, at least I wasn't aware, that there was much asbestos manufacturing activity. It turns out that after World War II, Chicago became one of the largest centers for manufacturing products using asbestos, including gaskets and other materials of this type. The excess of female cases in New York City, we think, may be related to the asbestos textile trade.

The next segment of this presentation is on survival. The 50 percent survival time for males is just under a year (approximately nine months). Male survival time seems to be unrelated to age at diagnosis.

For females, however, there seems to be a significant effect of age at diagnosis, such that women under age 50 at diagnosis seem to have 50 percent survival that is close to three years, which is much different than for the men. We can't explain this, but we've done some statistical modeling of these data, and this effect does seem to hold up, i.e., it doesn't seem to be an artifact of the data.

In summary:

- o The trends have been increasing only for pleural mesothelioma in older white males, consistent with occupational exposures during or just after World War II.
- o Because of the severe undercount problem, death certificates should not be relied on for estimates of total mortality.
- o The increase in incidence does not appear to be an artifact of changes in diagnostic criteria.

The next part of the presentation has to do with etiology, and is taken from the presentation I made at the Society for Epidemiologic Research meeting in Chapel Hill, North Carolina.

Although many studies have demonstrated the relationship between mesothelioma and asbestos exposure, there remains uncertainty as to the magnitude of the public health problem posed by asbestos and other materials. Dose-effect relationships have not been well quantified. The latent period remains uncertain, although known to be very long. Questions remain regarding histological diagnosis, and the industrial scope of biologically significant exposure is unknown.

The purpose of this part of the presentation is to examine the relationship between incident cases of mesothelioma and nine occupational or avocational groups thought to have been exposed to asbestos, taking into consideration tumor site, histology, and age first employed. Results presented today are of a preliminary nature.

Data are pooled from two population-based tumor registries, Los Angeles County and New York State, as well as from approximately 35 selected VA hospitals for the same time period. This study used a pair-matched case control study design with tight matching, where controls were taken from the death certificate files. Telephone interviews have been conducted with next of kin.

Respondents were asked whether the study subject ever performed or worked with nine activities, which will be enumerated on a later slide. In addition, respondents were queried as to whether the activity was job related or not, and years during which the activity was performed.

Other parts of the questionnaire not included in today's discussion are complete lifetime occupational history, a list of materials and processes, smoking history, medical history, and family cancer history. We asked whether the activity was related to a job or done on personal time, and if it was job related we then tied it back to the job history part of the questionnaire.

Of the 831 potential cases having mesothelioma mentioned somewhere on a medical record, 546 next-of-kin were located and interviewed. The main reason for not completing an interview was inability to locate a suitable next-of-kin, accounting for approximately 70 percent of the attrition. In approximately 20 percent of the attrition there was refusal to complete the interview.

Of the 546 interviewed next-of-kin of potential cases, approximately 50 percent were judged to be true cases of mesothelioma by a consensus of two expert pathologists. For 256 of the 275 confirmed cases, there were interview data available for a matched control. These 256 pure cases and their controls form the database for today's presentation.

The demographic breakdown shows most of the cases to be older white males. This again is consistent with our information incidence, that mesothelioma is a disease of older white males.

This slide of tumor site by histologic classification indicates that the large majority of cases were from the pleura, and were of the epithelial histologic type. This is the most predominant histologic variant.

Analysis was done by computing odds ratios with the 0.5 continuity correction. Confidence intervals of 95 percent confidence intervals were computed using the Woolf-Haldane method. Cases and controls with experience in each of the nine occupational/avocational groups were compared with cases and controls with no experience in any of the nine groups. In general there was an elevated risk in pleural mesothelioma cases relative to peritoneal mesothelioma cases. There was no clear pattern by histologic subtype.

This slide shows age first worked by age at death for the restricted group of cases and controls who reported occupational experience in one of the nine categories and gave dates for these jobs. Within each of these age-at-death categories, odds ratios for each age-first-worked category were computed by comparison with all cases and controls who never worked in any of the nine categories of jobs. The risk appears to be greatest 35 to 50 years after first employed.

The individual groups that I described before were as follows:

1. Furnace or boiler installation or repair
2. Shipbuilding, slip demolition, shipyard work
3. Insulation
4. Production of textiles
5. Plumbing or heating repair
6. Elevator installation or repair
7. Brake lining installation or repair
8. Building demolition
9. Production of paper products

Results for individual occupational/avocational groups were approximately as expected. For persons employed in furnace or boiler work, ship building, or insulation work, the risk was quite high. For other groups thought to have

potential exposure to asbestos, the elevation of risk was approximately twofold. Odds ratios and confidence intervals were not recorded if the number of cases with experience in the group was less than five.

While the risk was generally higher for pleural mesothelioma, larger odds ratios for peritoneal mesothelioma were reported for those groups thought to have relatively greater exposure to asbestos, that is furnace work, shipbuilding, and insulation. This is consistent with cohort studies of workers heavily exposed to asbestos done by Newhouse and Selikoff, et al. For the heavily exposed groups, the ones we think were heavily exposed, we're seeing a higher incidence, a higher odds ratio for peritoneal mesothelioma, but overall, we're seeing higher odds ratio for pleural mesothelioma.

Odds ratios by histologic category showed much variation and no consistent pattern. This is confirmed anecdotally by the study pathologist, who indicated that if they look long enough and hard enough at enough slides from a mesothelioma case, they are likely to see all three histologic patterns. We now think that histologic subtype is not an epidemiologically important marker or factor.

In summary, this study confirms the strong association between mesothelioma and occupations or avocations with potential for asbestos exposure. The greatest risk appears to come 35 to 50 years after first employment for those in one of nine occupational/avocational groups selected for the study. In general the risk may be greater for pleural mesothelioma, except for those persons thought to have very heavy asbestos exposure, where there seems to be some weak evidence of a greater risk for peritoneal mesothelioma.

QUESTIONS AND DISCUSSION

DR. FRAUMENI: Any questions?

DR. COFFIN: David Coffin, EPA. One of the things that has intrigued me is that while mesothelioma appear asbestos-related there are certain areas where this association does not hold. For instance in one area in Turkey, a high incidence of mesothelioma appears related to a non-asbestos mineral termed erionite. Studies in our laboratory and others show that this fiber (a form of zeolite) has many times the potential of asbestos for inducing mesothelioma in rats. Erionite frequently occurs in volcanic tuff, a form of condensed volcanic ash. Such tuffaceous rock occurs in the Great Basin of Utah, Nevada, Oregon, and many other areas of the Western United States where, despite the low density of population, there is the possibility that mesotheliomas might result in resident populations. The material that we work with is derived from Rome, Oregon. A common site is also Needles Peak, Utah.

I wonder whether Dr. Spirtas would have any information on projects that might be considering this problem in the West.

DR. SPIRTAS: Dr. Rom at the University of Utah does have a proposal to study this, and I believe he has submitted his proposal once before, but because of lack of funding he has not been able to pursue it. From an epidemiological perspective it would be interesting to pursue the issue that Dr. Coffin has been working on in a laboratory setting, the results of which he presented, I think, a few weeks ago. It's a reasonable thing to pursue but so far it has not been funded.

DR. FRAUMENI: Bob, what proportion of your cases could not be explained by asbestos exposure?

DR. SPIRTAS: We don't know yet, since we are still assessing the likelihood of exposure. We are using the NIOSH-NOHS data to estimate probability of exposure for each job held. When this information has been entered into the computer, it will be possible to answer your question.

DR. BRESNICK: Edward Bresnick, University of Nebraska Medical Center. What about the spouses of patients with mesothelioma? I mean, there probably is asbestos in the home or on clothes or whatever that they have been working with. Do you have enough of a cohort to be able to get at that issue?

DR. SPIRTAS: Let me back up a little. This is not a cohort study. It is a case-control study, and the people we interviewed were the spouses, primarily. We did ask the spouses whether there was any washing of clothes. We also asked whether any family member other than the case worked with asbestos.

DR. FRAUMENI: What did you find?

DR. SPIRTAS: We haven't looked at that . . .

DR. FRAUMENI: You have not analyzed that aspect of the study.

DR. SPIRTAS: We're just beginning. This is the first preliminary analysis of the etiology, and it's going to be delayed when I look at the pathology data, which is the next thing that I'm presenting.

DR. FRAUMENI: Okay, Dr. Shy?

DR. SHY: Carl Shy, University of North Carolina. The risk of mesothelioma seems to be much affected by the type of asbestos fiber, with evidence that chrysotile asbestos has a low risk. Are you able to make any differentiation through the history of the work and the time at which workers were involved between the different forms of asbestos?

DR. SPIRTAS: Yes, but only to the extent that certain jobs may have been more likely to use one type of asbestos or another. There is a conference on the health effects of chrysotile asbestos in two weeks that I'm going to be attending in Wales, and it may be that some answers will come forward on that issue. It's an important issue because chrysotile asbestos is produced in Canada. It's the only kind of asbestos they produce, as I understand it, and the economic questions hinging upon whether chrysotile is as dangerous as the other types of asbestos are enormous. So Dr. Shy's question is quite important, and we've been unable to give specific answers, but the issue is being addressed.

DR. STONER: Gary Stoner, Medical College of Ohio. Do your data on the relative frequency of pleural versus peritoneal mesothelioma suggest that if you're working in an occupation where you have high-dose exposure at any point and have better clearance, that you are more likely to get tumors in the peritoneal region, whereas if you're working in an occupation where you have chronic low-dose exposure, that there's a problem of clearance and you have a greater chance of getting pleural mesothelioma?

DR. SPIRTAS: I don't know if we can tease that out of our data. Preliminary information shows that there does seem to be some differential by site, and we hadn't stratified the data beforehand into those jobs or industries where there was heavy exposure versus light exposure. That was something we noticed after we looked at the data, so it's a little unfair to say we found something, but having seen that in the preliminary look at the data, that's certainly something we will address--trying to break the exposures into heavy exposures versus light exposures and see if there's some relation to site of disease.

DR. STONER: But in those occupational groups where you have a higher occurrence of peritoneal versus pleural mesothelioma, are those people exposed to higher doses of asbestos?

DR. SPIRTAS: We don't know that. We can look at how long they were in a given industry, which may provide useful information, but remember, we talked to the next-of-kin. To interview next-of-kin for information on a person's work history is a very tenuous thing. To then try to get an idea of how intense the exposure was, is impossible.

DR. FRAUMENI: Yes, sir.

DR. STERN: Frank Stern, NIOSH. Bob, I have a couple of questions. First of all, when you look at the mesothelioma you see it increasing. Could that possibility be

that it's just better reporting? Could you give a reason as to why you see the mesothelioma increasing? Secondly, you showed it higher in the white males than in any of the other race/sex groups. Is that because white males are more exposed than the other race groups? Or is this a phenomenon of white males?

DR. SPIRTAS: Okay, the first question, I thought I answered that. I thought that we showed that we don't think it's a change in reporting, that we see the same percentage of correct diagnoses.

DR. STERN: You said diagnosis. I'm saying is it better reporting to the registry? Maybe the states are reporting it more often now, and diagnosis might be better or the same, but there might be more reporting to the registry.

DR. SPIRTAS: Well, I think some studies have been done in this direction. I don't think that there've been changes in reporting of cancers in general, and I don't know how this would slip past. It's pretty obvious that this is cancer when you look at an autopsy.

DR. FRAUMENI: But it's a trickier diagnosis . . .

DR. SPIRTAS: Yes.

DR. FRAUMENI: . . . in terms of differential diagnosis of cancer.

DR. STERN: Well, if that's the case why would the mesothelioma be increasing? Could you give an explanation?

DR. FRAUMENI: Let me answer that if I can. I think much of the increase that we're seeing in the mortality data, as well as the incidence data, seems to be related to shipyard work, particularly the widespread exposures occurring during World War II. Because of the long latency period cases are still arising today. So, I think, much of the increase is real, and the increase is seen among white males because virtually all employees at that time were white males.

DR. SPIRTAS: Let me add to that. The asbestos use didn't stop after World War II was over. It increased until approximately 1970. Maybe that will help to explain why there are many people who were exposed occupationally to asbestos, and that exposure began in World War II, and the increase in numbers of persons at risk continued until approximately 1970.

The second question had to do with white males, I think, did it not?

DR. STERN: Yes.

DR. SPIRTAS: The most likely explanation is that it's occupational. There is a differential in survival, but that should not affect the measures of incidence.

DR. FRAUMENI: Dr. Farland?

DR. FARLAND: William Farland, EPA. The data on the misclassification were very interesting. I wonder if you might comment on whether you have looked at the types of reporting that was done that would indicate other types of tumors that were reported, and whether or not one can sort of tease that out to understand a little bit more about the underreporting?

DR. SPIRTAS: Oh, yes, we look at all the misclassifications on the death certificate. It was just too long a chart to present. For the pleura, there were a lot of cases misclassified as lung cancer. A lot were classified as other and unknown cancer. For the peritoneal cases, a lot of them were classified as ill-defined or unknown site.

DR. FRAUMENI: Time for one more question? Yes?

DR. MANDULA: Barbara Mandula, EPA. Just a comment on the question of chrysotile versus other kinds of asbestos. My recollection is that even in the Canadian mines, which are almost all chrysotile, there's at least some tremolite contamination of that asbestos. I think when pathology studies have been done on the lungs of miners, at least tremolite has shown up. So it may actually be that the mesotheliomas are possibly caused, not by the chrysotile, but by the tremolite, which is normally considered just a minor contaminant.

DR. SPIRTAS: Yes, the possibility exists that the tremolite contaminant may be more hazardous than the chrysotile itself.

