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

MAY 6-8, 1980

SHERATON/POTOMAC, ROCKVILLE, MARYLAND
The papers included in these Proceedings were printed as they were submitted to this office.

Appropriate portions of the discussions, working groups and plenary session were sent to the participants for editing. The style of editing varied, as could be expected. To the extent possible, we have attempted to arrive at a consistent format.
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Proceedings were developed from a workshop on the National Cancer Institute's, the Environmental Protection Agency's and the National Institute for Occupational Safety and Health's Collaborative Programs on Environmental and Occupational Carcinogenesis.
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Director
National Cancer Institute

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Deputy Assistant Administrator
Office of Health Research
Environmental Protection Agency

Anthony Robbins, M. D.
Director
National Institute for Occupational Safety and Health

OVERVIEW

H. F. Kraybill, Ph. D.
Scientific Coordinator for Environmental Cancer
National Cancer Institute
INTRODUCTORY REMARKS
Vincent T. DeVita, M. D.
Director
National Cancer Institute
Bethesda, Maryland

At the National Cancer Institute we are interested in knowing which chemicals exist in the environment, how they interact to cause cancer, and how we can take advantage of that knowledge to reduce the incidence of cancer. I am an optimist. I think that in our lifetime we are going to be able to make a significant impact on the reduction in the incidence and mortality from cancer. That is not because we expect to live an inordinate time but rather because we have reached a critical mass, in terms of opportunities for prevention. This accounts for the ferment, the controversy and the excitement in the field.

From the point of view of a person like myself, who grew up in the treatment area, I recall the same kinds of sensations and ferment going on as new advances, like the development of anticancer drugs for treatment of cancer, proved to be successful.

There are opportunities in at least three major areas for the prevention of cancer. One is the prevention of cancer causing chemicals from entering the environment. Our testing program to identify potential carcinogens is one of those in a great state of ferment. Investigators are now discovering the variables in the in vitro tests, like the Ames assay, which we thought were simple tests when they were first reported. For example, everyone's S-9 preparation is different from everyone else's. Still there are many opportunities in this area to exploit.

The second area is to reduce or eliminate chemicals in the environment that might cause cancer. This meeting will devote much of its attention to the epidemiology of identifying cancer causing agents. Our contribution in this area, represented by Dr. Fraumeni and his staff, is very great. Considerable opportunity exists for us to interact with NIOSH and EPA, because of EPA's capabilities to analyze the environment and NIOSH's authority in industrial settings, particularly in accessing records not normally available to us. These are examples of perfect collaborative opportunities.

The third area for the prevention of cancer involves lifestyle changes. This is the most controversial area. I could do very well at this meeting if I said that most cancers are caused by exposure to chemicals and then, before another group with a different orientation, to say most are caused by lifestyle. Without trying to be a great compromizer, I think the answer is obviously both chemicals and lifestyle. It is best not to think that a certain fraction is caused by lifestyle and a certain fraction is caused by chemicals, but rather to think that a certain fraction is caused by both. We are just beginning to learn about the multiplicity of cancer causation. Although the complexity might be discouraging, it has an encouraging side, because we may not need to get rid of all influences to prevent cancer. It may be quite possible to remove one influence, an initiator, for example, and then a lifestyle promoter may become unimportant, or vice versa. Although arguments about lifestyle versus chemicals have created a lot of tension, this is a healthy phenomenon.
As you may know, the National Cancer Institute is awaiting approval of a new division. We hope it will devote much of its activities to what we call applied prevention, using knowledge that may be generated from the kind of interactions that will be described here in the next two or three days, to approach the problem of application of knowledge to reduce the incidence of cancer. I am sure you will hear more about this activity.

I will conclude by showing a chart illustrating the area of carcinogen identification and regulation. I was reading a memo describing the process and the only thing I could do to save myself was to put it in the form of a chart. It illustrates the complexity of the problem. All of us are represented on this chart. Across the top is the process gone through in terms of identifying and regulating chemical carcinogens. Along the side are the committees and organizations that have an input into the process. It is a very complex process. We have a lot to do to make the complexity work a little bit better.

I am delighted to have the opportunity to meet those of you who work in this area and whom I have not had the chance to meet before. I hope we have a chance to do this on numerous occasions.
SCHEMATIC: BASED ON "REGULATION OF CHEMICAL CARCINOGENS"

THE REGULATORY COUNCIL
SEPTEMBER 28, 1979

OSHA
THE REGULATORY COUNCIL
SEPTEMBER 28, 1979

CPSC
EPA
USDA

REGULATORY ACTIVITY SEQUENCE

1. OFFICE OF MANAGEMENT AND BUDGET (OMB)
   - BUDGET PREPARATION AND LEGISLATIVE CLEARANCE
   - GUIDELINES ON ANALYTICAL REGULATORY DEPENDENCY

2. TOXIC SUBSTANCES STRATEGY CMTE. (TSSC)
   - COORDINATING FEDERAL RESEARCH AND REGULATORY PROGRAMS ON TOXIC CHEMICALS
   - ELIMINATING OVERLAPS AND FILLING GAPS IN DATA ON TOXIC CHEMICALS

3. REGULATORY COUNCIL
   - COORDINATES ACTIVITIES OF REGULATORY AGENCIES
   - COORDINATES GOVERNMENT INTERVENTION
   - ANALYZES COSTS OF REGULATORY ACTION

4. CPSC TO OMB: TOXICOLOGY ETC. (TOXIC ENVIRONMENTAL)
   - ADVISES OMB ON TOXICOLOGICAL ACTIVITIES AND COORDINATES DATA AND RESOURCES IN OMB

5. NATIONAL TOXICOLOGY PROGRAM (NTP)
   - TEST CHEMICALS, DEVELOPS TEST SYSTEMS, SUPPORTS RESEARCH AND NEEDS OF REGULATORY AGENCIES
   - MAY BE INVOLVED IN RISK ASSESS.

6. NATIONAL CANCER ADVISORY BOARD (NCAB)
   - COORDINATION OF CANCER RESEARCH INSIDE AND OUTSIDE GOVERNMENT

7. INTERAGENCY REGULATORY LIAISON GROUP (IRLG)
   - INTERAGENCY REGULATORY COORDINATION
   - CPSC
   - EPA
   - FDA
   - OSHA
   - USDA

8. RESEARCH PLANNING WORK GROUP
   - PLANNING & ADVICE ON TEST METHODS AND PRIORITIES
   - EVALUATION OF NCI TEST RESULTS AND RISK ASSESSMENTS

9. TESTING STDS & GUIDELINES WORK GROUP
   - DEVELOP UNIFORM PROCEDURES AND CRITERIA FOR RISK ASSESSMENT
   - PRIME COORD. OF RISK ASSESS. GROUP, OMB

10. SYNDROMOLOGY WORK GROUP
    - DEFINITION OF RISK CHARACTERS OF EPIDEMIC STUDIES

11. RISK ASSESSMENT WORK GROUP
    - DETERMINING WHETHER A CHEMICAL MAY CAUSE CANCER

12. INFORMATION EXCHANGE WORK GROUP
    - INFORMATION EXCHANGE WITH FEDERAL REGULATORY AND RESEARCH AGENCY STAFF

13. COMPLIANCE & ENFORCEMENT WORK GROUP
    - EVALUATION OF REPORTED VIOLATIONS

14. NATIONAL CLEARINGHOUSE FOR ENV. CANCER
    - RECOMMEND AND COORDINATE NORMAL PROGRAMS FOR PREVENTION/REDUCTION OF ENVIRONMENTAL CANCER, HEART AND LUNG DISEASE

15. INTERAGENCY TESTING CMTE.
    - ADVISORY TO THE PRESIDENT

16. OFFICE OF SCIENCE & TECHNOLOGY POLICY
    - COORDINATION THROUGH COMMON DATA UTILIZATION

17. CENTER FOR HEALTH STATISTICS
    - COORDINATES INFORMATION AND DATA SYSTEMS

18. NAT. TOXIC SUBSTANCE DATA OFFICE
    - REVIEW OF VOLLUCTION MONITORING

19. COUNCIL ON ENV. DUAL USE TASK FORCE
    - RECOMMEND AND COORDINATE NORMAL PROGRAMS FOR PREVENTION/REDUCTION OF ENVIRONMENTAL CANCER, HEART AND LUNG DISEASE

20. TASK FORCE ON ENVIRONMENTAL CANCER, HEART AND LUNG DISEASE
INTRODUCTORY REMARKS

Vilma Hunt, B.D.S.
Deputy Assistant Administrator
Office of Health Research
Environmental Protection Agency

Since 1978 the relationship between the National Cancer Institute and the Environmental Protection Agency on this particular program of environmental carcinogenesis has included the Office of Pesticides and Toxic Substances and the Office of Research and Development. The interactions have been quite close during that period. There has been a gradual development of the relationship to the extent that we now have ongoing the projects that you will be hearing about over the next several days.

One thought I had in terms of Dr. DeVita's comments is almost light years away from a meeting I was at yesterday in Buffalo. I thought you would be interested in hearing the kind of presentation that was given there. It was an international conference on cancer in blacks. The first day - today is the second day and deals more with the American scene - was virtually restricted to cancer in blacks in Africa. There was a very heavy representation of speakers from medical schools or former medical schools in Africa. The intent for most of them was to present quite descriptive comments on the main clinical experiences that they had had in Africa over the years. It became quite evident that if there is ever a meeting of environment and lifestyle, that is where we can see it. The development of that meeting today, I am hoping, will move more to the American reality in which certainly the black population living in this country is somewhat different to what it is in Africa.

To get back also to Dr. DeVita's chart, we are going to offer to him some additional advice. I think the Task Force on Environmental Cancer and Heart and Lung Disease is missing. I do not see it in casting my eye rapidly over it. That particular report, which I just signed off on this morning in fact, is now going to the heads of agencies and will reach Dr. DeVita before very long. I am hoping that it will go through your office expeditiously.