**BODY BURDEN OF TOXIC SUBSTANCES IN
SUPPORT OF THE TOTAL EXPOSURE ASSESSMENT
METHODOLOGY (TEAM) STUDY**

**Presented by Dr. Lance Wallace
U.S. Environmental Protection Agency**

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I would like to present the results of a five-year EPA study of personal exposure to volatile organic compounds (VOCs). The basic concept of the study, called the Total Exposure Assessment Methodology (TEAM) Study, is to measure the actual amount of material that a person is exposed to through all the major routes of exposure, which in general are air, food, and water. Following a pilot study showing that VOCs are normally not present in food to a significant extent (with the exception of chloroform, present in sodas and other cold beverages) we limited our measurements to personal air and drinking water.

Phase I: Measurement Method Development

We wanted to look at the air that a person breathed, so we had to develop equipment that the person could carry around with him—a personal monitor—to measure his air. We also measured the outdoor air to see what fraction of the exposure was due to that. We measured drinking water out of his tap at home or at work, and we also wanted to look at body burden as a check on whether we were in fact measuring all the important exposure pathways. We looked at blood, breath, and urine of 12 people in a pilot study, and breath was far superior to blood and urine for looking at the compounds of interest. About 100 compounds could be indentified in a single breath sample, compared to fewer than 10 in a corresponding blood sample from the same person.

The personal monitor is a glass tube filled with Tenax-GC[®], which adsorbs organics from the air. Compounds are analyzed by thermal desorption and GC/MS. A Dupont pump runs for 12 hours and collects about 20 liters. A vest was designed with a Velcro flap to prevent protuberances that might cause safety problems. We wanted to get participants who worked in chemical plants and petroleum plants, since we believed they might have high exposures.

The other device that was developed was a spirometer, which is really not much more than a tube—a cylinder of pure air that fills up a Tedlar bag of 40 liter volume. The person breathes from it through the two-way valve, exhales into a second tedlar bag, and then that is pumped across two more Tenax cartridges. These cartridges are then analyzed by GC/MS in precisely the same way as the air sample. The collection of the breath sample takes about 10 or 15 minutes. The system is in a van and the van is driven to the people's homes, where they can give their breath samples conveniently. The precision for most of the compounds varied between 20 and 30 percent relative standard deviation for the air measurements, with breath a little worse than the air (30 to 40 percent).

Phase II: Estimation of Urban Exposures

The objective of the second phase was to apply this to a city and to try to estimate the exposures of the population of that city, or at least of a sub-segment of the city. The approach was similar to the Gallup poll technique of selecting a stratified random sample of people to represent a much larger population. Their personal air, drinking water, and breath were measured during a single 24-hour period.

Also, a questionnaire was administered on their activities during the day and we tried to relate their exposures to their activities using that questionnaire. A total of eight visits to five locations were made.

Back in 1979-80, when the study was being planned, the thought was that the exposures would not be great except in areas of heavy chemical manufacturing such as northern New Jersey, and Los Angeles and Contra Costa County (just northeast of Oakland in California).

In Bayonne, New Jersey, which is just east of Newark and just west of Staten Island, we drew 1-1/2 kilometer rings around the major petrochemical and chemical plants, and then selected people who lived within the rings and outside the rings. The idea was to see if we could detect a difference in the exposure of these two groups of people.

A total of 5,000 homes were screened in New Jersey to determine age, sex, smoking status, and occupations of the population. From these screening results, a sample was selected that was weighted toward people that we thought would be very heavily exposed--gas station attendants, the petroleum plant workers, chemical plant workers, and so on. Knowing their probabilities of selection allowed us to estimate the exposure distribution of the entire population.

The completion rate of 40 to 60 percent seems to be rather typical for these monitoring studies, where participants have to carry monitors and otherwise carry out several tasks. The rates are lower than in a Gallup poll, and there is a question whether our respondents have different exposures than non-respondents. We don't know the answer to that question. One fact in our favor is that exposure may not depend on personal attitudes as much as, say, political affiliation would.

Following is the schedule each respondent went through. The technician would go there in the afternoon or in the evening and set up the equipment and explain what was going on. He would give the participant an operating personal monitor with the charged pump. That person would carry it around for the evening, and then when he went to bed, set it on his bedside table, so that first overnight sample was, in most cases, really an indoor sample--a sample of the bedroom air. In the morning the technician would come by again before the person went to work, collect the first cartridge, give him the second cartridge, and the person would take it to work or school, and come back home. Finally, in the third and last visit, the technician would collect the second air cartridge, collect the water samples, collect the breath sample, and then give the participant \$15.00 for his work.

Some of the 20 target chemicals in air and breath are present nearly all the time, while others, particularly the brominated chemicals, are seldom present. We found that the agreement between air and breath frequencies is quite good. That is, what people are breathing in, they're also breathing out.

For drinking water, of course, at least in New Jersey, which has a surface water supply, there were only three chemicals--the three trihalomethanes, including chloroform--present all the time, and the others hardly at all. California, which had some ground water supplies, also had some bromoform.

Eleven compounds were present 60 to 90 percent of the time in the air and in the breath of the participants. These included one human carcinogen (benzene), several animal carcinogens (chloroform, trichloroethylene, tetrachloroethylene, carbon tetrachloride, and para-dichlorobenzene), and several mutagens (styrene, III-trichloromethane) as well as some common toxic aromatics (xylenes and ethylbenzene).

The ultimate aim of the study was to estimate the frequency distributions of exposures of 130,000 people residing in Bayonne and Elizabeth, New Jersey. Several observations can be made about the resulting estimations of exposure for 11 chemicals in New Jersey. The night and day personal air samples aren't very different from each other, but the median exposures are usually two to five times the medians of the outdoor air samples. For people who are highly exposed, the ratio of exposures to outdoor concentrations is much higher, often a factor of 10 or more. The breath levels follow the personal air exposures more closely than the outdoor concentrations.

One example of a strongly indoor-related chemical is p-dichlorobenzene. Its main uses are as moth crystals and air fresheners or toilet deodorants, which are designed to maintain a high concentration in the home. This chemical had mean values 50 times the outdoor means in New Jersey.

Following the New Jersey study, we went to another site, Greensboro, North Carolina, which is similar in size--population of 130,000--but does not have chemical or petroleum refining or anything of that sort. Outdoor concentrations declined sharply, but the indoor concentrations only went down by perhaps a factor of two. The ratio of indoor to outdoor is even larger.

The next visit was to a rural wheat farming area in North Dakota. The visit took place in October, with Arctic winds sweeping down from Canada, and outdoor levels almost disappeared. However, the indoor levels were similar to those in Greensboro, North Carolina. Benzene and styrene were also present, but they failed the quality assurance tests.

The final visits were to California. Los Angeles in wintertime was considerably different from all the other team study sites. The outdoor air was comparable to indoor air. This was also the first time we ever saw large day-night differences--nighttime increased concentrations by a factor of 50 percent over the daytime. Nighttime inversions form in winter in Los Angeles and the air flows back in from the hills and settles over the city.

But in springtime, Los Angeles looked similar to the other sites, with personal air exposures well above the outdoor air concentrations. At Contra Costa County in June of 1984, the personal air exposures for nearly all compounds continued to exceed the outdoor air concentrations.

Data Analysis: Sources of Exposure

In preparing the questionnaire on personal activities, we had selected 14 occupations that we thought might have higher exposures, and indeed 11 of the 14 did have higher exposures to at least one chemical. For example, chemical plant workers showed significantly higher breath concentrations of five chemicals.

Persons exposed to auto or truck exhaust in the last 24 hours showed no increased levels in their breath except for benzene, which is in gasoline exhaust. Persons who had gone to the dry cleaners had measurably higher levels of tetrachloroethylene in their breath.

Persons who had gone to the service station to fill their tank with gasoline had levels twice as high of benzene in their breath several hours after filling their tank. Many compounds were increased in smokers' breath: p-xylene, benzene, ethylbenzene, o-xylene, styrene, and all five aromatics.

In homes with smokers, the benzene level in the air was elevated by about 30 to 50 percent over the air in homes without smokers. About 60 to 70 percent of the homes in the country have at least one smoker.

Thus, children in homes with smokers may be breathing more benzene, based on these observations, than children in homes without smoking parents. And a recent study by Sandler (American Journal of Public Health, May 1985) comparing the lifetime mortality history of people in homes with smokers and without smokers found there was a difference in only one cause of death: leukemia, which can be caused by benzene.

If the indoors is an important source, we should be able to prove that by measuring the organic emissions from certain common items--paint, carpet, wallpaper, glue, cleansers. We used a clean-air chamber at Yale, aged the materials, put them in the chamber, and took samples of the air in that chamber. It had a known air exchange rate so we could determine the emission rates. We observed that almost all our target chemicals were being emitted from common materials that are in everybody's home.

It cost about \$50,000 to do that chamber study to measure five materials. Thousands of building materials and consumer products exist, so it would be an immense task to try to measure the emissions. But we do have an existing database, a space program spinoff. The astronauts suffered "sick building syndrome" on one of their early trips, caused by an adhesive that was put in just before liftoff.

Since then, NASA has tested a total of 5,000 materials, all of which were taken into the shuttle, for organic emissions. Marking pens contained the highest amount of benzene per gram of materials.

For styrene, 175 materials out of the 5,000 emitted styrene in measurable amounts. In fact, large numbers of materials were found to emit essentially all of the target chemicals.

Conclusions

We conclude that the personal monitor is a useful tool for measuring exposure of people. The breath measurement method was extremely sensitive. It is non-invasive, unlike a blood sampling technique. For these volatile compounds that preferentially come out in the breath from the blood as it passes through the lung, breath measurements appear to be much more sensitive than blood measurements. However, unfortunately, there is not much activity in the area of breath sampling, at least by environmental scientists. Industrial hygienists are doing more. These team studies convince one that the methodology is there for measuring people's breath, and environmental scientists should take advantage of it.

We also conclude the indoor exposures are often much greater than outdoor concentrations. The major sources appear to be building materials and consumer products--not the traditional sources (chemical plants, industry) that EPA is worried about. We did not find a difference between people living close to the plants and people living far away. We had sufficient numbers of people--150 people close by and 150 far away--to make it possible to see a rather small difference, but we saw none.

Another major source of exposure is personal activities--smoking, visiting the dry cleaners, filling your gas tank. If persons elevate their breath concentrations of tetrachlorethylene by a factor of two following a five-minute visit to the dry cleaners, the exposure in the dry cleaners must have been 1,000 times what it is outdoors. In fact, we measured a dry cleaners, and it was about 1,000 times the outdoor concentration.

The same calculation indicates that the exposure during filling of the gas tank is 100 to 1,000 times the average exposure. An EPA study found that at breathing level during refueling, concentrations of benzene were 100 times normal.

The main source of chloroform in the home may be the hot shower. At least 100 liters of water a day would have to release all their chloroform to the air in order to account for the observed concentrations, and the average person uses 200 liters of water per day. There has recently been a shower study showing that 100 percent of the chloroform is released from the time the water leaves the shower head to the time it hits the tub.

The final conclusion is that many new sources can be discovered by this technique, which should be extremely useful to the regulatory agencies.

QUESTIONS AND DISCUSSION

DR. FRAUMENI: Thank you very much. I think we have time for just about two questions. Dr. Kraybill and then Dr. Shy.

DR. KRAYBILL: Kraybill, NCI (Retired). The molecular species that are in water, if you look at them, compare fairly well with the molecular species in air. I don't know whether you looked into the water data, but could you get some estimate of the total exposure that you get from water contrasted with that in air for many of these compounds?

DR. WALLACE: Yes.

DR. KRAYBILL: Like the trihalomethanes for example, which had the greater concentration and which was less? In water or the air?

DR. WALLACE: For all except the trihalomethanes, 99 percent or more of the exposure was through air. For chloroform, there was a tremendous variation in the amount of chloroform in the air. For some homes, for some people, the air route appeared to be the most important. But for most people, the water route appeared to be most important, and we could estimate that based on our measurements for chloroform, that on the average something like 50 or 60 percent of the exposure was through water, and a smaller percentage through air. However, we did not measure beverages, and, as you know, sodas—even milk—have considerable chloroform in them. So, all things considered, for chloroform and probably for the other trihalomethanes, the water plus beverage is in general more important, but air is a significant portion of it, and I think the air exposure is from the hot showers--the basic source.

DR. FRAUMENI: Dr. Shy will have the final word.

DR. SHY: Carl Shy, University of North Carolina. My question is, what about the utility of breath sampling for estimating either human exposure or environmental exposure? In terms of human exposure, in order to relate adverse health effects to exposure, exposures of interest are usually those that occurred some years ago, not today's exposure. That's true for almost all the chronic disease that we'd be interested in. It might be relatable to acute irritation effects, but for most diseases, we're not interested in today's exposure.

In terms of assessing environmental exposure, if you're concerned about the indoor versus the outdoor environment, why not just measure the indoor or outdoor environment directly rather than going through the complicated process of having to get humans to be subjects for study and collect samples from them?

DR. WALLACE: Well, that's a complicated series of questions. As for the utility of exposure for health effects, there'd be two health effects of concern--chronic and acute. Let's take the acute first. The acute health effect of greatest interest, I think, is sick building syndrome, which the work of Mølhave has shown can be reproduced reliably by exposing sensitive persons to a mixture of compounds at concentrations ordinarily found in buildings. People who have

previously exhibited sensitivity to the building air show both subjective and objective effects—effects on the memory for example, typical sick building syndrome—from breathing a mixture of common compounds like xylenes.

So there the measure of today's exposure is relevant to today's health effect. In the other case, for chronic effects, these studies discovered certain sources of exposure. One can then calculate back and estimate previous exposures, which I think would be useful for those concerned with estimating chronic health effects.