It is quite important that that Task Force Annual Report, which is the third one, go through its appointed rounds to the agency heads and to the Secretary of Health and Human Services. The Task Force was mandated by Congress and the annual reports are directed to them. Again, it shows the very specific concern that Congress has developed that we do just the kind of things that Dr. DeVita mentioned earlier; that is, there are very marked concerns that the capabilities of all the agencies be brought to bear and focused on some of these areas of mutual concern rather than disparate efforts which go in their own directions. Certainly the program that we have been having on environmental carcinogenesis between EPA and NCI fulfills the expectations of that Task Force.
INTRODUCTORY REMARKS
Anthony Robbins, M.D.
Director
National Institute for Occupational Safety and Health

It is a pleasure for me to be here today at the onset of this historic collaborative workshop. The research conducted by our respective agencies has contributed much to our understanding of how factors in the working and the general environment contribute to the etiology of cancer, this knowledge ultimately contributing to the prevention of cancer. NIOSH is very pleased to be part of this cooperative effort and wishes to acknowledge the support of the National Cancer Institute, without which much of this important work would not have been possible.

The decade of the 1970's was characterized by a major concern for the problem of occupational and environmental cancer, which will surely continue in future years. This decade was also characterized in large part by the recognition that chronic exposure to chemicals posed a greater risk to the health of workers and the general population than previously realized. The decade of the 1980's has begun with the promulgation of an historic general regulation to deal with the problem of carcinogens in the workplace by OSHA, a regulation which many of the scientists in this room contributed to, in terms of research, testimony, and special analyses. It is envisioned that future collaborative efforts will continue to provide the often embattled regulatory agencies with the component scientific input necessary to formulate defensive public health policies.

If the Federal Government is to adequately deal with the problem of occupational/environmental cancer, it will only be through collaborative efforts such as our program in cancer research. This program cannot succeed without the collective strengths of our respective organizations working together. By working together, we build upon our strengths and complement our weaknesses. It is our hope that this program will also create an atmosphere of cooperation and friendship among individual scientists in our respective organizations. One of the best ways to foster such collaboration is to encourage joint program planning efforts as well as an exchange of scientists from our respective organizations; a proposal which we should consider to further strengthen our program.

As a result of this interagency program, NIOSH has initiated and/or completed over 60 research projects dealing with a broad spectrum of topics related to cancer in the workplace. Tables 1-4 illustrate the magnitude of our research program encompassing epidemiology, toxicology, and industrial hygiene studies. The results of these studies have potential applicability for protecting not only workers but members of the general population as well. This is because risks defined in working populations may also apply to the general population. At this conference, the results of nearly one-third of these projects will be summarized.

Among the highlights of our program are several rojects to be presented at this workshop including:

**Ethylene Dibromide/Disulfiram Interaction**

A carcinogenic interaction resulting from inhalation of ethylene dibromide (EDB), an industrial solvent, and oral doses of disulfiram, a drug used for treatment of alcoholism, was identified. Rats experienced inhalation exposures of 20 ppm EDB, which is the U.S. Occupational Standard,
for 5 days per week, 7 hours per day. The disulfiram dose was 0.05 percent by body weight. Significant histopathologic findings in animals receiving both treatments concurrently included: hemangiosarcomas of the liver, kidney and spleen; adenocarcinomas of the mammary gland in females, and atrophy of the entire genital tract in males. The incidence of tumors was substantially increased in animals receiving both EDB and disulfiram compared to animals receiving EDB alone or disulfiram alone. Results from this study led to the issuance by NIOSH of a Current Intelligence Bulletin (CIB) and notices in the scientific literature. This study clearly suggests the potential deleterious combination of workplace chemicals and certain drugs.

Benzidine-Based Dyes

Work performed jointly (through Interagency Agreement) with the National Center for Toxicologic Research showed that the benzidine-based azo dye, Direct Black 38, is extensively metabolized in the hamster to yield metabolites known to be carcinogenic. Hamsters given purified Direct Black 38 were shown to excrete benzidine, N-acetyl benzidine, and 4-aminobiphenyl - all known carcinogens in the urine. Another azo dye, Direct Yellow 12, was not metabolized to carcinogenic metabolites in the hamster. As part of the same study, urine from workers occupationally exposed to Direct Black 38 was also found to contain benzidine. NIOSH recently published a Special Hazard Alert (SHA) on benzidine-based dyes which included some of the results of this study. Industrial hygiene studies to determine extent-of-exposure among workers to azo dyes were also used as input to the SHA.

Workers Exposed to Polychlorinated Biphenyls

Results of a retrospective cohort mortality study showed an observed excess mortality risk of cancer of the liver and cirrhosis of the liver among exposed workers, although these observations were not confirmed by latency of exposure analyses. These data are among the first to investigate a possible increased risk of death from certain causes among individuals exposed to PCBs.

Retrospective Cohort Mortality of Workers Exposed to Talc

Two studies were published (Vermont and New York talc workers) under this project. These reports will be used as input to the Talc Criteria Document which is under preparation by NIOSH. The Vermont Study is unique in that it showed an excess nonmalignant respiratory disease mortality risk in a population exposed to nonasbestiform talc; while the New York study showed an excess mortality risk due to nonmalignant respiratory disease and bronchogenic cancer. NIOSH has also published an industrial hygiene study documenting extent-of-exposure among workers to talc.

Other highlights of our collaborative efforts which will not be presented at this specific workshop include:

Radon Daughters from Foundry Sands

As part of a larger project investigating the co-carcinogenic effects of foundry particulates, zirconium silicates are being evaluated. We have
recently discovered that commercially available zirconium silicates, which are used extensively in foundries as a replacement for silica sand, emit large quantities of radiation through release of radon daughters. This has led NIOSH to initiate follow-up field investigations of foundry workers. The U.S. EPA has been informed of our finding and is also investigating the situation.

Proportionate Mortality Study of Foundry Workers

Results of these studies already published showed a significant increase in mortality due to lung cancer and nonmalignant respiratory disease. These results are being evaluated in more detail through a case control study.

Carcinogenicity of 2-Nitropropane

Inhalation exposure (200 ppm) of rats and rabbits to 2-nitropropane (2-NP), a nitroparaffin once used widely in a variety of industrial applications, induced liver neoplasms, indicating that 2-NP is a potent carcinogen. The exposure time was 6 months, an unusually short time to demonstrate carcinogenicity in the rat. A verification study performed by the major producer of 2-NP yielded similar results as obtained by NIOSH. A Current Intelligence Bulletin (CIB) alerting the occupational health community to this finding was issued by NIOSH, and a Health Hazard Alert was also prepared by OSHA.

This study contributed to the control of a commonly used substance involving exposure to more than 100,000 workers.

Retrospective Cohort Mortality of Workers Exposed to Beryllium

Results of this study were published in Environmental Research, suggesting an excess mortality due to lung cancer among workers exposed to beryllium. This study also contributed to the overall OSHA assessment of beryllium during a public hearing on a proposed Beryllium Standard.

Retrospective Cohort Mortality of Workers Exposed to Benzene

Results of this study were used to support the OSHA promulgated standard and were published in The Lancet. This work, which represents the pivotal epidemiology study of workers occupationally exposed to benzene, showed a five-fold excessive risk of all leukemias and a ten-fold excess of deaths from myeloid and monocytic leukemias combined in the study population compared with controls.

Retrospective Cohort Mortality of Workers Exposed to Styrene-Butadiene Rubber

The results of this study suggested an excess mortality (although not statistically significant) from neoplasms of the lymphatic and hematopoietic tissues.
Our program this week includes not only an opportunity to share the results of ongoing studies, but also an opportunity to discuss new methodologies, future cooperative ventures, and how our programs can be more responsive to the needs of the regulatory agencies. To have the greatest impact, research conducted must meet certain criteria: (1) it must be of sound quality and able to meet the test of critical peer review; (2) the results must be published in refereed journals; and (3) the results must be summarized in language that is understandable to the workers and to the general population who are to be protected. In this regard, it is important that the results from this workshop be published and widely disseminated. We may also desire to develop a summary of the proceedings which would be useful to non-scientists who have an interest and concern about the problem of occupational and environmental cancer. We may also desire to expand the audience at future workshops to include not only government scientists but representatives from academia, labor, industry, and public interest groups.

In reviewing the overall accomplishments and future directions of our program, it is clear that a reasonable balance needs to be established between the amount of research characterized as problem identification (such as toxicological and epidemiologic studies) in contrast with research devoted to problem solution (such as development of improved control technology or a safer substitute material). NIOSH is committed to expanding its own base program efforts in the area of control technology to protect workers. Hopefully in the future, we can consider control technology to be a candidate for research under this collaborative program as well.

As we look forward to the 1980's, it is important that our agencies continue our vigorous efforts to identify cancer hazards in the general and in the working environment. It is also extremely important that Federal agencies expend a greater effort finding solutions to these problems as well. I am confident that this workshop will mark an important step in our overall efforts to effectively deal with the problem of environmentally related cancer. Hopefully this will be the first of many such workshops.