But, as I mentioned in the beginning, this is not an epidemiology study, and did not have health effects as an endpoint. An ultimate endpoint for a regulatory agency is determining these sources of exposure and prioritizing them for study.

DR. SHY: Why not measure the environment?

DR. WALLACE: Well, that's a very interesting point too. If we had measured the environment and not measured breath, it would have been harder to discover the main source of benzene, because the personal air monitor did not pick up the mainstream smoke from cigarettes. For any compound that anybody gets through a cigarette, neither the indoor monitor nor the personal monitor is useful. Only the breath, in this case, allowed us to see where the main source of benzene was. A smoker is taking in about two milligrams of benzene a day, whereas the rest of us get only 0.2 or so milligrams a day. So it just overwhelms other sources, and this is the first time, I believe, that that has been put on a quantitative basis.

DR. FRAUMENI: Thank you very much.

DR. ROBINSON: Philip Robinson, EPA. If I may elaborate just a little on those comments. With regard to today's exposures, I think we at EPA are interested from a prospective stance in trying to control today's exposures to prevent future health problems. The other side is that we look at body burden as we look at the body as sort of the integrator of multiple exposure routes. And we are trying to develop the capabilities to look at body burden and to predict the risk associated with those body burden levels, and to employ these as sort of a screening tool to focus our attention on the more problematic compounds.

The other side of body burden is that it does give us definitive information that exposure has, in fact, occurred, which is another area that we in EPA are concerned about because historically we've taken a lot of leads from the toxicological side, where we've done quite a few investigations on chemicals for which there are no exposure data, either because they degrade very rapidly in the environment or whatever the reason might be. So we do believe that body burden data is a very good indicator of exposure, and if we can push the science along, it will give us some quick way of assessing the relative risks of those exposures.

WORKSHOP WRAP-UP

**Dr. Edward Bresnick
Dr. William Farland
Dr. Frode Ulvedal
Dr. Carl M. Shy
Dr. Joseph F. Fraumeni, Jr.
Dr. Roy M. Fleming**

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DR. FRAUMENI: We now come to the climax of the meeting, which is called workshop wrap-up.

DR. CAMERON: Yes.

DR. FRAUMENI: Perhaps I could take the prerogative of the chairman and start off the discussions. It is my impression that the papers presented here, particularly in the field of epidemiology, quite well illustrate the value of interagency collaboration, involving both intramural and extramural scientists. With the decline or plateauing of funding and positions available to the federal agencies, it is obvious that collaborative projects now take on added significance, as the need increases to share existing technical and data resources and to utilize complementary types of expertise.

The major thrust of the interagency epidemiology program to date has been in the area of analytical studies, which are mainly case-control or cohort investigations, sometimes including multidisciplinary approaches that incorporate laboratory probes in an epidemiologic context. In recent times, we have tried to give special attention to studies that clarify the effects of widespread population exposure, that resolve matters of important public health or regulatory concern, or that contribute to a better understanding of carcinogenic mechanisms and host-environment interactions.

These points are illustrated by the studies we have heard described of painters, of leather workers, of formaldehyde workers, of auto workers, and the like. The specific types of collaboration that are needed by epidemiologists may take various forms. A top priority, in my mind, is the utilization, modification, and preservation of routinely collected data resources, such as those located in various federal agencies, including the Social Security Administration, the National Center for Health Statistics, the Census Bureau, the Internal Revenue Service, and the Health Care Financing Administration. Especially valuable are the data resources located in private industry, in labor unions, in insurance plans, in health care delivery systems, and in mortality systems for occupational surveillance in various states as presented by Ms. Burnett of NIOSH.

The utilization of record-linking systems and the National Death Index should be encouraged in tracing high-risk individuals and clearing the rosters for mortality. We'd like to see more efforts taken now to extend the National Death Index retroactively, to cover at least 10 years prior to 1979, when the registry took effect.

Another area of collaboration is needed as epidemiologists join forces with environmental and industrial hygienists, and information specialists, to facilitate the gathering and the characterization of exposures involving specific physical and chemical agents. This type of effort obviously enhances the power of epidemiology to link specific exposures with cancer risk. With the sensitive and systematic ways being developed to measure exposure, we can obviously achieve studies that will have much greater power toward this and we need better methodology as illustrated by the papers given on workers exposed to hydrazines, to organotropin compounds, and now, as we just heard, with the TEAM study.

Finally, collaboration is needed with laboratory investigators, to clarify not only environmental exposures, but also to identify the subclinical or preclinical effects of environmental agents through measurements in body fluids and tissues, or to detect host susceptibility factors and the actual mechanisms of carcinogenesis.

Many of the limitations of cancer epidemiology may be overcome by the innovative approaches that we now call biochemical or molecular epidemiology. The effective conduct of multidisciplinary studies will require regular communication between scientists in different agencies, and an awareness of the expertise and resources that are available.

Meetings such as this workshop are crucial in exchanging information and in identifying areas of common interest to help uncover special opportunities and stimulate collaborative research that will help settle many of the remaining key issues in environmental and occupational carcinogenesis.

I'd now like to call on my colleagues, and first, Ed Bresnick.

DR. BRESNICK: First, before I make a few comments, those of you who know me know I'm involved in hard science using animal systems, not so much humans. After a day and a half of hearing some epidemiology, I'm kind of glad that I'm still in animal systems. That's a very, very hard area. Those of you that are in there have my greatest respect.

The comments I would make relate to papers that were in the session which I chaired. Three-fourths of them dealt with the business of aquatic animals, and the aquatic animals were being used like the canary in the coal mine, as beacons, or as tools for studying carcinogenesis. I think in both those areas, they have even greater utility. If we find clams and mussels have a fair amount of polycyclics or cadmium or trichloroethylenes or all these bad actors, then I think they're going to be a pretty good indicator that the bottom on which they're feeding or the little creatures that they take in have this stuff, and it's probably a pretty good indication to go look for a potential source of pollution, really, and to try to do something about it.

The good part about those mollusks and other creatures is that they don't move really very great distances. Therein lies the problem, I think with using certain fish as beacons, because some of them do move fairly sizable extents, so it's like looking for the key under the light when you dropped it a block down the road where it's dark. I think one's going to have to know a heck of a lot more about the migrations of some of these aquatic species that we're proposing as potential beacons.

Now in terms of models for carcinogenesis studies, I think here might be even their greatest utility. If we can get some of these creatures to reproducibly breed and do nice things "in tank," then we have a pretty good system for studying carcinogenesis because, as has been established in several model systems, these aquatic beasts apparently are very, very sensitive to carcinogenic substances. You'll see the first lesions earlier than has been reported for animal systems. Even the senear mouse, which provides a host for the skin tumorigenesis experiments, had a slower response time.

The third comment I would make relates to the paper on asphalt mixtures. As sort of a pure scientist, and I use that in the most catholic of senses, I always used to denigrate research in which people used mixtures. I've gone a 360 degree turn on this issue. I'm less convinced that studies on absolutely purified compounds have anything to do with reality. I think much, much more effort is going to have to be done in studying the effects of mixtures, because components of mixtures often have pro-effects, anti-effects, and they can negate out.

It's nice to know what the absolute composition of emission is, and it's nice to know that bezo(a)pyrene is a reasonable carcinogen in animal model systems, but I think it's nicer to know what the total emission is that we actually breathe and handle in a pharmacokinetic sense. And I'll leave it there.

DR. FRAUMENI: Thank you, Ed. I'd like to call on Dr. William Farland.

DR. FARLAND: Ed's given us a good summary of his session. I think what I would like to do is to make a couple comments about the papers that were in my session, the second experimental study session, and then make a couple general comments about the collaborative program, and the benefits that we see emanating from it.

One of the things that I would want to stress is the title of this workshop, that is "Progress on Joint Environmental and Occupational Cancer Studies," and I think that what we're seeing is progress. What we're seeing are studies that are asking more questions, and I commend the investigators who are undertaking these studies right now, and make the strong recommendation that we all look to a number of different ways to find mechanisms for funding studies that would come out of these initial studies that are being funded under this collaborative program.

More specifically, with regard to the work on cultured human and other mammalian tissues, I think that this is a field of study that we will continue to rely more on as we're encouraged, especially in regulatory agencies, but certainly as scientists in general, to use all of the data that we have on hand as we start to evaluate chemicals for potential hazards, or risks; to carcinogenicity, and other end points. Trying to understand the metabolism across species, trying to understand the relevance of toxicity end points that are found in animals, and their relationship to humans--these are the types of studies that provide us with those stepping stones for answering some of the questions in terms of extrapolation. Certainly, there are a number of very interesting questions that have been developed simply from the data that are being generated out of this particular study, which I hope will be continued with future support.

With regard to the discussion of transformation of human cells, transformation of cells in general, we are looking for means for setting priorities on what types of chemicals should go through the very costly, labor-intensive work of long-term bioassays in animals. These transformation assays, I think, are very strong, very reasonable candidates for those types of studies.

A lot of years of scientific effort have gone into working with these systems and our feeling is that we're getting closer and closer to the point where we are validating them for use in evaluating chemicals. Perhaps not as we originally thought, as an overall screen for any chemical that you might want to put in, but certainly, with the development of characteristics for individual transformation assays, we have the ability to select the transformation assay and the types of chemicals to which it might be most beneficial, and to continue to use those assays in setting priorities for chemicals going on into long-term studies.

Again, international workshops, as the one that Tom reported on this morning, go a long way to bring the scientific minds together, and bring the data together to answer some of these questions with regard to the balance of the systems. What types of systems might we rely on for making decisions with regard to setting priorities for further testing? From the regulatory standpoint, that becomes an extremely important issue.

Just a brief comment on the experimental studies section that occurred yesterday. Not only are we looking at fish as sentinel organisms, as Ed Bresnick mentioned, we're also looking at them as, perhaps, the system of choice for beginning to screen chemicals because of the characteristics that were discussed--the ability to deal with large numbers of animals, the ability to control exposures reasonably well, and as Ed said, to see our effects in a reasonably short period of time.

I think that it is important to note that a large effort in the use of aquatic systems for long-term studies of this type began years ago under this collaborative program. Certainly to a large extent, it was Dr. Kraybill's doing, and I think that we're starting to see this area blossom. The studies that were discussed yesterday are good examples of the application of these studies to specific problems where the methodologies fit very well with the question being asked.

I would also remind people that there is a request for applications on the street right now that the NCI has initiated for use of these types of systems in evaluating both the fish and lower invertebrate models as potential systems for looking for potential human carcinogens, both as sentinels and also as an experimental system.

As Dr. Bresnick said, I think we've all changed to a certain degree our thinking on the complex mixture, and the magnitude of the problem that one has when one starts to take apart a complex mixture is illustrated very well in the discussion yesterday with regard to the asphalt fumes. It's important that we develop those data, that we look for reactive components, but again, when it comes right down to it, it is the individuals who are exposed to the fumes that we need to try to protect, and we need to know something about the composition of those fumes in order to be able to deal with that.

In general sense, I guess, I would like to just reiterate something that was mentioned in Dr. Fraumeni's opening remarks, and that is that we're moving into a time when collaboration becomes extremely important with the budget constraints. In this program particularly, we will attempt to continue our collaboration with a very severe limitation on the funds, if not a complete limitation on funds, for the next several fiscal years.

The way that we can do that, I would hope, is that we can introduce projects that would be of interest to the topic of environmental and occupational cancer that we are funding as Agencies or Institutes just to maintain the awareness and maintain the scientific interchange as we have in meeting like this today. And I would hope that we continue to do that and support this program over the next few years, even if we don't have any funding coming forth specifically for these types of projects.

I'd like to wrap-up by saying, being involved with this program for a number of years, I've seen the continuity. I've seen these types of studies develop, be completed, and lead to more definitive studies, all of which provide us with answers that, from my perspective, become immediately applicable in the regulatory forum, and from the scientific standpoint, certainly increase my knowledge of carcinogenicity and, in that sense, are extremely important from both of those view points. Thank you.

DR. FRAUMENI: Thank you very much. I'd now like to introduce Dr. Carl Shy.

DR. SHY: Thank you, Joe. I want to center my remarks around two presentations that were made yesterday, the study of painters and the evidence that painters have excessive lung cancer, and the study of the formaldehyde-exposed workers, which showed an absence of an excess of lung cancer.

I think these two studies illustrate fairly well some of the strengths and limitations of epidemiology. First of all, in the painters' study, one of the things that came out is that there was an excess of lung cancer in painters and that that excess was modified by a number of characteristics, including whether the painters were smokers, whether they used a mask or didn't use a mask for painting, and to some extent, I guess, some age factors were in there as well.

These are the kinds of things that are very difficult to replicate in a laboratory setting. We might not even think of having replicated those exposure situations, you know, of adding smoke to another carcinogen or using something like a protective device, which would be rather difficult to do, I guess, with an animal experimental system.

The limitation of that study, from an epidemiologic point of view is that, you know, we don't know what agent may have caused the lung cancer excess, because the painters are obviously using all kinds of different paints, and there are a lot of different chemicals in the paints. So what good is it to get that information?

We can protect painters without knowing what chemical they were exposed to. We can protect cigarette smokers without knowing what chemicals they're exposed to in getting their excess lung cancer. There are a lot of kind of generic things that can be done in order to interrupt the causal chain, and in the case of painters, simply using a mask or increasing the ventilation in which they usually work could very well reduce across the board the risk of lung cancer for painters.

It may take decades before we find out what agent has caused the excess lung cancer for painters, so it's both the strength and limitation of epidemiology. We could certainly have information to proceed to protect the painters, but from a scientific point of view, we don't know what's going on.