Thank you.
Table 1. - Cancer Projects Related to Epidemiology/Industrial Hygiene Project (Performing NIOSH Division)

- Mortality and IH Study of Gold Miners (DSHEFS)
- Mortality Study of Pesticide Formulators (DSHEFS)
- Kepone Registry (DSHEFS)
- Mortality, Medical and IH Study of Talc Workers (DSHEFS)
- Mortality and IH Study of Styrene-Butadiene Rubber Workers (DSHEFS)
- Mortality, Medical and IH Study of PCBs (DSHEFS)
- Mortality and IH Study of Perchloroethylene Workers (DSHEFS)
- Mortality, Medical and IH Study of Chlorinated Hydrocarbons (DSHEFS)
- Mortality Study of Beryllium Workers (DSHEFS)
- Mortality and IH Study of Nitrosamines/Cutting Oils (DSHEFS)
- Mortality, Medical and IH Study of Painting Trades (DSHEFS)
- Mortality and IH Study of Printing Trades (DSHEFS)
- Mortality and IH Study of Phosphate Workers (DSHEFS)
Table 1.- Cancer Projects Related to Epidemiologic/IH (Continued)

Project (Performing NIOSH Division)

- Morbidity and IH Study of Fibrous Mineral Workers (DRDS)
- Mortality and IH Study of New Agents (DSHEFS)
- Mortality and IH Study of Workers Exposed to Styrene (DSHEFS)
- Mortality, Morbidity and IH Study of Brakeline Workers (DSHEFS)
- Mortality and IH Study of Benzene Workers (DSHEFS)
- Mortality and IH Study of Foundry Industries (DSHEFS)
- Mortality Study of Workers in Plywood, Paper, and Pulp Industries (DSHEFS)
- Mortality, Medical and IH Study of Workers Exposed to Ethylene Oxide (DSHEFS)
- Mortality Study of Crushed Stone Exposures (DRDS)
- Mortality and IH Study of Workers Exposed to Toluene (DSHEFS)
- Mortality and IH Study of Leather Industry Workers (DSHEFS)
- Mortality and IH Study of Workers Exposed to Dyes (DSHEFS)
- Mortality Survey of a Chemical Plant in Kanawa Valley (DSHEFS)
- Cancer Surveillance of Occupational Cohorts in a Western SMSA (DSHEFS)
Table 2. - Cancer Projects Related to Industrial Hygiene Project (Performing NIOSH Division)

- Occupational Cancer Surveillance (DSHEFS)
- IH and Medical Study of Workers Exposed to Azo Dyes (DSHEFS)
- IH Study of New Agents (DSHEFS)
- Utilization of Full File For Survey/Sampling Decisions (DSHEFS)
- Mapping Chemical Exposures (DSHEFS)
Table 3. - Cancer Projects Related to Toxicology

<table>
<thead>
<tr>
<th>Project (Performing NIOSH Division)</th>
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<tr>
<td>Carcinogenicity of Inhaled 1,2-Dibromomethane (DBBS)</td>
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<tr>
<td>Chronic Inhalation of Short Asbestos Fibers (DBBS)</td>
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<tr>
<td>Perform Intratrachael Testing of Copper and Lead Smelter Dusts + Fractional Smelter Dust to Determine Their Carcinogenicity (DBBS)</td>
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<tr>
<td>SO₂ Effect on Smelter Dust Carcinogenesis (DBBS)</td>
</tr>
<tr>
<td>Metabolism of Azo Dyes to Carcinogenic Amines (DBBS)</td>
</tr>
<tr>
<td>Foundry Mold and Sand Pryolysis Effluent-Carcinogenesis (DBBS)</td>
</tr>
<tr>
<td>Roofing Asphalts and UVL Carcinogenesis (DBBS)</td>
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<tr>
<td>Determine Whether Thallium Trioxide and Antimony Trioxide Are Carcinogenic in Animals (DBBS)</td>
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<tr>
<td>Machine Oils and Nitrosamines (DBBS)</td>
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<tr>
<td>Tumorigenicity of Nitroaliphatics (DBBS)</td>
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<tr>
<td>Fibrogenic Responses and Pulmonary Carcinogenesis (DBBS)</td>
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<tr>
<td>Workshop on the Role of Metals in Carcinogenesis (DBBS)</td>
</tr>
<tr>
<td>Co-Carcinogenicity of Foundry Particulates (DBBS)</td>
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<tr>
<td>GI Absorption of Be &amp; Pt Complexes (DBBS)</td>
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<tr>
<td>Interaction Between Drugs and Industrial Chemicals (DBBS)</td>
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<tr>
<td>Carcinogenicity of Dimethylformamide (DBBS)</td>
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<td>Project (Performing NIOSH Division)</td>
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<td>----------------------------------</td>
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<tr>
<td>o Sampling and Analytical Methods for Four (4) Carcinogens (DPSE)</td>
</tr>
<tr>
<td>o Develop Behavioral Approaches For Improving Work Practices Directed Toward Reducing Occupational Exposure to Carcinogenicity (DBBS)</td>
</tr>
<tr>
<td>o Develop Protective Equipment and Determine Protective Clothing Permeability For Carcinogens (DPSE)</td>
</tr>
<tr>
<td>o Development of Trade Name Ingredient Data Base (DSHEFS)</td>
</tr>
<tr>
<td>o Validation of NOHS and RTECS Risk Ranking Algorithm (DSHEFS)</td>
</tr>
<tr>
<td>o Physical and Chemical Properties of Foundry Particulates (DBBS)</td>
</tr>
</tbody>
</table>
The collaborative program on occupational cancer between the National Cancer Institute and the National Institute for Occupational Safety and Health for the conduct of cooperative research in this area had its inception in late 1976. In the U. S. Congress House Record of 1976, it was noted that "considerable wide public attention has been drawn to the number of known and potential environmental carcinogenic hazards, such as vinyl chloride, pesticides, water pollutants and certain gene combinations that could result from certain types of research." This interest on the part of the U. S. Congress gave rise to a Congressional mandate to set up a collaborative program between the National Cancer Institute and the National Institute for Occupational Safety and Health. This program had as its main thrust the identification of cancer hazards in the general work environment. The objective of this congressional directive was to encourage interagency collaboration with a view towards increased efficiency and the widest possible utilization of staff capabilities and expertise. During the earliest phase of this program there was simply a transfer of funds to NIOSH from the NCI annual budget allocation; the funds to be used essentially at their discretion. Since then, however, the program has evolved into a truly excellent collaboration, with NCI becoming a partner in the decision-making process on the type and scope of projects which would be of mutual interest to both agencies. More recently, the NCI staff has begun to initiate proposals in this general area. Clearly these types of interfacing reflect more closely the initial intent of the Congress.

The present agreement allows that proposed projects receive technical, relevance, need and priority reviews by senior scientists in the Division of Cancer Cause and Prevention of NCI and representative senior staff from NIOSH. It is ultimately planned to have some of the contract project supervision by an equal representation in project officers from NCI and NIOSH. Of the total of 71 projects initiated to date, as of March 1980, 19 have been either completed or satisfactorily terminated. For any one year the dollar ceiling has been set at four million. This level of funding seems to provide for the mutual needs for achievement of goals and the attainment of a quality program which achieves excellent collaboration. The first interagency agreement for this collaborative program was signed by the sponsoring and accepting agencies on September 23, 1976.

The National Cancer Institute/Environmental Protection Agency Collaborative Program on Environmental Cancer had similar beginnings to the NCI/NIOSH agreement previously described. This program was implemented in early 1977 based on a mandate from the Office of Management and Budget as a result of interest shown by the U. S. Congress. The stipulation was that $4 million included in the NCI budget be set aside for projects of mutual interest to both EPA and NCI. The early planning of this program took place between the top staff of the Division of Cancer Cause and Prevention of NCI and the Office of Research and Development of the Environmental Protection Agency. Later the Office of Toxic Substances of EPA joined in these planning meetings.
In November, 1977 a list of 16 projects was approved conjointly by an NCI/EPA coordinating and advisory group. This initial list of projects soon expanded to 20 projects. Final approval of these projects and plans for a continuing program were secured by signatures of the Director of NCI and Assistant Administrators of the Office of Toxic Substances and the Office of Research and Development of EPA. These plans, finalized in late 1977, soon developed into a Memorandum of Understanding signed by Drs. Upton (NCI), Dr. Gage (EPA) and Mr. Jellinek (EPA) on January 20, 1978. In essence, the Environmental Protection Agency looked upon this collaborative effort as supportive to their research in environmental programs which is responsive to the requirements set forth in the Toxic Substances Control Act.

An interagency agreement for commitment to long-term continuance of such a program was signed by representatives of NCI and EPA on June 22, 1978 for funding for the period June 1978 to June 21, 1984. For any one year the dollar ceiling has been set at four million. This level of funding seems to provide the mutual needs of each agency for achievement of goals in effective collaboration and the attainment of a high quality program. The NCI/EPA collaborative program now has, for fiscal year 1980, thirty projects. Projects scheduled for completion with FY80 funding are seventeen unless a few are extended.

To monitor the progress in this program, biannual and annual reports are required from each project officer. The first edition of the annual report was recently mailed out to all participating project officers and program staff of each agency. If there are any omissions on our distribution list then the respective NCI and EPA coordinators for the program should be notified.

The program coordinators for the NCI/NIOSH and NCI/EPA collaborative programs considered it timely to sponsor a workshop which, of course, is why we are here today. In our view, we considered that the interfacing of all program staff and project officers, limited to federal representation, would serve the purpose of orienting all representatives in both programs of the activities and accomplishments in all technical areas. With reference to areas of emphasis in the NCI/EPA collaborative program, and perhaps the same holds for the NCI/NIOSH program, one is essentially dealing with the following areas: a) Information/Monitoring and Data Resources, b) Experimental Studies/Mechanisms, c) Methodological Approaches and Developments, and d) Epidemiological Studies.

In order to provide adequate coverage within the timeframe of three days, we have scheduled concurrent working sessions A, B and C. It is hoped that all participants at the workshop will identify the working session they will attend so that we can achieve active participation for each working session.

We look forward to a successful workshop and through your participation and support we believe that this objective will be achieved. The Organizing Committee wishes to express their thanks and appreciation for your efforts in this first workshop. We have a compendium of abstracts and, in due course of time, Proceedings for the Workshop will be made available.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Tuesday Morning, May 6

EPIDEMIOLOGICAL/STATISTICAL SESSION

SESSION CHAIRPERSON

Dr. Roger Cortesi,*
Environmental Protection Agency

(Note: Dr. Vilma Hunt, EPA, substituted for Dr. Cortesi until his arrival.)
CANCER MORTALITY IN AN INDUSTRIAL AREA OF BALTIMORE

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Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, SW
Washington, D.C. 20460
Cancer Mortality in an Industrial Area of Baltimore

Arsenic has long been known to be a poison when ingested in large quantities by man, animals or plants. It is known that continued ingestion of high natural levels of arsenic in water or food will produce skin lesions (Tseng et al., 1968; Braun, 1958) including cancer. Consumption of arsenic as a therapeutic agent is also known to cause skin lesions (Neubauer, 1947).

The risk of inhalation of arsenic has not been as extensively investigated. Workers exposed to arsenic in the manufacture of pesticides have an increased risk of lung cancer and lymphomas. Less is known about the chronic health effects in the general population exposed to arsenic in the air. It is known that children around smelters may have high arsenic levels in nails and hair but it is not clear whether these observed indications of absorption of the agent also indicate long-term toxicity. Blot and Fraumeni have suggested that there may be an association between excessive lung cancer mortality and the existence of non-ferrous smelting industries in several counties in the U.S. It is not known whether some by-product of this industry such as arsenic is associated with these carcinogenic effects.

The purpose of the current study is to determine whether there is an excess mortality from cancer in the population which resides near a chemical plant in the inner city of Baltimore and whether any observed excess can be associated with previous exposure to arsenic. The plant has produced insecticides, herbicides, and other arsenic products from 1897 until early 1976. In 1952, the original plant was torn down and a new one erected with better hygienic conditions for the workers. The plant produced arsenic acid, calcium and lead arsenate, Paris green (a cupric acetoarsenite), and sodium arsenite. In the past, all products were dried and packaged except sodium.
arsenite which was shipped as a liquid. Paris green was not produced after the early 1950's and no dry arsenicals after 1973. Other pesticides such as chlorinated hydrocarbons and organophosphates were not produced at this facility but were made into formulations on-site since 1947. There are several other industries which are currently located in the area or have been manufacturing in that vicinity in the past.

METHODS

The census tracts which were selected as having had possible environmental exposure to arsenic from the point source of the pesticide plant were defined empirically as those for which at least 50 percent of their area lay within a 3/4 mile radius of the plant. This distance was chosen so that large tracts which lay across the river and in which the majority of the population did not reside within a one mile radius of the plant would not be included. The four index census tracts which fit these criteria were 2303, 2302, 2404 and 2301. The tract in which the plant was located was 2303.

The comparison group of census tracts consisted of all tracts which matched the index ones on age distribution, race, sex and socioeconomic factors. Index tracts 2303, 2302 and 2404 were similar in these matching characteristics and were compared to the same set of comparison tracts designated as Match I. Index tract 2301 differed in age and race distribution from the others and was compared to a second set of tracts, Match II. Death rates for the index tracts in 1958 through 1962 were compared to tracts selected for matching through information available from the 1960 census. The matching criteria used to select control tracts for comparison of death rates in all other years were derived from the 1970 census. The age distribution differs in the tracts across time intervals, so to compare rates between years, the figures have been age-adjusted.
The initial matching criteria for the 1970 census were:

- Age distribution ± 10% for each age
- Race ± 15%
- Sex ± 5%
- Median income ± $1,000
- % below poverty level ± 10%
- % head of household over 65 years ± 20%

The matching criteria based on the 1960 census were the same except that the variation in median income was reduced to reflect current inflation, and information on the last two characteristics was not available in the earlier decade.

The matching tracts in 1970 and 1960 are shown in figures 1 and 2. A total of 18 Match I control tracts was identified from 1970 census data and 45 tracts from 1960 data. A total of five Match II tracts was found in both census periods. The variation in the numbers of Match I tracts between the two periods is the result of changing racial distribution especially in middle income census tracts over the past ten years. The three index tracts have a predominantly white population and fewer census areas have that racial distribution in the later time period.

The index area was stable with an increasing proportion of individuals living in the same household for five to seven years from the 1960 to the 1970 census. This stability is also reflected in the slight increase in age of the population of the area.

The scattered distribution of the control tracts has placed them in areas which may also have had different risks. The adjacent tracts contiguous to the index ones may have had minimal exposure to the same agents as in the major area. Southern tracts are in heavy industrial areas as are the central tracts.
but the characteristics of the populations and their stability are different. The northern area consists of mainly residential dwellings with little industrial exposure. For these reasons the controls were divided into four groups for comparison - adjacent, south, central, and north.

Cancers were identified by examining all certificates of deaths which occurred within the city for the years 1958-62 and 1968-74. The death was selected for study if cancer appeared as a cause listed anywhere on the death certificate, with the exception of the years 1973-74 where only cancers listed as underlying causes were chosen. Deaths of city residents were selected from the total cancer list. This procedure would not include the deaths of city residents which occurred outside the city. In order to determine the extent of these differences we abstracted information on out-of-city deaths of city residents for the three years, 1970-72. The proportional increase in deaths for the index tracts was five percent and for the control tracts 13 to 15 percent. This difference is not large enough to account for the variation in cancer rates observed. Deaths were included only once using either underlying cause or first cancer listed. Adjustments were made in the changing codes in the 7th and 8th revision so that data by site of cancer were compatible for the total period.

The hospital records of a sample of cancer deaths were reviewed to verify the accuracy of death certification of cancers in Baltimore, to identify any possible differences in diagnosis by area in the city, to determine any variation in pathological characteristics of cancers in index and comparison areas and to investigate differences in personal characteristics such as smoking as described in hospital charts. The review specifically focused on unusual cell types of lung cancer and possible arsenic-associated symptoms and diseases
in cancer patients from the index and control areas.

The soil was sampled for the presence of arsenic in the areas near the chemical plant. The original selection of sampling sites was determined both by distance from the plant and by direction from north through south coordinates. We intended to collect about half the samples within the 1/4 mile radius and 40 percent at the next 1/4 mile distance with the remaining samples collected further out on the radii. Control samples would be taken from two parks nearby but a distance greater than 1 mile from the plant. The field survey team had problems adhering to the sampling design since the sources of soil were limited in the area. We attempted to take samples near residences whenever possible as long as there were no obvious problems of tree-cover, water run-off, or redevelopment. For those few samples taken at private housing, the residents were interviewed concerning the use of herbicides or pesticides in the area and the sample was avoided if the soil had been treated. After collection of the original 101 samples taken at 35 sites under these directives and including additional samples in the park, a second set of samples was collected in a north and north-west direction to determine how far distant the high levels could be detected. Special emphasis was placed on sampling from the park which was adjacent to the plant. This park has a central grassed area which had been recently re-sodded. Surrounding the park was a dirt-track which had been undisturbed. Part of this path was adjacent to the fence along the plant boundary and near the areas where railroad cars were filled. Another portion bordered on the water and the last was adjacent to railroad tracks.

Samples were collected at one, two and four inches at each location unless otherwise noted. A core sampler, with a 3/4 inch bore and marked at one inch intervals, was driven into the ground and samples were removed down to the
appropriate depth marked. A one foot circle was marked off around a selected site and a set of samples was collected according to the described technique until the 30 ml. polyethylene sample bottle was filled with soil from the appropriate depth but from different core samples. Initially we had tested four sites using consecutive one inch samples down to a depth of four inches. We found that samples at three inches were usually close to those at four and thus it was elected to take the extreme depth and discard the three-inch level. Samples in control areas were all obtained from two city parks, Riverside or Federal Hill.

All instruments used in collecting samples were free of arsenic. The analysis was done using either conventional flame or flameless atomic absorption spectrophotometry depending on initial level of arsenic.

RESULTS

Mortality by Tracts

The crude rates for cancers at four specific sites, oral, pancreas, lung and prostate as well as for all cancers are presented for males in tables 1 and 2. The first table includes data for the five-year period around the 1960 census and the second table for a seven-year period around the 1970 census. As can be seen the risk for lung cancer and for all cancers is excessive in the period around the 1970 census for tract 2303 compared to any of the control groups. This is not consistently true in the earlier period. The weighted relative risk of lung cancer in white males from index tract 2303 as compared to north controls which had the highest control rate is 2.5 as shown in table 2 with a probability of .0005 as determined by the chi square calculated by the Woolf-Haldane method. The black males in tract 2301 also have a higher rate of lung cancer but this was not true for white males in the same tract. In the 1960
census period, although the lung cancer rate is higher in white males in tract 2303 than the north and south controls, there is very little difference between the rates for all index tracts and for the adjacent and central controls. There are no differences in rates for males in tract 2301 and their control groups.

If we examine the comparable crude mortality rates for females in tables 3 and 4, we can find no excess risk of cancer at any site for census area 2303. In fact, the overall cancer rate appears somewhat low especially in the 1958-62 period. The mortality from breast cancer is slightly high in the early period and there are no deaths from cervical cancers. The lack of an observed increase in lung cancer in women in 2303 might be the result of a small population size. This will be discussed later in the report.

**Adjusted Rates**

The age distribution of the index tract changed with time and these differences were reflected in similar changes in the control group. In order to have appropriate comparisons the mortality rates for each cancer site and for all cancers have been adjusted using the method of standardized mortality ratios. The average annual Baltimore City mortality rates were calculated from all deaths in the 1968-74 period and these values were used as standards to adjust the mortality in each time period. As seen in table 5, the mortality ratios for white males in the tract 2303 were high for cancers of the lung, pancreas, stomach, prostate, oral cavity and all sites. The numbers of deaths except for lung and all sites were small but the pancreas cancer rate was still significantly higher than that for the city. White females in 2303 had an unremarkable overall cancer rate with excesses noted only for oral and rectal cancers of which only the latter ratio is significantly greater than unity.

In figure 3, we examine the lung cancer mortality in two or three year time intervals. Using rates adjusted by the direct method to the 1970
Baltimore City population as a standard, we find that the death rate for this cancer has always been higher in males from tract 2303 than from most controls but that it has been rising rapidly. The "all cancer" rates have also shown higher values than among controls. A preliminary look at the lung cancer rates for 1950-51 indicated that the adjusted rates for that period were high for tract 2303 with a rate of 253 per 100,000 population as compared to rates ranging from 35.8 to 87.4 in other index tracts and controls.

Employees of an industry may live in close proximity and could have accounted for an increased mortality in the census tract due to occupational exposure. With the cooperation of the company and the investigators studying the employees we reviewed lists of all employees to match with known deaths. Four employees were found among the cancer deaths in tract 2303 but removing these individuals did not change the significance of the rates.