The formaldehyde study which failed to show an excess of lung cancer is also interesting in pointing out a limitation of epidemiology. That is, if you think something is a human carcinogen, it isn't necessarily the epidemiologic study that's going to confirm or deny that. Formaldehyde is probably a human carcinogen, and everything points in that direction, but if you don't have very much exposure to formaldehyde, you're not going to find a cancer excess in a human population.

For instance, if you studied the survivors of the atomic bomb and limited your follow-up to those who were exposed to between 10 and 50 rads, you would not have found a cancer excess in those people. All the excesses are manifested in the survivors, who had quite a bit in excess of 10 rads. Most of them had in excess of 100 rads, yet there's no question that radiation is a human carcinogen and that any radiation increases the risk of cancer.

But this may be the very thing that's going on with formaldehyde workers. Formaldehyde is a human carcinogen, but at low doses, you can't find it unless you have huge populations exposed to a moderately high dose. When you fractionate the population of 25,000 workers into dose categories, you come down with relatively small numbers of people exposed to the higher dose; and with small numbers at an even relatively high dose, you're not going to find an effect.

But that doesn't mean there is no carcinogenic effect of formaldehyde. There are some things that Dr. Blair pointed out that were suggested there, like nasopharyngeal cancer excess, perhaps, but the numbers were small. There was also a possibility of a nasal cancer excess, but again he had only two nasal cancers in as many as 25,000 workers.

I think in order to determine whether something is a human carcinogen, you should rely on model systems, assays, and possibly in vitro tests, because we'll always be stuck with that same problem of not having enough workers exposed at a high enough dose to draw a definitive conclusion as to whether an agent is a carcinogen.

If it's a strong carcinogen, we find it in human studies. We did it with cigarettes, we did it with asbestos, with bis-chloromethylether, and a bunch of other things. If they're strong carcinogens, they're easy to find. If they're carcinogens that produce a rare disease like angiosarcoma of the liver, we can find it too. But if they're weak carcinogens or if they're carcinogens that affect a lung cancer site or a site that is also affected by many other factors, like lung cancer, our chance of finding that effect in excess in a human population is small.

So we shouldn't rely on human studies to give confirmatory evidence as to whether something is or is not a human carcinogen. I think we should make that decision on other bases. We can use human studies to tell us whether it is a strong carcinogen, whether there's an epidemic of cancer caused by that agent. I think the formaldehyde study says from the best of our evidence there isn't an epidemic

of cancer in that working population; but the study does not show, and I don't think Dr. Blair drew the conclusion, that formaldehyde is not a human carcinogen simply because he didn't show an excess in his study.

DR. FRAUMENI: Thank you, Carl. I'd now like to introduce Dr. Roy Fleming from NIOSH.

DR. FLEMING: Thank you. Instead of commenting on some of the individual studies, I thought in the final wrap-up session I might look at things a little bit more globally. NIOSH and NCI have been involved in this collaborative effort for nearly 10 years. During that time there have been over 110 projects conducted, and the list of publications from them is quite impressive. The types of projects that were reported on in this workshop are representative of both the experimental and epidemiologic studies that we have been pursuing collaboratively. I believe that everyone involved with the whole process knows that the level of collaboration in the past couple years has strengthened tremendously.

But there's one thing that's really been missing all this time, I feel; something that maybe a lot of us don't deal with. It's something that NIOSH has been struggling with for a couple years now, and we'd like to begin to try to have others think along these lines as well. That has to do with the concept of a national strategy.

We've heard reported here surveillance activities that are used for not only identifying and monitoring populations, but also for follow-up. Also reported were experimental studies which can lead us into understanding the mechanisms of carcinogenesis, and giving an indication, perhaps of biological monitoring mechanisms. In epidemiology, even with the limitations Dr. Shy mentioned, it is possible to obtain an indication of the effects on humans. However, there has not been an integrated approach to looking at these various research activities.

Last May, NIOSH held a symposium to begin the process of trying to define what might be a national strategy that would blend the various resource-limited activities of our organizations, ones that you represent, NIOSH, and NCI--try to blend those into a cohesive strategy that would lead us towards some objectives in terms of preventing occupational carcinogenesis in a broader spectrum.

So I would just like to make that notation from the standpoint of where this collaborative program might be headed to try to give it a little more definition of direction, and likewise, the organizations that you all are affiliated with to begin to think in terms of doing things that would fit into a national strategy for prevention.

We hope to have this strategy available very soon, and if any of you would like to take a look at it and make comments about it when you see it, we'll be happy to work with you on that. It is not designed to be a NIOSH strategy, it was designed to be something that would be useful to the entire field.

DR. FRAUMENI: Thank you. Dr. Fleming. I'm not sure how much more time we have left, Tom. Is it just about time now to wrap up?

DR. CAMERON: Just about.

DR. FRAUMENI: I'd just like to make a couple of comments. First it's a real pleasure to see Dr. Herman Kraybill, who has contributed so much over the years to interagency cooperation, participate in this meeting.

I'd also like to thank the panel for their very stimulating comments, and on behalf of the panelists and the participants, I'd like to express our thanks to Dr. Tom Cameron and Mrs. Inge Blackwood of NCI for their successful efforts in organizing this meeting and enhancing interagency collaboration. Thank you.

APPENDIX A
POSTER PROJECTS

**SURVEY OF COMPOUNDS WHICH HAVE
BEEN TESTED FOR CARCINOGENIC ACTIVITY**

**Presented by Anthony Lee
Technical Resources, Inc.**

SURVEY OF COMPOUNDS WHICH HAVE BEEN TESTED FOR CARCINOGENIC ACTIVITY

*A. Lee, B. Campbell, and T. Varley
Technical Resources, Inc., Rockville, MD*

The *Survey of Compounds Which Have Been Tested for Carcinogenic Activity* (USPHS Publication No. 149) is a database on the tests of substances in experimental animals for carcinogenic activity. The data are compiled from literature published since 1906; and it is the largest and most comprehensive survey of chemicals that have been tested for carcinogenicity worldwide. The original issue of PHS-149 was compiled by Dr. Jonathan L. Hartwell and included literature published through 1947. Supplement 1, covering 1948-1953 literature, and Supplement 2, covering 1954-1960 literature were prepared by Dr. Hartwell and Dr. Philippe Shubik; the latter volume, published in 1969, was edited by Dr. James A. Peters. In 1969, responsibility for compiling and publishing the carcinogenicity data was assumed by the Carcinogenesis Area, Division of Cancer Cause and Prevention, National Cancer Institute. Since then, 10 supplements have been published, including literature for the following years: 1961-1967, 1968-1969, 1970-1971, 1972-1973, 1974-1975, 1976-1977, 1978, 1979-1980, and 1981-1982. Under the present contract the 1983-1984 supplement has been prepared and is awaiting printing. The preparation of the 1985-1986 supplement is presently underway.

The PHS-149 Series has been employed as a major information tool in scientific research, research planning, and technology transfer programs in the carcinogenesis field. It is one of the major data sources used in the selection of chemicals for the NCI Bioassay Program. PHS-149 has also been recognized as a very important data source by the Occupational Safety and Health Administration in the Agency's development of the OSHA Cancer Policy, as well as in the activities of the Environmental Protection Agency Cancer Advisory Group. The PHS-149 database has also been employed as a source of information for other databases such as the Chemical Carcinogenesis Research Information System (CCRIS).

The database currently consists of 13 volumes covering literature published from 1906 to 1984. It contains data on approximately 7,500 chemical substances for which long-term bioassay tests have been conducted. Over 10,500 literature references are included, selected from screening over 520,000 articles published in 56,600 journal issues. In addition to the 13 volumes, a separate volume of Cumulative Indexes contains approximately 13,923 chemical names and 3,874 CAS Registry Numbers along with the accession numbers of each volume of PHS-149. The Cumulative Indexes as well as the various supplement indexes serve an important function in directing researchers to previously published experimental results. This information can then provide a basis for comparative studies, prevent unnecessary and costly duplication, or be used in evaluating the carcinogenicity of a chemical substance.

For literature inclusion, over 600 scientific journals of the published world-literature, are screened for articles relevant to the PHS-149 publication. The data are extracted in units of data lines reflecting the test protocol for each bioassay experiment. This involves analysis of the article in order to identify the

pertinent data for inclusion in PHS-149, and recording of the data onto work-sheets for later editing and typing. Although PHS-149 does not evaluate the experimental design or the resultant data, the extractors must attempt to enter all relevant information from the article clearly, accurately, and concisely in order for the user to draw their own conclusions from the data. The execution of this, the most critical task in the project, requires painstaking care and attention to detail. Each data unit incorporates: a) literature reference, b) animal species and strain information, c) methods of preparation and dose level of the chemical substance(s) administered, d) the route and site of administration, e) level of pathology examination, f) total number of animals with tumors for each experimental group, g) the number and histopathological description of the tumors, h) survival data, i) duration of experiment, and j) information on control groups.

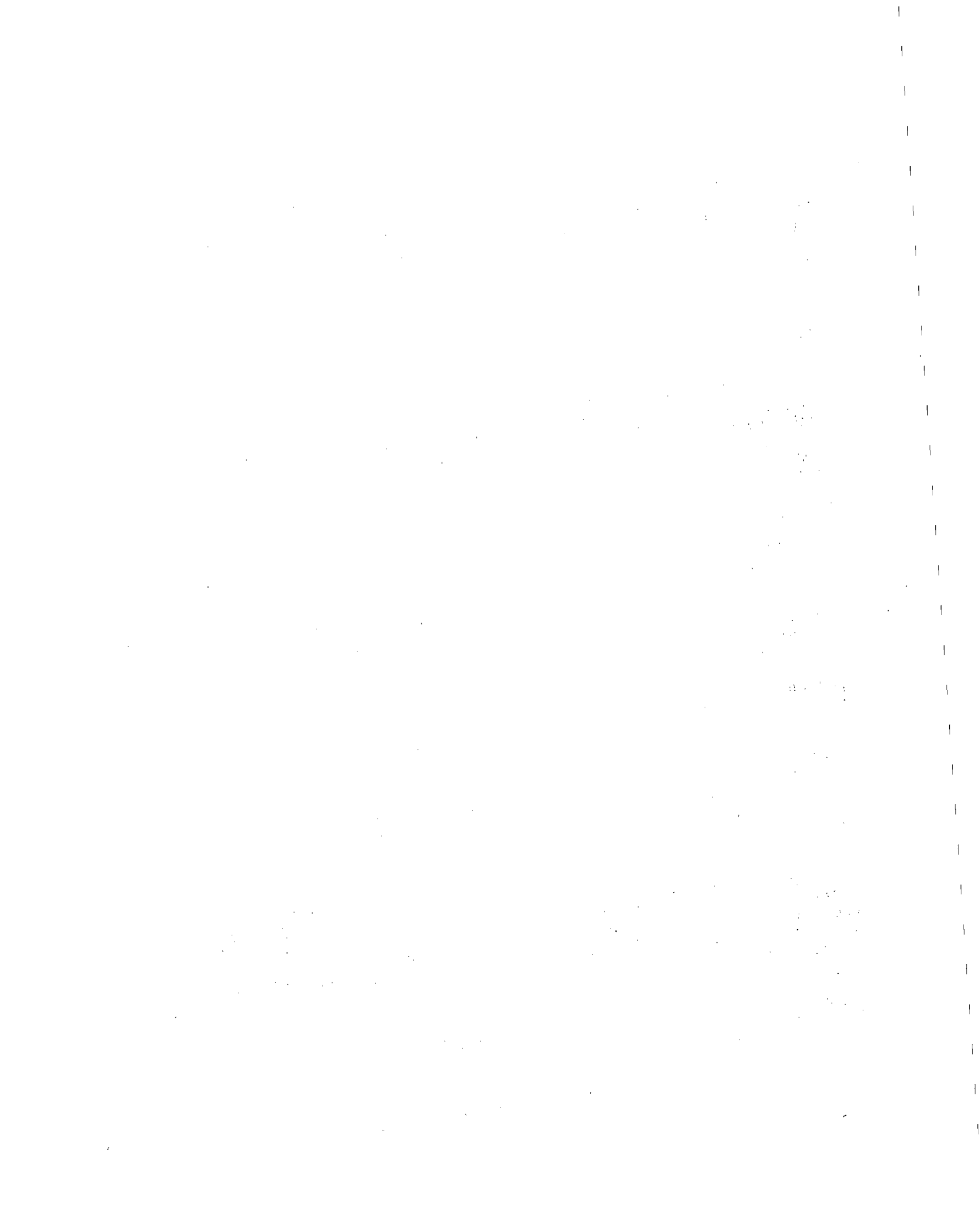
The volume is organized according to chemical structural categories (inorganic, aliphatic, monocyclic, bicyclic, tricyclic, tetracyclic, pentacyclic, hexacyclic, azo compounds, steroids, heterocyclic, plant oils, miscellaneous, plants and plant extracts). Each compound is assigned to its structure category, and within each category, the test compounds are entered alphabetically by the preferred name of the compound which is assigned according to the Chemical Abstracts Service (CAS) 9th Collective Index. The PHS-149 employed the CAS based nomenclature (beginning with the 1961-1967 volume) to organize and consolidate the extracted data. The CAS nomenclature reconciles synonymous chemical names and serves to sort the data sheets into publication sequence order, and thereby to consolidate the data for publication in the PHS-149. The chemicals must be traceable by their CAS registry number, their CAS-approved chemical name, and their synonyms or common acronyms. The chemical editor prepares files for the chemical names so they can be tracked and rearranged in the proper structure-related sequence for publication. The structures are determined and verified and then drawn in a uniform style appropriate for inclusion in the PHS-149.