The geographic distribution of cases was plotted on spot maps as shown in figures 4 and 5 for the two census periods. For both periods, lung cancer appears to be concentrated in an area about eight blocks wide and nine to twelve blocks long lying to the north and east of the plant. The area encompasses all of tract 2303 and parts of 2302 and 2301. If one takes all of tracts 2303, 2301 and 2302 which lie within a 3/4 mile radius of the plant and calculates the proportion of lung cancers to all cancers in this area compared to the remaining census areas on the figure 4, the proportion is 44.4 percent near the plant and 16.8 percent in the outer areas. For figure 5, the proportions show similar differences for 1973-74 as in the previous three years. For the area within the defined census tract and 3/4 miles of the plant, lung cancer represents 47.8 percent of the total cancers whereas in other areas it is only 33.3 percent. The northerly direction of this lung cancer excess is
not compatible with the strong wind directions in that area. These winds arise in the northwest and west and should have carried contaminants to the east and southeast of the plant. The particles may have been moved by gentler winds and deposited nearby.

**Soil Sampling**

The highest arsenic levels are shown in figure 6. In most cases these levels occurred at 2 inches suggesting higher contamination in the past. Occasionally, as in the area adjacent to the plant, the levels were highest at one inch. In general, arsenic levels were highest where lung cancer mortality was also highest. The mean arsenic level from 20 sample sites in tract 2303 was 63 ppm of arsenic. Even the omission of samples from the park adjacent to the plant only reduced the mean arsenic level to 38 ppm. Tracts 2301 and 2404 had means of 6 ppm and 2302 a mean of 4 ppm based on only 2 to 4 sample sites. All sites in the park had high levels except for an area which has been turned over and resodded and in which low arsenic levels were present. The one inch levels near the fence were as high as 695 and 226 ppm whereas at the opposite side of the park the values were only 29 to 97 ppm at one inch but as high as 46 to 161 ppm at two inches deep. From the soil levels of the original samples, the data indicated high levels within a 3/8 mile radius of the plant. It was also apparent that higher levels were found in a northerly direction along the railroad lines.

**Hospital Validation**

The deaths of the total 14 years of study were included in the sample and stratified by control and index census tracts. The sample for hospital record review included all deaths for residents in the index tracts. Deaths for control tracts were stratified by age, race, and sex and three time periods,
1958-62, 1966-67 and 1968-74. Four control deaths were selected randomly from each stratum for each index tract death within the same stratum. For the following analyses no attempt was made to expand the sample to the original population size.

The hospital abstract form included information on the following variables:

1. Cancer diagnosis
2. Final diagnosis other than cancer
3. Source of information for cancer diagnosis
4. Arsenic-associated symptoms
5. Personal characteristics as smoking and occupation
6. Description of pathological specimens; operative or autopsy findings

Verification of the identification of the correct individual on the hospital record was done by name, birthdate, residence, and date of death.

Records were reviewed in eleven of the hospitals in Baltimore City. The remaining five non-cooperating hospitals were small and did not limit substantially the number of records reviewed.

All possible medical conditions found on record review were listed and coded by the same nosologist who coded all the death certificates. The cell types were classified, in general, according to the Manual of Tumor Nomenclature and Coding. Since this coding scheme does not appropriately classify the cells of several tumors, especially those of non-solid origin, a revision of the scheme was made to include these cancers if we felt that their frequency was sufficient to warrant specific classification.

The causes of death were grouped into two time periods which represented the use of the 7th and 8th ICDA codes and grouped into causes as listed on the
certificate by the first two digits of the code. These causes were then compared to the first four medical conditions or diagnoses as noted on the hospital records.

The problem of validation of death certificate information was reviewed further by a physician who examined the data on the abstract forms. As indicated in table 6, there was complete agreement in diagnoses to four digits in the ICDA code in only 75.0 percent of the cancer deaths. If classification to three digits only is used we will correctly verify 80.7 percent of the cases listed on the certificate. In 1.8 percent of cases metastatic lesions were identified on the death certificate as underlying and in another 5.5 percent multiple cancers were listed on the certificate and the primary site varied from that listed on the hospital record. There was no cancer diagnosis listed anywhere on the hospital record for 2.7 percent of deaths. A further examination of the method of diagnosis of cases was attempted in order to demonstrate whether differences in the methods might have changed the accuracy of death certification. Data from autopsy and histological examination of tissue were used for the diagnosis of 82 percent of the cases with complete agreement in records and 88.9 percent of cases where the agreement was less than perfect. Therefore, the consistency of cancer diagnosis on hospital record and death certificate is not related to the method by which the cancer was identified.

The method of cancer diagnosis differed only slightly in the larger hospitals with 69.7 to 91.4 percent of cases diagnosed by autopsy or histology. An examination of the differences in diagnosis by census tract has not been completed, but it is unlikely that there will be variations in results since we have included all hospitals used by individuals from the index area in the above evaluation.

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An examination of the hospital records for possible arsenic-associated symptoms included gastrointestinal signs, skin lesions, Mee's lines on nails, neurological or neuromuscular symptoms, cardiovascular disease, stroke and asthma. Only respiratory symptoms were slightly higher in the index tract but since there were so many records in which there was no comment about these symptoms, it is difficult to interpret the small variation. We also sought information on diseases for which arsenic might have been used as a treatment, such as syphilis, trypanosomiasis and amebiasis and the results indicated no higher frequency of these conditions among residents of index tracts.

Both smoking and drinking histories were abstracted from hospital records. Drinking habits were rarely recorded and smoking histories were also frequently missing. Table 7 indicates the smoking characteristics of lung cancer deaths in index and control tracts as determined from the hospital records. For 46.6 percent of the patients, the smoking histories are unknown. Despite that fact, we attempted to compare the smoking levels in index versus the control tracts. The percent of smokers is slightly higher in the index tracts but the difference is not impressive. If one includes only those charts with a recorded history, almost all cases are positive for smoking in both index and control tracts.

The original hypothesis was that if arsenic had caused the lung cancers, the cell type of lesions from the index tract might differ compared to other areas with an expected predominance of small cell or oat cell tumors in the exposed tracts. The data in table 8 would indicate that the cell types differ very little from index to control tracts.
DISCUSSION

An excess mortality from lung cancer has been demonstrated among men living in a highly industrialized area of South Baltimore over a period from 1966 through 1974. The death rate is significantly higher than in control tracts in the later years.

The area surrounding the pesticide plant has high levels of arsenic in the soil which corresponds generally to the same areas where a high proportion of lung cancers to other cancers has occurred. There was no attempt to correlate directly arsenic levels to residences of lung cancer deaths.

The review of hospital records did not indicate that the excess of lung cancer deaths had occurred because of variations in diagnostic practices, cell types or other factors. The information on other risk factors was poorly ascertained from hospital records.

There are some definite questions which arise in regard to the data. Why did the excess risk appear primarily in the late 60's and early 70's when the plant had existed and produced arsenical products since the early 1900's? The discrepancy could indicate that the plant did not account for the excess but some other local industry or occupational group accounts for the excess. It is also possible that the men in the area had a higher frequency of smoking and smoked a higher dose of cigarettes than did populations in the rest of the city. It is possible that selective mobility of younger, healthier males has left the area with a high risk among the remaining group.

The sudden rise in lung cancer might be related to the destruction of the old plant in 1952. Such an undertaking could have spread dust diffusely throughout the community. Under these circumstances we must ask why the concentration of lung cancer in the area does not coincide with the assumed wind spread of
particles. It is necessary to further examine the mortality in the 1950 period to determine whether an excess existed at any time before the destruction of the old plant. It would be interesting to see if the appearance of the excess risk of lung cancer in the community coincided with that found in the workers within the pesticide plant. If we presume that arsenic may not be causing the excess then it would be necessary to examine the mortality experience of workers in other industries in the area, especially the natural gas plant, to see if they have an excess lung cancer mortality. In almost all cases, workers within an industry should have higher exposure and a greater risk of disease than the general public. It is necessary to investigate whether the increase in lung cancer can be related to a change in production or methods of operation of any of the businesses. For example, differences in handling arsenic, changes in formulation of pesticides or the conversion of the gas plant from carburetted water gas to oil gas production could have created variations in level of type of pollution.

The fact that the excess lung cancer mortality has occurred only in men raises the question as to whether another environmental factor, differences in smoking characteristics, or occupation has caused the increased death rate in tract 2303. It is possible that smoking plus an environmental pollutant are required to produce the excess of cancer. The rates in older women then could be lower because they did not smoke and the possible synergistic effect of cigarettes and the environmental factor were not observed. Many of these questions might be determined by a community survey.

Further sampling for arsenic should be done to determine at what distance the levels actually returned to background. It was first thought that the high levels along railroad lines might indicate a relationship to previous coal use. However, further investigation showed that the arsenic content of coal
in local use did not reach levels as high as those measured along the tracks. Rail transport of materials from the plant may have been related to the high levels. Further investigation of this possibility is needed. It appeared that use of herbicides did not explain the arsenic levels in the rail beds.

In summary, men living in close proximity to a chemical plant which produced arsenicals have a higher risk of lung cancer than comparable individuals in other areas of the city. The distribution of arsenic in the soil near the plant and along the railroad line is higher than in control areas.
Table 1

Average Annual Crude Death Rate per 100,000 for Index and Control Census Tracts
Deaths 1958-62

Males

Match I

<table>
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<tr>
<th>Cause</th>
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Table 2
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Deaths 1968-74
Males
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Census Tracts

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Match II

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Table 3  
Average Annual Crude Death Rate per 100,000 for Index and Control Census Tracts  
Deaths 1958-62 
Females  
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Match II  

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Table 4
Average Annual Crude Death Rate per 100,000 for Index and Control Census Tracts
Deaths 1968-74
Females

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* Average annual Baltimore City rates (based on deaths in 1958-1962) were applied to the 1960 match population and were weighted for five years. Average annual Baltimore City rates (based on deaths in 1968-1972) were applied to the 1970 match population and were weighted for nine years.
## Table 6

Level of Agreement between Death Certificate Cancer Cause and Hospital Diagnosis

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Total 740
Table 7
Smoking History in Lung Cancers from Index and Control Tracts by Sex

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<tr>
<td><strong>Control Tracts</strong></td>
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<td></td>
<td></td>
</tr>
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<td>5</td>
<td>0</td>
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<td>Adenocarcinoma</td>
<td>Epidermoid</td>
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Figure 1. Map of Baltimore City showing location of 1970 index and control census tracts.