To facilitate users' access to the database, each volume contains a number of indexes: bibliography, author cross-reference, chemical name, Chemical Abstracts Service (CAS) registry number, route of administration, site of application, animal species/strain, tumor site, and vehicle. A machine-readable version of the index material is also available.

The database has continued to evolve in response to the needs of the scientific community and regulatory authorities. Scientists in carcinogenesis research throughout the world have continued to express their support to NCI for the PHS-149 publication because it provides ready access to the world's literature on the subject. It provides a structured and detailed presentation of data as well as an overview of research activities in the field of chemical carcinogenesis. It also enables researchers to gain insight in understanding the mechanisms of chemically induced carcinogenesis and enhances the predictability of anticipating the effects of untested chemicals. Results indicated in the publication serve as a guide to areas of additional biomedical research needed to determine positive correlations, or lack thereof, between chemical exposure and cancer. Chemicals tested and included in the publication are those found in the environment as well as the workplace. Regulatory authorities have also found it an important information resource in the development of regulations and in responding to hazard assessment issues associated with chemical waste and spill problems.

**NATIONAL DATABASE ON BODY BURDEN OF
TOXIC CHEMICALS**

**Presented by Philip E. Robinson
U.S. Environmental Protection Agency**



NATIONAL DATABASE ON BODY BURDEN OF TOXIC CHEMICALS

Philip E. Robinson¹, Anna S. Hammons², M. Virginia Cone², Marialice Wilson², C. Donald Powers², Cindy R. Stroup¹, and Thomas P. Cameron³

INTRODUCTION

Continuing reports of toxic chemicals being found in human tissues and fluids led scientists to recognize the need for a centralized, comprehensive, and readily accessible source of data concerning human body burden of xenobiotics. The National Database on Body Burden of Toxic Chemicals was established to satisfy this need. Because body burdens reflect exposures to environmental contaminants, a systematic collection of body-burden data facilitates assessments of the impacts of such exposures on human health. The body-burden database is a particularly valuable tool in identifying chemicals of concern and in setting priorities for nomination and selection of chemicals for carcinogenicity and toxicity bioassays.

CONTENT OF THE DATABASE

The database contains two files: Chemicals Identified in Human Biological Media, established in 1978 and Chemicals Identified in Feral and Food Animals, established in 1980. Information for the database is collected from published, scientific literature, retrospective to 1974 for the human file and to 1978 for the animal file. Approximately 65 periodicals, selected for relevancy, are routinely searched manually for current data. Contacts with federal agencies and private investigators and periodic searches of computerized databases also help to identify pertinent body-burden data. The database currently contains information on about 1800 chemicals.

Information on animals was added to the body-burden database for several reasons: 1) animals are significant components of the human food chain, 2) body burdens of chemicals in animals provide early warning of potential human exposure, and 3) various species can be good indicators of potential hazards of exposures to contaminants because adverse effects may be detected after exposure to concentrations which would cause no demonstrable effect in humans.

The database contains over 16,000 records, each containing information on a specific chemical/tissue or chemical/tissue/animal combination. Some of the specific data elements which may be included in a record are: bibliographic information; data source (report, journal, letter); language (other than English); Chemical Abstracts

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Service (CAS) preferred name; CAS Registry number; synonyms; chemical formula, properties; source of chemical exposure; route of exposure; animal, species; number of cases; organ, tissue, or body fluid; concentrations measured (mean, range); analytical technique; half-life; demography; toxicity, health effects; pathology, morphology; explanatory comments or caveats; and keywords.

AVAILABILITY

New records, arranged alphabetically by CAS preferred name and in tabular format, are published annually. Author, corporate author, tissue and keyword indices, as well as several appendices and a directory of chemicals, are included. These publications are distributed internationally to libraries of government agencies, medical schools, public health institutions, and to various universities. The volumes are also available from the National Technical Information Service, Springfield, Virginia. All records can be accessed on-line through Lockheed's DIALOG.

USES

Body-burden data provide 'de facto' evidence that exposure to a toxic chemical has occurred. Such information is important to the Environmental Protection Agency's Office of Toxic Substances (OTS) because, in the absence of toxicity data, it is sufficient to require that tests be performed. Also, priority setting, historically done on a toxicological basis, can now be performed by focusing initially on those chemicals which have already been identified in tissues and body fluids.

The availability of the body-burden database facilitates the early identification of human exposure to environmental contaminants and aids in assessing its significance. For example, the OTS currently uses the database in identifying chemicals in the Chemical Substances Inventory, mandated by the Toxic Substances Control Act, that pose potential risk to the general population. Subsequent actions may include requiring additional toxicological testing if available data are not sufficient for an assessment, or placing the chemical in the Existing Chemical Assessment Process for a more detailed evaluation of exposures and risks. Surveys of other users show that the database is important to those involved in assessments of risk associated with exposure to toxicants, in toxicological research and testing, and in disease prevention and treatment.

Information in the database can be valuable in identifying not only populations at increased risk from exposures to chemicals but also probable sources of exposure. This information is important in planning activities necessary to obtain statistically valid estimates of exposure and risk.

The database is also used by medical and health professionals in teaching and by investigators in planning research in toxicology, epidemiology, and monitoring. Body-burden data often serve as a baseline for comparing experimental data on concentrations and frequency of detection of selected chemicals. Managers of other databases concerned with toxicology and effects of chemicals use the information from the body-burden database to augment their files.

CONCLUSIONS

Responses to user surveys have emphasized the importance of having a comprehensive, easy-to-use, centralized source of body-burden information. As indicated by these surveys, the database aids primarily in: nominating and selecting chemicals for testing; assessing the hazards and risks associated with exposures to chemical contaminants; teaching; planning research and comparing results; and in augmenting related databases. Major users are researchers, regulators, and medical and public health professionals and students.

Improvements to the body-burden database are ongoing, therefore, continuing dialogue between users and database managers is encouraged. Responses from users are always welcome and carefully considered.

Future plans for the database project include providing other products such as specialized reports and bibliographies.

ACKNOWLEDGMENT

This database was established under the aegis of the Interagency Collaborative Group on Environmental Carcinogenesis, National Cancer Institute. The work is performed by Science Applications International Corporation, Oak Ridge, Tennessee, under the direction of the U.S. Environmental Protection Agency's (EPA) Office of Toxic Substances and through an agreement between EPA and the U.S. Department of Energy.

**INTERACTION BETWEEN NORMAL HUMAN
DIPLOID CELLS AND CHEMICAL
CARCINOGENS/MUTAGENS IN VITRO**

**Presented by Shiu Huang
Environmental Health Research and Testing, Inc.**

FOURTH NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
INTERACTION BETWEEN NORMAL HUMAN DIPLOID CELLS
AND CHEMICAL CARCINOGENS/MUTAGENS IN VITRO

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The objective of the investigations presented here was to relate the process of DNA alteration to mutation frequency and enzyme activity at the hypoxanthine phosphoribosyl transferase (HPRT) locus in cultured human cells. Two types of DNA alteration were examined, mutagen-DNA adduct formation with benzo(a)pyrene [B(a)P], and chromosomal rearrangement and deletion generated by ionizing radiation (gamma rays).

Human fibroblasts were treated for 4 and 16 hours with B(a)P using S-9 activation. One group of cells was used for the detection of mutations and the other for the extraction of DNA. The extracted DNA was subsequently used for detecting and quantifying B(a)P-DNA adducts using a ^{32}P -postlabeling method. ^{32}P -postlabeling analysis is a new technique for detection and quantification of DNA adducts that does not require radiolabeled chemicals (Randerath et al., 1981). Because of the high specific radioactivity of ^{32}P (6000-9000 Ci/mole), a high sensitivity of detection can be achieved for aromatic carcinogen-DNA adducts.

When subjected to microsomal activation, B(a)P was found to be strongly mutagenic, although direct dose-dependent mutation responses were not observed. B(a)P-DNA adducts were found in one major spot and occasionally a minor one, whereas control DNA produced no adducts. Mutagenic activity was shown to be highly correlated with the number of B(a)P adducts per nucleotide.

The straight line fit for the induced mutation frequencies versus DNA-adducts is: $\ln(Y) = 1.71 + 0.48 \ln(X)$; where Y equals the induced mutation frequency and X equals the number of recovered DNA-adducts. The data set gives an estimated slope of 0.48 ± 0.06 . The slope is significantly different from zero at the 0.1% level.

The nature of the mutational events at the molecular level was explored using Southern Blot analysis, which can detect deletions or insertions of large fragments of DNA, as well as the loss or gain of restriction enzyme cleavage sites. A total of 15 spontaneous and 21 gamma-ray-induced mutants were analyzed. 40% of the spontaneous mutants and 52% of the induced mutants carried gross changes at the HPRT locus, that are detectable by Southern Blot analysis.

Nine gamma ray induced mutants, that represent different classes of intragenic alterations, have been examined by Southern Blot analysis employing three different restriction endonucleases: Hind III, MspI and EcoRI. Of these, Hind III was most efficient at detecting DNA rearrangements, although all three were equally able to detect gene deletions.

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INTRODUCTION

The mechanisms by which environmental mutagens and carcinogens act on macromolecular targets to produce mutations or initiate tumors remain a central focus of cancer research. Chemical or physical alterations in DNA as a result of the interaction between genotoxic agents and the genome are thought to be an initial step in carcinogenesis (Brookes and Lawley, 1964; Perera et al., 1982; Doerjer et al., 1984). Such modifications in base sequences in DNA and formation of DNA-mutagen adducts are strongly linked to mutagenic activity in a variety of *in vivo* and *in vitro* systems (Newbold et al., 1977; Huang et al., 1978; Aust et al., 1980; Strom and Michalopoulos, 1982; Arce and Grunberger, 1983; and Turner et al., 1985).

Most mutagenic and carcinogenic chemicals require metabolic activation before molecular reactivity can be observed (Newbold et al., 1977; Jongen, 1982; Sebti et al., 1982; Huang and Waters, 1983). It is likely that mixed-function oxidase activity in plant and animal tissues provide the metabolism to produce reactive metabolites; however, it is now clear that culturing human cells through several passages reduces mixed-function oxidase activity (Newbold et al., 1977; Huang and Waters, 1983). Several processes are involved in metabolic activation, including adsorption, metabolism, detoxification, and excretion of the carcinogens. Due to these multiple processes occurring concurrently, the relationship between exposure and delivered dose becomes less apparent (Aaron, 1976; Lee, 1976; Sega, 1976). Therefore, one cannot assume linearity between exposure concentration and delivered dose for those chemical mutagens requiring activation. The distinction between dose and exposure to a chemical by cells or organisms is essential in developing a dosimetry concept for comparative mutagenesis/carcinogenesis and risk estimation (Lee, 1976). Lee (1976) has defined the

dose for chemical mutagens as the amount of a chemical that reacts with a target molecule generally assumed to be DNA, even though the genetically significant moiety of the DNA may not be known. By studying target molecules that react with the test chemical, one can measure the accumulated stable products, obtain a measure of the dose delivered to DNA, and thus establish valid relationships between mutagenesis, DNA adducts, and changes in DNA at the molecular level.

The hypoxanthine phosphoribosyl transferase (HPRT) locus has been used to study these mutational mechanisms because of the ability to select for mutations at this locus in cultured mammalian cells. Protein sequencing of mutant HPRT has led to the identification of single amino acid changes that lead to altered enzymatic activity (Nalbantoglu et al., 1983; Nussbaum et al., 1983; Tindall et al., 1984). This approach, however, is limited to the study of mutants that produce significant amounts of enzymatically active proteins. Examination of mutational changes at the DNA level is not subject to these limitations. Southern blotting techniques allow for analysis of changes in DNA at this locus (Southern, 1975). There is evidence that a very high proportion of HPRT mutations, involve gene alterations, which are not detectable by cytogenetic methods (Yang et al., 1984). The major gene alterations include deletions and DNA rearrangements based on Southern Blot analysis. These alterations are detected in a large number of patients with Lesch-Nyhan syndrome (Nussbaum et al., 1983; Yang et al., 1984).

A major limitation on the effective characterization of these molecular interactions has been the requirement that carcinogens/mutagens must be detectable after adduct formation. The limited availability of certain radioactive chemicals has restricted the scope of carcinogen-DNA binding studies. Determination of DNA adducts by the ^{32}P -postlabeling assay method,

developed by Randerath et al., (1981), permits the detection of carcinogen-DNA adducts using non-radioactive test chemicals, rendering the quantification of DNA adducts more applicable to a much larger number of chemicals.

The objective of the investigations reported here was to relate the process of DNA alteration to mutation frequency and enzyme activity at the HPRT locus in cultured human cells, both for a chemical mutagen [benzo(a)pyrene] and for ionizing radiation (gamma rays). DNA-B(a)P adduct formation was quantitatively compared to mutation frequency in treated human cells by means of ³²P-postlabeling techniques. Southern Blot analysis was used to evaluate the relationship between structural changes in the HPRT gene and modification of HPRT enzyme activity for spontaneous mutants and mutants recovered after gamma ray treatment. Further objectives of this study were to document microsomal activation of B(a)P and to evaluate the efficiency of various restriction enzymes in Southern Blot analyses for detecting DNA rearrangements.

MATERIALS AND METHODS

Cell Cultures and Media

Human fibroblast lines used in chemical mutagenesis and adduct formation experiments were derived from neonatal foreskins. A chemically-transformed human cell line (HT) used in gamma-ray-induced mutation experiments was kindly supplied by Dr. T. Kakunaga. All cells were grown in 85 vol. of Ham's F10 medium (without hypoxanthine) and 15 vol. of fetal bovine serum (FBS) with 100 units penicillin per ml and 0.1 mg kanomycin per ml at 37°C in a 5% CO₂ atmosphere. 6-Thioguanine selective medium (FBS-F10-TG) consisted of FBS-F10 and 7.5×10^{-5} M 6TG.