Figure 2. Map of Baltimore City showing location of 1960 index and control census tracts.

Figure 3. Age- and time-adjusted rates per 100,000 for tract 2303 and North and Adjacent Controls. White males. All cancer and lung cancers. (semi-logarithmic scale)

Figure 4. Spot map showing cancer deaths for 1970-72 by residence at death, excluding chemical plant employees.

Figure 5. Spot map showing cancer deaths for 1973-74 by residence at death.

Figure 6. Arsenic level in soil - ppm. Highest value at each site. Summer 1976 and Spring 1977
References


DISCUSSION

DR. HUNT: I think we do have about five minutes for questions. We can proceed on that basis. Are there any questions for Dr. Matanoski?

DR. O'CONOR: It is a very impressive study. I have two questions. One has to do with the characteristics or definition of the population in the indexed census tracts and how that compares with the control census tract. The other has to do with similar industries to the one that is now apparently defunct in your area. What kind of controls or regulations are in effect now for plants to benefit from the kind of experience which you have described?

DR. MATANOSKI: The control tracts were actually matched for the several characteristics to the index tract, namely age, race, sex, socioeconomic status and percent at poverty level. So they were similar in these characteristics with very small deviation. One thing I did not emphasize is that the data that you have seen was subsequently examined with employees excluded, and that did not change the observed difference, the epidemic still persisted. We had a list of all employees provided through the industry which allowed us to accomplish this task.

Your second question related to what my advice would be for control of this situation. The problem in this plant was apparently from dust. At least when we observed the operation externally, it had a very high dust level and the material was circulating very close to the ground. The plant did not have high stacks. Thus, the material was not moved very far away. We had not anticipated this distribution of arsenic. We expected the material perhaps to be carried by wind currents away from the plant and further out into the population. Instead, the arsenic remained in the very community close to the plant. If one could manage local dust problems, this type of spread could be avoided.
EPIDEMIOLOGIC STUDY OF A POPULATION PREVIOUSLY EXPOSED TO HEXACHLOROBENZENE

A. Gocmen 1, D. Cripps 2, H. Peters 2, G. T. Bryan 2, and C. R. Morris 3

1 Hacettepe University Medical School, Ankara, Turkey; 2 University of Wisconsin Center for Health Sciences, Madison, Wisconsin; and 3 U. S. Environmental Protection Agency, Washington, D. C.

In several areas in Eastern Turkey during a 3-year period from 1956-59, approximately 4,000 people were inadvertently exposed to hexachlorobenzene (HCB) which was utilized as a herbicide for seed grains. These exposures occurred during periods of austerity when individuals ingested seed wheat unintentionally diverted to replace edible wheat stores.

Original Turkist government records for precise areas of distribution of wheat have been obtained. The seeds that were treated with HCB to control a wheat fungus were distributed mainly to South Eastern Anatolia between 1954-1959.

Distribution of the seed wheat included the areas surrounding the cities of Diyarbakir, Urfa, Siirt, Mardin, Gaziantep, Mus, Elazig, Nigde, and Adapazari.

Several unique clinical features were noted during the initial exposure period from 1956-1959. The interval between HCB ingestion and development of symptoms was calculated to be approximately six months. The initial symptoms mentioned by most subjects were weakness, loss of appetite, and sunlight sensitivity. Hyperpigmentation was maximum in exposed areas of skin and hypertrichosis occurred principally on the forehead, cheeks, arms, and legs and was sufficiently distinctive in the five to fifteen year age group for them to be described as "monkey children." Development of bullae often up to 5 cm in size occurred frequently, healing with severe mutilating scars. The porphyria was known as Kara yara (black sore). During the early period of active porphyria the affected persons continued to be irritable with colic, loss of appetite, weakness, and they excreted red or brown urine (porphyrinuria). Many adults and children died from this initial acute toxicity. It was estimated that nearly 14 percent of the exposed individuals died during the initial acute exposure phase. Survivors developed porphyrinuria with concomittant neurologic, cutaneous, hematologic, and mental aberrations characteristic of a syndrome called porphyria turcica. Arthritis was common in the younger age group with swelling and spindling of the fingers but very little pain. In addition, children born to mothers who had ingested the HCB-treated grain developed a condition called "Pembe yara" (pink sore) that was followed by a high infant mortality within two years. It has been estimated that more than 2000 children died from this condition in which many were breast-fed by mothers who had been exposed to HCB.

Preliminary data on a sampling of over 100 members of this population, 20 years after their initial exposure, indicate continued clinical symptoms and signs such as hyperpigmentation, hirsutism, cutaneous scarring, small stature and hands, painless arthritis, enlarged liver, and enlarged thyroid.
This is a picture of two sisters. The younger sister who is age 17 years appears on the left. She has a normal appearance and is of normal stature. The sister on the right was exposed to HCB during her childhood. As you can see, the HCB exposed individual had scarring on the face and hands. You can also see the smaller hands, the short fingers, and the arthritis of the hands. These characteristics are common among many of the HCB survivors.
This picture depicts the enlarged thyroid observed in several of the HCB exposed individuals.
This picture depicts another example of an HCB-exposed individual with an enlarged thyroid. The presence of enlarged thyroids in the HCB exposed population are presently being viewed with suspicion in light of recent experimental studies in which thyroid tumors have been observed in hamsters exposed to HCB. We are presently trying to determine whether the enlarged, goiter-like symptoms are endemic to this Turkish population or are a manifestation of the HCB exposure. We plan to request the pathologic findings on those individuals who elect to have their thyroid condition treated surgically and have requested thyroid scan data on all individuals whether surgically treated or not. As necessary, individuals with thyroid adenoma will be studied in more detail at the Hacettepe Hospital in Ankara.
This picture depicts the extensive facial scarring, the shortened fingers, and the painless arthritis observed in several of these HCB exposed individuals.
This study represents a unique opportunity to observe the human effects of HCB exposure. The current health status of previous exposed individuals may provide us with additional information relevant to the potential chronic effects of HCB. We have a particular interest in the status of youngsters born of mothers who were 6-12 years of age at the time of their initial exposure.

We have been most fortunate and very appreciative of the cooperation shown by the Turkish authorities and clinicians. Dr. Ayhan Gocmen, a Turkish clinician from Hacettepe Medical School of Ankara, Turkey, has been indispensable in his efforts to assist us in the clinical aspects of the study. Dr. Gocmen was initially involved in this problem when he was a resident working in Eastern Turkey on this particular problem shortly after the initial incidence was observed and has been associated with evaluating the problem ever since. He has been able to assist us in visiting a total of 10 villages in which over 100 individuals have been identified as survivors of their initial acute exposure some 20 years ago. Records indicate that nearly 5,000 villages may have been involved to various extents in this epidemic area suggesting a sizable population for study. Dr. Gocmen continues to expand our investigation and has acquired the services of several paramedical personnel to assist him in identifying HCB exposed individuals. Indoctrination of personnel at various medical centers throughout the epidemic area has also made them more aware of these disorders.

Preliminary information to date indicate that fecal and urinary porphyrins are still being excreted in significant amounts by several individuals. Urine and stool porphyrins have been obtained on 100 individuals with clinical evidence of porphyria. These data are being compared with Turkist and U. S. control individuals. The preliminary results indicate that five subjects are still porphyric after 20 years. Four of these subjects have moderately increased excretion of porphyrins whereas the fifth subject had a urinary uroporphyrin of 1,607 micrograms/liter compared with Turkist and U. S. controls of only 5.17 and 9.0 micrograms/liter, respectively. This latter individual also had a stool uroporphyrin of 189.2 microgram/gram dry weight compared with controls of 2.09 and 2.80 micrograms/gram, respectively.

Further follow-up studies are under way including the identification of additional exposed individuals and quantitative analysis of HCB and porphyrin levels.

In addition, animal studies are also in progress in an attempt to correlate animal and human symptomology as a result of a range of HCB exposures. These correlations will be based upon both acute and chronic animal exposures and those estimated in HCB exposed human populations.

In summary, we have observed clinical symptoms of porphyria including the excretion of urinary and stool porphyrins as well as HCB in maternal milk from HCB exposed individuals. These data suggest that HCB was accumulated in body tissues and fat stores for at least 20 years from initial exposure. These findings would support our continuing concern regarding the potential chronic effects of chlorinated hydrocarbons and, in particular, the chlorinated benzenes, on human health and the environment.
Discussion

Dr. Kraybill, NCI: Is there a parallelism in the United States, because there was an episode, I believe in Louisiana or Texas, where cattle got quite an insult from HCB? Would this be a population that one would be looking at?

Dr. Morris, EPA: There is a strong possibility that some of the clinical data that we develop in the Turkish incident can be correlated to other situations here in the United States. Certainly, the Louisiana incident is a prime example. The Agency does have a fairly sizeable data base on some of that and we are going to be looking at that data base as we proceed in this particular study.

Dr. Plotnick, NIOSH: Is there any indication of the levels of HCB in the original grain that was eaten. Also, are there samples still available and have impurities been analyzed. Some of these things appear to me to be more related, or possibly related, to the impurities than to the unchanged HCB itself.

Dr. Morris, EPA: Dr. Gocmen's brother-in-law is a member of the Turkist Department of Agriculture. In fact, he was very helpful in getting us the records of distribution. We are attempting now to methodically go through the list and identify storage facilities at distribution points to see if there might be any residual sacks of grain which might be made available to us for analysis.

In terms of your question about contamination, we would agree with you. A variety of potential contaminants with which many of us are aware may be of equal concern. I am hopeful that when our research team returns from their next visit that we might have some samples to analyze. Of course, after 20 years, I do not know exactly what this is going to mean either, but it certainly needs to be evaluated.

Dr. Fraumeni, NCI: Are there some clinical or experimental observations suggesting a relationship to cancer with this agent?

Dr. Morris, EPA: Yes. There are animal experimental data showing an oncogenic response in hamsters and mice when they are exposed to hexachlorobenzene.

Dr. Fraumeni, NCI: Do you know what type of cancer?