Mutation Induction

Mutagenesis was accomplished by two methods, chemical induction using benzo(a)pyrene (B[a]P) and gamma-ray induction. Human fibroblasts were treated for 4 hours or 16 hours with B(a)P at concentrations ranging from 0 - 24 ug/ml, using S-9 activation (as described below). Gamma ray exposures were performed using a ¹³⁷Cesium dual source providing an unattenuated dose rate of 21 Gy/min. (2100 rad/min.). During each exposure, the cumulative delivered dose. Cell monolayers in plastic flasks were placed on a 12 rpm turntable, with the monitor probe centered above, during the periods of exposure.

Metabolic Activation

The S-9 fraction was prepared using 8 to 10-week old rats (Sprague-Dawley, Madison, WI) primed with Aroclor 1254. Each 10 g of liver was homogenized in 20 ml of 0.25 M sucrose solution and centrifuged at 9000 x g. The supernatant fraction (S-9) was collected and stored at -70°C. S-9 batches were prescreened for toxicity, with the least toxic batches being used in the experiments. For treatments, S-9 was used at a concentration of 0.5%, which exhibited an average cell mortality rate of 60% in method control experiments. The cofactors used

caused no detectible cell mortality at experimental concentrations (G6P, 2.3 mg/ml; TPN, 1.15 mg/ml).

Estimation of Mutation Frequency

After treatment with B(a)P or gamma irradiation, each flask of cells was cultured in FBS-F10 medium for 7 days with 2 passages. After a 7 day expression, cells were plated in dishes to detect mutants and to estimate cloning efficiency. For estimation of mutation frequency, 10^4 cells were plated per 60-mm dish; 120 dishes were prepared for each dose. All the dishes received 6TG at a final concentration of $7.5 \times 10^{-5}M$ within 3 h. The selective was renewed once every week. The selective medium was renewed once every week. For determination of cloning efficiency, 100 cells per 60-mm dish were plated and grown in FBS-F10 medium. Dishes for estimation of cloning efficiency were stained at the end of the second week and mutagenesis dishes at the end of the third week. Mutant colonies were counted and the mutation frequencies were determined according to the Poisson expectation (Albertini and DeMars, 1973; Maher and Wessel, 1975; Jacobs and DeMars, 1977, 1978; Huang and Lieberman, 1978).

Individual mutant lines were isolated with sterile cotton swabs, grown in selective medium, and frozen in liquid nitrogen for use in subsequent experiments.

Isolation of DNA

Human cells in 175 cm² flasks were harvested, washed, suspended in 10 mM Tris.HCl pH 7.6, 5 mM MgCl₂, 0.32 M sucrose, 1% Triton X-100 and homogenized gently. Nuclei were recovered after centrifugation at 4000 rpm for 20 minutes and suspended in 75 mM NaCl, 25 mM EDTA pH 8, adjusted to 1% SDS. The solution was extracted with an equal volume of phenol, dissolved in 2 ml of 10 mM Tris.HCl pH 8.0, 1 mM EDTA, adjusted to 2X SSC (SSC is 0.15 M NaCl, 0.015 M Na

citrate) and incubated at 37°C for 1 h in the presence of 0.05 mg/ml ribonuclease. DNA was precipitated with ethanol, washed, dried and dissolved at about 1 mg/ml in sterile TE, and stored at -20°C.

Identification and Quantification of Non-radioactive Aromatic Carcinogen-DNA Adducts

Determination and quantification of carcinogen-DNA adducts was conducted using the ^{32}P -postlabeling assay method developed by Randerath et al. (1981). Control and carcinogen-modified DNA were enzymatically digested to deoxyribonucleoside 3'-monophosphates, which were then converted to [5'- ^{32}P]phosphate transfer from [γ - ^{32}P]ATP. The ^{32}P -labeled nucleotides were resolved by anion-exchange thin-layer chromatography on polyethyleniminecellulose and detected by autoradiography.

Southern Blotting

Ten μg of DNA was digested in 50 μl of a buffer specified by the supplier with 40 units of restriction endonuclease at 37°C for 6 to 18 h. Three different restriction endonucleases were used and compared: Hind III, EcoRI and MspI. The DNA samples were separated by electrophoresis in 0.8% agarose in Trisborate-EDTA buffer (108 g Tris base, 55 g boric acid, 0.3 g EDTA per liter adjusted to pH 8.3) for at least 12 h at 1 V/cm. The slab was then photographed, equilibrated in 0.25 N HCl, then in 0.5 M NaOH, 1.5 M NaCl and in 0.5 M Tris.HCl pH 7.5, 3 M NaCl before overnight blotting onto a nitrocellulose sheet in 20X SSC solution.

The nitrocellulose sheet was then manipulated after baking at 80°C for 2 h. A plasmid pHPT30 generously provided by Professor C.T. Caskey (Baylor College of Medicine, Houston, Texas) was cleaved with PstI to release a 0.9 Kb fragment, 0.2 μg of which was nick-translated in the presence of 65 μCi of (^{32}P)dCTP to a level of 10^9 c.p.m./ μg DNA. The nitrocellulose sheet was

immersed at 42°C for 24 h in 10 ml of a 50% formamide solution containing 75 mM NaCl, 50 mM sodium phosphate pH 7, 4.5 mM EDTA, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.1% SDS and 0.1 mg/ml sonicated denatured salmon sperm DNA. It was then transferred to a second 50% formamide solution containing 20% Dextran sulphate, 10^6 c.p.m./ml of labelled DNA and 45 mM NaCl, 30 mM sodium phosphate pH 7.4, 3 mM EDTA, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 0.1% SDS and 0.05 mg/ml of sonicated denatured salmon sperm DNA and incubated at 42°C for 24 h. It was washed at 60-65°C in 1 x or 0.2 x SSC and autoradiographed in the presence of an intensifying screen for 4-5 days at -70°C.

Cell Lysis and HPRT Activity

A near confluent monolayer of cells on a 10-cm dish was washed free of medium and lysed in 0.3 ml of 10 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$, 3 mM KCl, 1 mM dithiothreitol, 0.5% Triton X-100 and stored at -70°C. HPRT activity was measured by the method of Huang and Lieberman (1978). Here, 12 μ l enzyme extract was mixed with 4 μ l [^3H] hypoxanthine ($2 \times 10^{-4}M$; 6.2 Ci/mmol), and 6 μ l of a mixture of phosphoribosylpyrophosphate ($10^{-4}M$; $MgCl_2$ ($5 \times 10^{-3}M$) and tris-HCl buffer (0.5 M, pH 7.4). After incubation at 37°C for 10 or 20 min., 5 μ l aliquots were mixed with 2 μ l of 4 N formic acid and spotted on a MN 300 cellulose thin-layer plate (Analtech) and cochromatographed with 3 μ l of unlabeled hypoxanthine, inosine and inosinic acid solution ($10^{-2}M$ each) using 5% Na_2HPO_4 as the solvent. The spots were visualized with a UV light and cut from TLC plate individually. The radioactivity was determined in Aquasol using a liquid scintillation counter. The HPRT activity of the parental cells was determined to be 100 nmole/mg of protein/hr.

Protein was assayed with a Bio-Rad protein assay kit and based on a γ -globulin standard; lysates generally contained 1 mg protein/ml.

RESULTS

Mutagenic Activity of Benzo(a)pyrene

The mutagenic activity of B(a)P in cultured human cells with S-9 activation is demonstrated in Table 1. In contrast to previous experiments with similar mutagens (Huang et al., 1982; Huang and Waters, 1983), dose-dependent mutation responses did not consistently appear (Friedman test, $p > 0.15$). However, the mutagenic activity of B(a)P was clearly demonstrated (Wilcoxon test, $p < 0.05$). Induced mutation frequency increased monotonically with dose in the 16 h exposure. The correlation between mutation frequency and concentration of B(a)P was low, but exposures of 16 h consistently produced higher mutation frequencies than those of 4 h. The relatively high variability in background mutation frequencies in these experiments probably resulted from the fact that 4 h and 16 h treatments were conducted at different times.

Mutation Induction by Gamma Radiation

Survival and mutation frequencies in human cells exposed to a single dose of gamma radiation are presented in Table 2. Gamma radiation at 330 rad induced approximately a nine-fold increase over background mutation frequencies. Gamma ray-induced mutant cells were isolated and used in subsequent experiments.

B(a)P-DNA Adduct Formation

To relate mutation frequency to B(a)P adduct formation, the ^{32}P -postlabeling technique (Randerath et al., 1981) was used to detect and quantify B(a)P-DNA adducts in human cells. As indicated in Figure 2, control DNA produced no adducts, whereas DNA from B(a)P-treated cells yielded significant amounts of adducts. Adduct production rates in treated cells ranged from 0.24×10^{-6} adducts/base at an exposure of 4 h to 3 $\mu\text{g/ml}$ B(a)P, to 9.23×10^{-6} adducts/base at an exposure of 16 h to 24 $\mu\text{g/ml}$ B(a)P (Figure 2).

When the numbers of B(a)P adducts per nucleotide were compared to calculated mutation frequencies from the same treatments, a highly significant correlation resulted. Figure 3 shows the linear regression analysis of this relationship ($\ln Y = 1.71 + 0.48 \ln(X)$, where Y is the induced mutation frequency and X is the DNA-adduct formation rate). Statistical analysis on the computed slope of 0.48 ± 0.06 showed that this slope is significantly different from zero at the 0.1% level.

Southern Blot Analysis of Alterations at HPRT Locus

Cells from 15 spontaneous and 21 gamma-ray-induced mutant lines were analyzed for alterations in the HPRT locus. When the restriction enzyme Hind III was used, heterogeneous changes in Southern Blot patterns were identified in 6 of 15 spontaneous mutants, and in 11 of 21 gamma-ray-induced mutants (Table 3). The increase in the number of detectable changes in restriction fragment patterns of mutants from treated cultures is not statistically significant ($\chi^2 = 0.538$, $df = 1$). The restriction patterns observed, both within and between the spontaneous and treated mutants, were not identical. Pseudogenes residing on the autosomes showed no change, whereas the changes observed in mutant clones included a variety of deletions and other intragenic alterations (Figure 4).

Other restriction enzymes tested were not as efficient in detecting intragenic alterations as Hind III (Table 4). Although both EcoRI and MspI were able to detect all three gene deletions detected by Hind III, neither could detect all three DNA rearrangements. DNA alterations, not detected by Hind III, were not detected by any other restriction enzyme.

HPRT Enzyme Activity

DNA rearrangements of various types were expected to affect production of HPRT enzymes (Yang et al., 1984). The HPRT activity of parental cells was

determined to be 100 mmole/mg of protein/h. Mutant cell lines ranged in activity from 0% to 20% of the parental line. Generally, mutants with DNA rearrangements or deletions exhibited no HPRT activity, while those having normal DNA restriction fragment patterns displayed small amounts of HPRT activity (Table 5.).

DISCUSSION

The interactions between environmental mutagens/carcinogens and target macromolecules, such as DNA, and the relationships between adduct formation and other DNA alterations and mutagenesis/carcinogenesis are not well understood. This study was conducted to clarify the effects of alterations at the HPRT locus generated by a chemical mutagen (benzo(a)pyrene) and ionizing radiation (gamma rays) on mutation frequency.

A major finding of this investigation was the strong correlation between the frequency of mutations induced by B(a)P and the formation of DNA-B(a)P adducts. This result indicates that adduct formation is directly linked to the mutation process for this particular mutagen in our particular cell system.

This investigation also demonstrated the effectiveness of metabolic activation by S-9 microsomes from rat livers in our cell system. Metabolic activation is necessary to obtain dose-dependent mutation responses in bioassays of many mutagenic or carcinogenic substances (Miller, 1970; Heidelberger, 1975; Aust et al., 1980; Michalopoulos et al., 1981; Huang and Waters, 1983). The departure from a direct, dose-dependent response observed here at the highest concentration tested suggests that more complex relationships occur, and that better prediction of mutagenic activity could be accomplished if bioactive metabolites were used instead of an exogenous activation system (Strom and Michalopoulos, 1982; Arce and Grunberger, 1983). The pertinent metabolites for B(a)P are probably diol epoxides (Newbold et al., 1977; Brookes et al., 1982). Another, failure to obtain simple dose-dependent response curves was reported previously for B(a)P (Strom and Michalopoulos, 1982).

The second major finding of this study was that mutant cell lines with DNA rearrangements or deletions generated by gamma irradiation showed either an

absence of or greatly decreased HPRT enzyme activity. This result suggests that such DNA alterations are directly responsible for lost enzyme activity and are a specific component of the mutation process. The frequency of occurrence of various types of alterations (deletions, rearrangements) was found to be similar among spontaneous mutants and gamma-ray-induced mutants. This similarity contrasts with previous studies of radiation-generated mutants at the adenine phosphoribosyl transferase (APRT) locus in Chinese hamster ovary cells (Grososky et al., 1986), so genic size may contribute to the observed differences in response to radiation. In this study, many mutants with reduced enzymatic activity were isolated which could not be ascribed to a particular type of DNA alteration. Such mutants probably reflect base substitutions and other localized genomic alterations that are not detectible under the conditions of the Southern Blot analysis with the restriction enzymes used.

The investigations reported here demonstrated the advantages of the Southern Blot analysis, and the relative performance of three different restriction enzymes at detecting genomic alterations. Among all restriction enzymes tested, Hind III was clearly more efficient for at detecting DNA alterations; Hind III provided for the recognition of all alterations detected by any of the enzymes. Obviously, different restriction enzymes cleave target DNA at different points. DNA alterations that occur on a scale too fine to be detected cannot be evaluated by this technique. Hind III provided the highest detection efficiency among those restriction enzymes tested.

CONCLUSIONS

Benzo(a)pyrene was demonstrated to be an effective mutagenic agent in cultured human cells when it was subjected to metabolic activation by S-9 rat liver microsomes. Although dose-dependent response curves were nonlinear, a very strong direct relationship was demonstrated between mutation frequency at the HPRT locus and the formation of B(a)P-DNA adducts.

Southern Blot analysis demonstrated that mutant cell lines often contained major chromosome alterations at the HPRT locus, either deletions or rearrangements. Among three restriction endonucleases evaluated, Hind III was most efficient at detecting DNA alterations. Spontaneous mutants and gamma-ray-induced mutants both displayed similar proportions of DNA alterations of various kinds, suggesting that mutation events are approximately random at this locus. All mutant cell lines which are determined to have major DNA alterations had not or very low HPRT enzyme activity. Those mutant lines which could not be demonstrated to have substantial rearrangements or deletions had low, but measureable, enzyme activity.

These investigations show clearly that environmental mutagenesis at the HPRT locus in cultured human cells is mediated by modifications of the genome, either by adduct formation (B[a]P) or by base-sequence alteration (gamma-radiation).

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TABLE 1

CALCULATION OF INDUCED MUTATION FREQUENCIES AFTER TREATMENT WITH B(a)P

| Period of treatment (h) | Concn. (ug/ml) | Cloning effic. (%) | P ₀ | Number of mutants per dish | Number of viable cells per dish | Mutation freq. (X 10 ⁻⁶) | Induced mutation frequency (X 10 ⁻⁶) |
|-------------------------|----------------|--------------------|----------------|----------------------------|---------------------------------|--------------------------------------|--|
| 4 | 0 | 38.5 | .966 | .035 | 5780 | 6.06 | |
| | 3 | 42.8 | .942 | .060 | 6420 | 9.35 | 3.29 |
| | 6 | 41.5 | .939 | .063 | 6225 | 10.12 | 4.06 |
| | 12 | 33.3 | .926 | .077 | 5000 | 15.40 | 9.34 |
| | 24 | 46.0 | .924 | .079 | 6900 | 11.40 | 5.34 |
| 16 | 0 | 48.3 | .858 | .153 | 7241 | 21.13 | |
| | 3 | 34.5 | .875 | .134 | 5180 | 25.87 | 4.74 |
| | 6 | 34.7 | .833 | .183 | 5201 | 35.19 | 14.06 |
| | 12 | 20.3 | .897 | .108 | 3041 | 35.61 | 14.48 |
| | 24 | 21.6 | .868 | .141 | 3240 | 43.31 | 22.18 |

The average number of mutant colonies (X) per dish was obtained according to $P_0 = e^{-X}$, where P_0 was the fraction of dishes bearing no mutants. Multiplication of the initial number of cells (1.5×10^4) per dish by the cloning efficiency of the cells gave the number of surviving cells per dish. Mutation frequencies were calculated by dividing X by the number of surviving cells per dish. Induced mutation frequencies were obtained by subtracting the background mutation frequency from the calculated mutation frequencies.

TABLE 2

GAMMA RAY INDUCED MUTATION IN HUMAN CELLS

| <u>DOSE (RAD)</u> | <u>RELATIVE SURVIVAL</u> | <u>MUTATION FREQUENCY</u> |
|-------------------|--------------------------|---------------------------|
| 0 | 1.00 | 3.56×10^{-6} |
| 330 | 0.58 | 27.94×10^{-6} |

Gamma ray induced mutants, used to determine molecular changes in the HPRT gene, were isolated from mutation experiments performed with a chemically transformed cell line (HT: kindly provided by Dr. Takeo Kakunaga). Gamma ray exposures were performed using a $^{137}\text{Cesium}$ dual source providing an unattenuated dose rate of 21 Gy/min. (2100 rad/min.). During each exposure, a gamma-ray monitor (MDH Industries, series 2025) was employed to measure the cumulative delivered dose. Cell monolayers in plastic flasks were placed on a 12 rpm turntable, with the monitor probe centered above, during the periods of exposure. Following irradiation, cells were cultured in FBS-F10 medium for 7 days with 2 passages. After expression, 10^4 cells were plated per 60 mm dish and medium containing 6-Thioguanine ($7 \times 10^{-5}\text{M}$) added to the cultures to select HPRT mutant cells.

TABLE 3

ALTERATIONS IN THE HPRT GENE DETECTED BY SOUTHERN BLOT ANALYSIS DIGESTED WITH HIND III

| <u>ALTERATION</u> | <u>MUTANT TYPE</u> | |
|-------------------|--------------------|-------------------|
| | Spontaneous | Gamma-Ray Induced |
| DNA rearrangement | 2 ^a | 3 |
| Gene deletion | 4 | 8 |
| Not detectable | 9 | 10 |

^aNumber of mutant lines positive for alterations. The increase in the number of detectable changes in restriction fragment pattern of induced mutants over that of spontaneous is not statistically significant ($\chi^2=0.538$, $df=1$). However, it was observed that the restriction pattern, both within and between the spontaneous and induced mutants, were not identical. This indicates that the lesion in the HPRT gene takes place in a random fashion.

TABLE 4

SOUTHERN BLOT ANALYSIS OF SPONTANEOUS(S) AND GAMMA-RAY INDUCED(I) HPRT- MUTANTS

| Clone | S or I | Enzyme for Detecting Alteration | | | Alteration |
|-------|--------|---------------------------------|--------------|-------------|----------------------|
| | | <u>Hind III</u> | <u>EcoRI</u> | <u>MspI</u> | |
| 19 | I | + | + | | DNA
rearrangement |
| 20 | I | + | | + | |
| 26 | S | + | + | + | |
| 21 | I | + | + | + | Gene
deletion |
| 22 | S | + | + | + | |
| 28 | I | + | + | + | |
| 14 | I | | | | Not
detectable |
| 25 | I | | | | |
| 30 | I | | | | |

Nine mutants, representing different classes of intragenic alteration, were examined by Southern Blotting techniques.

TABLE 5

RESTRICTION FRAGMENT PATTERN, HPRT ACTIVITY OF SPONTANEOUS(S) AND GAMMA-RAY INDUCED(I) HPRT⁻ MUTANTS

| Alteration | Clone | S or I | HPRT Activity
as % of Wild Type |
|----------------------|-------|--------|------------------------------------|
| DNA
rearrangement | 11 | S | 0 |
| | 19 | I | 0 |
| | 20 | I | 4.6 |
| | 26 | S | 0 |
| Gene
deletion | 13 | I | 0 |
| | 21 | I | 0 |
| | 23 | I | 0 |
| | 28 | I | 0 |
| | 43 | S | 0 |
| | 44 | S | 0 |
| Not
detectable | 24 | I | 0 |
| | 25 | I | 0 |
| | 27 | S | 5.5 |
| | 41 | S | 1.5 |
| | 42 | I | 19.6 |
| | 45 | S | 9.2 |
| | 46 | I | 4.6 |
| | 47 | S | 9.3 |
| | 48 | S | 1.0 |

For HPRT assays, 12 μ l enzyme extract was mixed with 4 μ l [³H] hypoxanthine (2×10^{-4} M; 6.2 Ci/mmol), 6 μ l of a mixture of phosphoribosylpyrophosphate (10^{-2} M), MgCl₂ (5×10^{-3} M) and tris-HCl buffer (0.5M, PH7.4). After incubation at 37°C for 10 or 20 min., 5 μ l aliquots were mixed with 2 μ l of 4N formic acid and spotted on a MN 300 cellulose thin-layer plate (Analtech) and cochromatographed with 3 μ l of unlabeled hypoxanthine, inosine and inosinic acid solution (10^{-2} M each) using 5% Na₂HPO₄ as the solvent. The spots were visualized with a UV light and cut from TLC plate individually and the radioactivity was determined in Aquasol using a liquid scintillation counter. The HPRT activity of the parental cells was determined to be 100 nmole/mg of protein/hr. Generally, mutants with DNA rearrangements or gene deletions exhibited no HPRT activity, while those having normal DNA restriction patterns exhibited reduced HPRT activity.

LEGENDS TO FIGURES

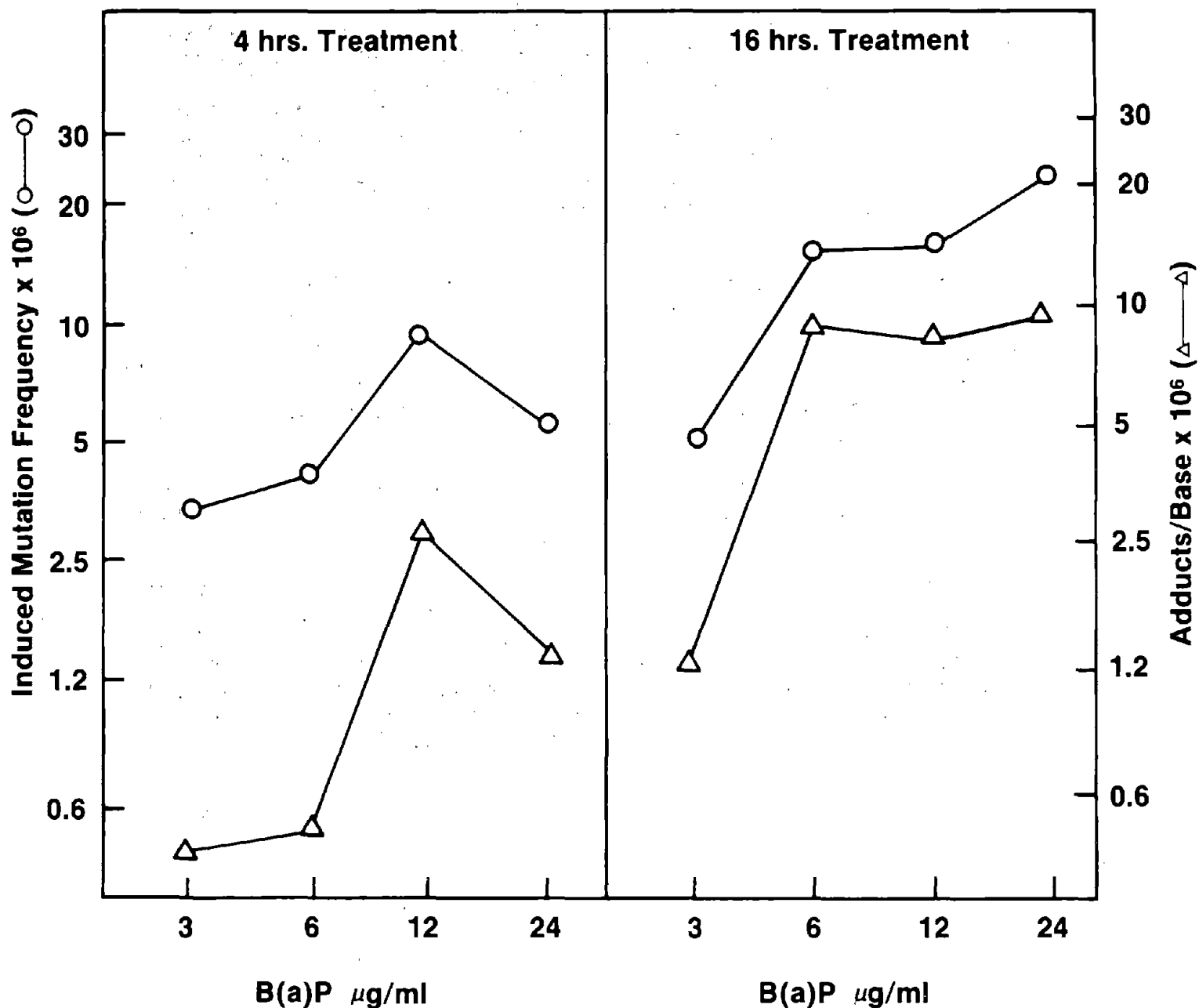
Fig. 1 Relationship between concentration of B(a)P induced mutation frequencies and DNA adducts.

Fig. 2 Fingerprints of digests of human DNA preparations from fibroblast cells treated with B(a)P for a period of 16 h. DNA was digested to dNp's which were subjected to ^{32}P -labeling and mapping by 4-directional (d) PEI-cellulose t.l.c. About 39-46 uCi of labeled digest was chromatographed. Development was in 1 M LiCl (D1) and 2.5 M ammonium formate, pH 3.5 (D2), resulting in the removal of normal nucleotides and $^{32}\text{P}_i$, while the adducts were retained at or close to origin. [^{32}P]adducts were then resolved by development in 3 M lithium formate, 7 M urea, pH 3.5 (D3), followed by 0.6 M LiCl, 0.5 M Tris-HCl, 7 M urea, pH 8.0 (D4). Screen-enhanced autoradiography was at -80°C for 16-24 h. Control DNA (top) produced no B(a)P-DNA adducts.

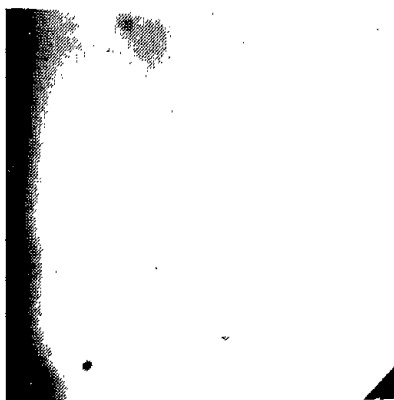
Fig. 3 Relationship between B(a)P induced mutation frequencies and DNA adducts. When the mutation frequencies were plotted, the mutagenic activity was shown to be highly correlated with the number of B(a)P adducts per nucleotide. The straight line fit for the induced mutation frequencies versus DNA-adducts is: $\ln(Y) = 1.71 + 0.48 \ln(X)$; where Y is induced mutation frequency and X is DNA-adducts. The data set gives an estimated slope of 0.48 ± 0.06 . The slope is significantly different from zero at the 0.1% level.