Dr. Morris, EPA: The thyroid gland appears to be a major target organ. This is why I indicated in my talk that we are interested in these particular patients with enlarged thyroids. I believe there has also been liver involvement.

Dr. Fraumeni, NCI: There is a condition called porphyria tada, which is associated with cirrhosis, which in turn predisposes to liver carcinoma. Do these patients have cirrhosis?

Dr. Morris, EPA: I do not recall seeing it. We have noted enlarged livers in some of these patients; that is true. During my last visit in October 1979, we had one of the patients die of leukemia. I do not know what that means, because it is just one patient and he was only 26 years old. I think there may be a lot more pathology once we have identified the population. I appreciate that the study presently funded through the NCI/EPA activity is a five year study, but we are just now at the breakpoint of 20 years. I think in the next 20 years this particular population may provide us with a lot more data. So it is possible that this may be one of those activities, which Dr. Kraybill mentioned, that we might consider extending.
Cancer in Southern Louisiana: Progress Report of a Case-Control Study of Lung, Stomach, and Pancreas Cancer

Linda W. Pickle, Ph.D.

Maps of U.S. cancer mortality by county for the period 1950-69 pointed to southern Louisiana as a high risk area for cancers of the lung, pancreas, and stomach. This interview study of newly diagnosed cases of cancer at these sites and their appropriate controls was designed to investigate the causes of the high rates and to follow up leads generated by a recent study of 10,000 death certificates in the area. Information is being gathered by face-to-face interviews on diet, ethnic background and alcohol consumption, occupational, medical, and residential histories, as well as other factors related to the unique Acadian culture of the study area.

Interviewing began in several large hospitals in June, 1979, and has been expanded to include 22 hospitals covering the entire southern half of the state. Since the great majority of lung, pancreas, and stomach cancer cases are diagnosed by anatomic pathology and cytology specimens, the primary source of cases has been through pathologists' reports. In addition, though, the abstractor/interviewers work closely with one physician on the regular staff at each hospital to ensure that no cases are missed. Controls are selected from hospital admissions lists or medical records matched to the cases by age, sex, race, and hospital.
Interviewing teams are based in both New Orleans and Baton Rouge to reduce travel time. As of April 1, 1980, 998 interviews have been completed, including 450 cases of lung cancer, 68 stomach cancers, and 48 pancreas cancers which represent an average of 83% of available study subjects. We anticipate continuation of the study in order to complete interviews of 1200 lung cancer cases, 150 pancreatic cancer cases, 200 stomach cancer cases, and an equal number of controls.

In addition to the information obtained through interviews, manufacturing industries with over 50 employees in the area were located and mapped for future comparison to the residences of cases and controls. It may be possible to utilize EPA maps of various emission levels in the study area to further define the residential exposures of the study subjects.

Interviewing will continue for at least one more year. No results are available at this time.

Acknowledgement:

This research is being conducted by Louisiana State University, Dr. Pelayo Correa, Principal Investigator, under NCI/EPA Contract #NO1 CP 91023.

NO DISCUSSION FOLLOWING THIS PAPER
SUPPORT SERVICES FOR STUDY OF BLADDER CANCER IN NEW HAMPSHIRE, VERMONT, AND MAINE (NEW ENGLAND)

Dr. Robert Hoover
National Cancer Institute

Abstract

Field studies are being carried out in seven New Hampshire, nine Vermont, and seven Maine counties to determine environmental and possibly host factors which may be responsible for the high rates of bladder cancer in both men and women. The study involves a case-control next-of-kin survey of at least three hundred persons in each group, a case-control incidence survey targeted for one hundred cases, characterization of industrial and other relevant features of the study area, and collection and analysis of air and water samples. The interviewing, recording, and other field work are carried out under the support services contract with Westat, Incorporated of Rockville, Md. A considerable amount of time has been devoted to professional contracts and discussions for carrying out the study. The questions asked are those used for the national saccharin/bladder cancer survey, plus investigation of the importance of edible bracken fern for possible carcinogenic factors and possible importance of French Canadian background.

NO MANUSCRIPT RECEIVED
A Case-Control Study of Lung Cancer Near A Zinc Smelter

Linda M. Pottern, William J. Blot, and Joseph F. Fraumeni, Jr.

Background and rationale

In 1969 a cohort study of employees of a large copper smelter in the Western United States revealed a 3-fold increased risk of lung cancer, reaching 8-fold among workers most heavily exposed to arsenic trioxide (1). This finding was substantiated by other studies of copper smelter workers in the U.S. (2,3), Japan (4), and Sweden (5). Although many suspect chemicals are present in the smelter environment, inorganic arsenic has been implicated as the respiratory carcinogen. This is also the case in other industries, including the manufacturing of arsenical pesticides (6,7), where exposures to inorganic arsenic are relatively heavy. The consistent epidemiologic evidence linking occupational arsenic exposure to lung cancer has been sufficient to label arsenic a carcinogen, even though the agent has not induced tumors in laboratory animals.

In the 1970's environmental measures of stack emissions from copper smelters indicated high airborne levels of a number of pollutants including arsenic (8). Substantial amounts of inorganic arsenic were then detected in the soil and air near a copper smelter, and in neighborhood families the levels of urinary arsenic were as high as in smelter workers (9). The possibility that smelter emissions into the general community might pose a cancer risk was raised by the significantly increased mortality from lung cancer in male and female residents of counties with copper, lead, or zinc smelters and refiners (10). Inorganic arsenic is often a
component of these ores, more so for copper ores, and during processing it is released as an airborne or solid by-product. It was felt that work exposures alone would not completely account for the large excess mortality in male residents or the increased risk in females living in the communities.

To determine whether air pollutants such as arsenic from nonferrous smelters may contribute to the risk of respiratory cancer in surrounding communities, and to evaluate the confounding or modifying effects of cigarette smoking and work exposures, we planned a case-control interview study of lung cancer in areas of the U.S. where the smelters were located. The original study design called for parallel studies near several non-ferrous smelters (copper, lead, zinc) around the country. This was scaled down when the State of Montana initiated with the EPA an interview study centering about two copper smelters, and CDC-NIOSH developed related projects with respect to lead smelters. Therefore we decided to focus on a case-control study of lung cancer in a tri-county area of eastern Pennsylvania where a zinc smelter is located.

**Methods and data collection**

Since the survival rate of lung cancer is relatively short and there were no cancer registries or other means of rapidly identifying all newly diagnosed cancers, we selected cases and controls from a computer listing of death certificates supplied by the state of Pennsylvania. Death certificates were drawn for Northampton and Lehigh county residents who died of lung cancer during the years 1976-1977 and Carbon county
residents who died of lung cancer during the years 1974-77. A total of 447 lung cancer cases and an equal number of controls who died of other causes (excluding lung diseases and suicide) were identified. Medical records were sought on each lung cancer patient for further details on the disease, including the method of diagnosis and histologic type. Field operations were conducted through a support service contract with Lehigh University.

Interviews were conducted with the next-of-kin of cancer cases and controls, using a standardized questionnaire that solicited information on smoking habits, occupational and residential histories, associated medical conditions, and family history of cancer. To date, interviews have been completed on 430 lung cancer cases and 426 controls. This represents a remarkably high response rate of 96%. The medical records were available on 389 cases, with pathologic confirmation of primary lung cancer in 89%. The interview data have been entered into computer readable form and analysis will begin shortly. The residences of the cases and controls are being plotted on a grid map. For each individual it should be possible to determine residential proximity to the smelter's two stacks, and to estimate exposures to several pollutants including arsenic based on data from prior environmental surveys conducted primarily by EPA.

Summary

A case-control study of lung cancer is underway in a tri-county area of eastern Pennsylvania in the vicinity of a zinc smelter. The next-of-kin of approximately 430 patients who died of lung cancer, and
426 controls, have been interviewed. From these data we hope to clarify the role of occupational and neighborhood exposures to smelter pollutants as risk factors in lung cancer.
References


Discussion

Dr. Kraybill, NCI: Are you going to be measuring the ambient levels of these cations or elements in air, water, diet, et cetera?

Dr. Blot, NCI: Yes. One of the reasons for picking the Pennsylvania area was because CDC, together with EPA, conducted an environmental survey several years ago in the environs of the Palmerton zinc smelter. There are soil, air and household dust measurements available that we hope to be able to correlate with the information that we have on residences from the interviews.
Dr. Cortesi, EPA: If anybody wants to throw out a good subject for discussion, I think now is the time.

I would like to throw a subject out for people to consider. It is a very parochial point of view. EPA is in the number picking business. For epidemiology to be of much use to us, we need a little help in picking numbers, and "go/no go" is not it. I would like to hear any discussion on any subject, but on this I would like to say that in regard to epidemiology, where exposure is questionable or non-existent, it is of limited use to us. Epidemiological studies where you cannot reduce the relative risk, that you feel quite certain that you could have detected, is not apt to be very useful to us if we get a negative result. I think the recent studies on bladder cancer and saccharin, the three studies that have come out, are a good example. As you know, a couple of investigators talked about detecting a relative risk of 1.15. Another group, Wynder and coworkers, indicated a risk of 2.5. Relative risk value, being the smallest that could be detected for a lot of EPA type problems, cannot be constituted as an epidemiological study which tells us we do not have to worry about something when there is a widespread pollutant with very large numbers of people exposed.

Who else would like to make some comments?

Dr. Keefer, NCI: I have a question on a totally different subject. I wanted to ask the people involved with the HCB exposures how a thing like that could happen. It seems that for a large number of years a large amount of material was getting into the human food supply. More recently, Dr. Kraybill indicated the same kind of thing happened in the United States in cattle rather than people. I wonder if you could identify the factors which contributed to this disaster and comment on how we can prevent that kind of thing from happening again somewhere else in the world.