Fig. 4 Representative Southern Blot patterns of DNA from normal and mutant cells. Frame 1, Hind III digests; frame 2, EcoRI digests; frame 3, MspI digests. 10 μg of human DNA was used for restriction fragment analysis. Electrophoresis was at 40V for 16 hr in 0.8% agarose. Standard size DNA fragments are shown on the left in kilo base (Kb) units. Mutant lines (14,20,30,19,25,26,21,22,28) and the parental line (HT) are identified across the top of each frame.

RELATIONSHIP BETWEEN CONCENTRATION OF B(a)P, INDUCED MUTATION FREQUENCIES, AND DNA ADDUCTS



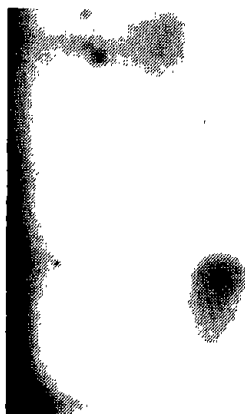
B(a)P-DNA ADDUCTS



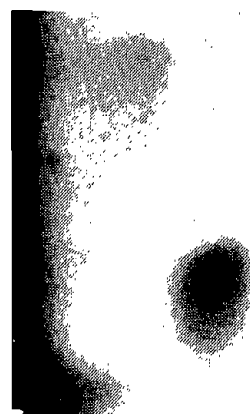
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1.5 µg/ml



3 µg/ml



6 µg/ml

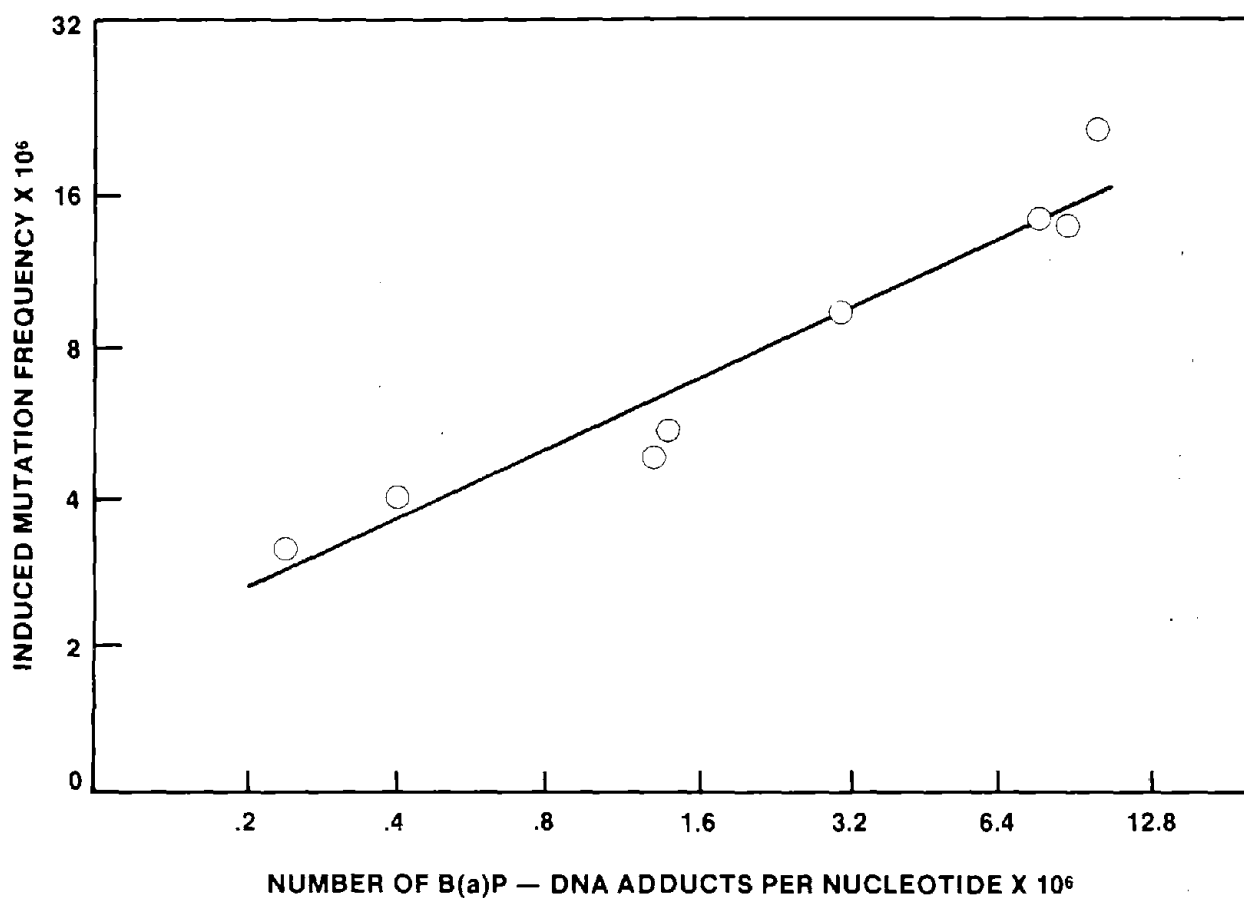


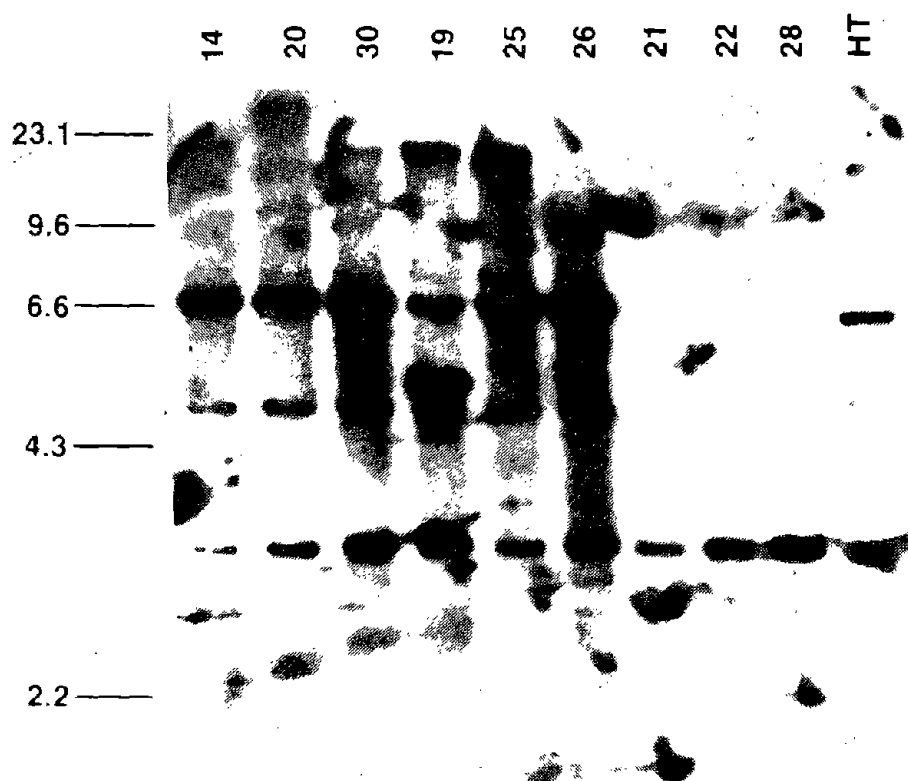
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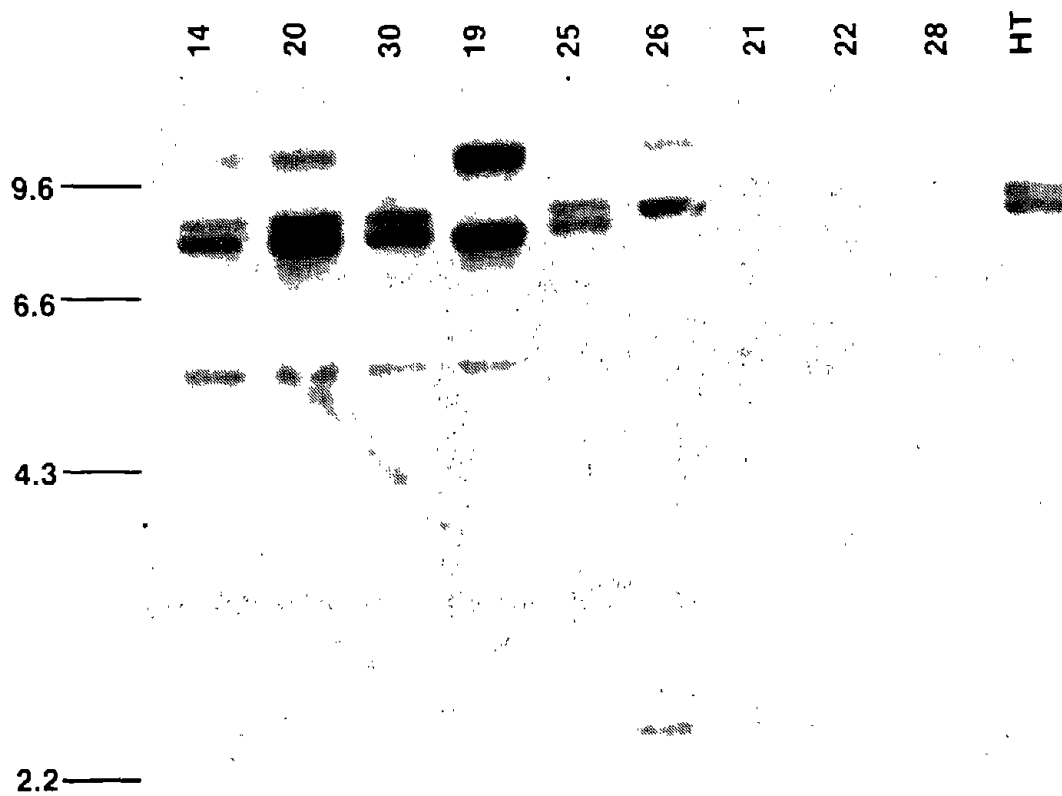
24 µg/ml

RELATIONSHIP BETWEEN B(a)P INDUCED MUTATION FREQUENCIES AND DNA ADDUCTS

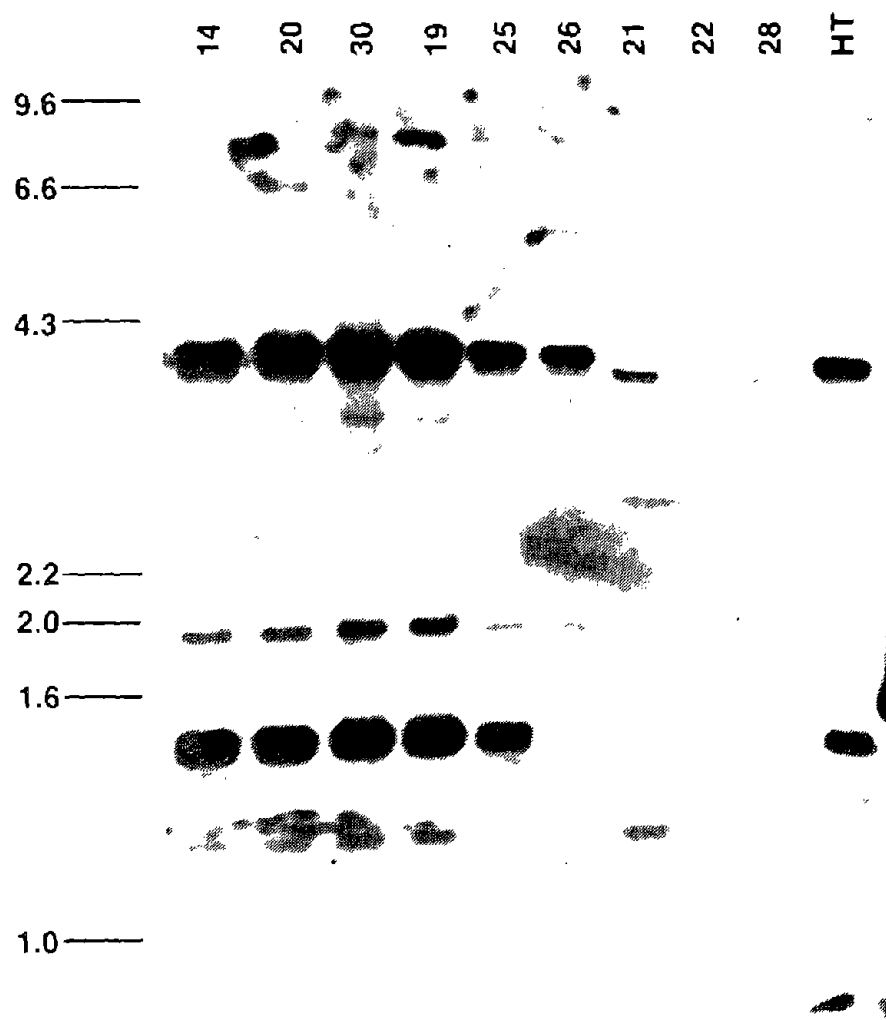




FRAME 1



FRAME 2



FRAME 3

APPENDIX B
PARTICIPANT DIRECTORY

**PROCEEDINGS OF THE
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PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES**

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