Dr. Morris, EPA: Actually, you have two situations in terms of the exposure. The Turkish situation was one in which there were starvation conditions. You are talking about an area of the country which is very poor. The government does subsidize them a great deal. It actually provides many of them the seed grain. In that case, when we had that situation, the people who received the grain had the choice of eating today and you do not worry about what the seed grain is going to do on your land a year from now. Even more importantly, the extent of information of the health and environmental effects of HCB were very limited, and for some effects, totally non-existent. So the people in this time of austerity did in fact substitute the grain, because they did not have other food sources. At the time, they were unaware that there was going to be a problem associated with it. Unfortunately, the communication at that time, compared with now, was a great deal different. A lot of these people up in the hill country of south-eastern Turkey can be lost and no one is going to get too excited, because no one knows what it is about. They died of natural causes and so on. But it certainly came to the attention of the Turkish government in the late 1950's and that is when they obviously put a stop to it and tried to do something about the situation.

In terms of the exposure in the Louisiana area, that was a result of HCB production. They were actually going to the dump with the HCB as a manu-
facturing by-product. A lot of our data comes from situations in which HCB was spilled from open trucks along the roadside on the way to the dump. The exposures were different in that sense because it got in the ground and in the water supply. These exposures were different but the end effects may not be different. That is what we are looking at.

Dr. O'Conor, NCI: Could you identify some of the contaminants that you suspect?

Dr. Morris, EPA: Certainly one of them is pentachlorophenol, which may well be a contaminant along with some of the other chlorinated benzenes. We have evidence that these compounds themselves are problems. I think those particular contaminants have a fairly extensive track record. That will always continue to plague EPA as we proceed through the rulemaking on various existing chemicals. We do have concerns about the effects of the contaminants of the chemicals which we are asking to be tested. This is encountered in the bioassay program, too. That is certainly the case with HCB.

We are doing some very interesting studies, which are very preliminary at this time, with the HCB that is now available to us. That is, we are trying to look at a series of purifications and then looking at the acute and subchronic toxicities to see if there are differences from a strictly toxic point of view. I do not have the data on that activity yet, but I would hope that I can tell you what we found in that area in our next progress report.

Dr. Cortesi, EPA: Before you go away, let me ask you another question. Don't chlorinated dioxins and chlorinated dibenzofurans always show up in these sorts of chemicals?

Dr. Morris, EPA: Well, I am reluctant to say "always." But oftentimes when you have the chlorination process there is the possibility of getting other chlorinated by-products. It is true that we have stopped making HCB, but HCB and other chlorinated hydrocarbons have been reported in drinking water following chlorination treatment. So that continues to plague us as well.

Dr. Bull, EPA: I have never heard of HCB being a by-product of chlorination. Can you expand on that?

Dr. Morris, EPA: Yes. I will provide you with my references on the presence of HCB in drinking water. The presence of HCB in chlorinated drinking water was reported in an earlier EPA publication. The extent to which chlorination contributes to the chlorination of organic compounds is certainly not clear. In fact, you might check with Diane Courtney down at RTP. Apparently, she has written a review paper on HCB which you might look at.

Dr. Bull, EPA: No, I meant as a HCB chlorination product of disinfection. That is a little harder to see.

Dr. Morris, EPA: I will be pleased to find you the reference and perhaps you can clarify the report for me. I would appreciate your thoughts on the matter.

Dr. Hoover, NCI: I would like to get back to your original question which you started the discussion with. I think in making a decision about whether a chemical is a carcinogen or not or whether it is a big or a little carcinogen, we cannot rely
on any one specific methodology. We cannot rely on chemical structure. We cannot rely on the bioassay. We cannot rely on epidemiology solely. I think we need to make a synthesis of the observations. The saccharin issue is a good case in point of the laboratory animal experimentation which did not identify saccharin as a carcinogen at the levels at which it is usually consumed by the American public. The problem of low level risk is not unique to the epidemiologists. It is just as much of a problem for the laboratory animal person.

The laboratory animal experimenters have the luxury of being able to give enormous doses in order to produce effects that then can make some assumptions about extrapolation to a low dose and some other people can make assumptions about interspecies extrapolation. I think the value of epidemiology is in doing the same thing, which is finding abnormally exposed people for whom elevations in risk may be detectable and for whom you do not have the interspecies extrapolation problem. You still do, however, have the dose response extrapolation problem, with the saccharin issue again being a case in point.

If the overall risk is 1.04, 1.06, 1.1, or some four to eleven percent excess, I do not think I or anybody else who does these kinds of studies would claim causality associated with that level of risk. In fact, chance is the least of our worries down in that zone. All you have to do is have the groups be different with respect to one cigarette a day and you get that kind of a difference. Most of the concern about saccharin from the large bladder cancer study was in the 50 to 60 percent excess risk for those who took upwards of 320 or more milligrams of saccharin per day as an average, which was the observation and which was some cause for concern.

So, I think that probably when the epidemiologist gets through looking at his high dose people, and when the laboratory animal experimenter gets done, probably the only way we can determine what is a logical risk is by trying to integrate the two results.

Dr. Cortesi, EPA: In reference to Dr. Hoover's remarks, I did not mean to be critical of epidemiology but to emphasize that a lot of what we want to do in EPA is thinking ahead in the design of the experiment and could you give us some help on what the dose was? It makes life an awful lot easier when it comes to telling people that they have to spend $500,000 a year to do something they do not want to do.

Dr. Spirtas, NCI: I have just one comment on your question. I think that the setting of standards is probably going to be done in one of two ways, either by the way that it is being done now, by formula or picking a magic number, or else by a panel of experts. I believe that the present tendency in regulations is to try to have this picking done by the government agencies and that is causing a lot of controversy. There is room to think about having independent panels of experts or relying on some sort of consensus standard. This may evolve eventually. But there are alternative ways to pick standards besides having a regulatory agency pick them.

Dr. Cortesi, EPA: That is a very good point. Even if you know everything about the health effects of a substance, the picking of a standard is a political process and I firmly believe that it should be a political process. I do not think that you ought to have a panel of experts do it. But I do think that this is something that is well worth a lot of consideration because it is at the core of the problem. There is
misapprehension by a lot of the public that when you set a health based standard that what you know about the health should or should not be, depending on your point of view, the predominant effect on where you set the level.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

Tuesday Morning, May 6

EPIDEMIOLOGICAL/STATISTICAL SESSION (CONTINUED)

SESSION CHAIRPERSON

Dr. George Burton
National Cancer Institute
INDUSTRIAL EMISSIONS AND CANCER INCIDENCE
IN CONTRA COSTA COUNTY:
PROGRESS ON THE EPIDEMIOLOGICAL STUDY

U. S. Environmental Protection Agency
Grant No. R806396010

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Presented at the First NCI/EPA/NIOSH Collaborative Workshop: Progress
on Joint Environmental and Occupational Cancer Studies
Sheraton/Potomac, Rockville, Maryland
May 6-8, 1980

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ABSTRACT

INDUSTRIAL EMISSIONS AND CANCER INCIDENCE IN CONTRA COSTA COUNTY: PROGRESS ON THE EPIDEMIOLOGICAL STUDY

This research effort contains the following tasks: cancer incidence analyses, occupational monitoring, case control studies, and industrial emission analyses.

Cancer incidence analyses compare the age, sex, race, and site specific cancer incidence rates in the industrialized areas with the non-industrialized areas of Contra Costa County. Analyses for years 1972-1977 are complete and analyses for years 1969-1971 are in progress.

Occupational monitoring determines whether any labor union or occupational group has higher incidence rates for any cancer sites than do other union or occupational groups in Contra Costa County. Names of union members, employed persons, and professional groups are being merged with the cancer incidence files of Resources for Cancer Epidemiology Section (RCE). This task has been completed for six (6) occupational groups totaling over 6,000 members.

Case control studies attempt to identify environmental factors associated with cancer incidence in Contra Costa County. The sample design and the number and selection of cases and controls depended on results of the incidence analyses and occupational monitoring. Selection of cases, controls, and design of questionnaire are about 75% complete. Cases and controls or their families will be interviewed to obtain length of residence, socio-economic status, smoking habits, occupation, and exposure to other pollutants.
Industrial emission analyses include the collection of air samples and inorganic chemical analyses to determine the levels of ambient air pollution by census tract. The Ames Salmonella test is being used to test for mutagenicity of industrial emissions. Collection of air samples and chemical analyses of inorganic fraction are complete.

Acknowledgment:

This research is supported by EPA Grant #R806396010 with the State of California, Department of Health Services, Resources for Cancer Epidemiology Section.
INTRODUCTION

Contra Costa County continues to be the major industrialized county in the San Francisco Bay Area. This industrial complex, in addition to five major petroleum refineries and many petrochemical plants, included Kaiser's major shipbuilding center during World War II. At present, 70 percent of the shipping going through the Golden Gate Bridge either enters or leaves ports in Contra Costa County. Sixty-eight percent of the total stationary air pollution emissions in the San Francisco Bay Area originates in Contra Costa County.

Considerable speculation exists on how much air pollution contributes to cancer mortality in urban areas. Previous studies have linked air pollution to four anatomic sites—lung, stomach, prostate, and lymphoma. Mortality study of U. S. counties with petroleum industries found other sites with greater than expected frequencies. These results raise the question of how much of the excess cancer mortality is due to occupational exposures, to ambient air pollution exposure, or to other relevant variables.

Investigators encounter several problems in trying to identify or quantitate the contribution of each variable to the greater-than-expected cancer mortality. These problems are:

- Small size of study population;
- Latent period for cancer development;
Population density;
Smoking effect; and
Other associated variables not measured or included in the study.

The monograph on cancer mortality by counties (1950-1969) by Mason and McKay\textsuperscript{1} revealed high site specific cancer mortality rates in industrialized counties with petroleum refineries and petroleum chemical plants.

As part of the NCI/EPA collaborative research program, EPA completed the review process and funded a grant request from Contra Costa County Department of Health. This coincided with the publication of Blot's report in Science. Blot et al.\textsuperscript{2} compared cancer mortality rates in 39 counties with petroleum refineries employing at least 100 people with 117 control counties. Both men and women had a significantly higher lung cancer mortality rate in the petroleum refinery counties than in the control counties. High lung cancer mortality rates among women suggests ambient or personal (smoking or in-door) rather than occupational exposure. Men also had a significantly higher cancer mortality rate for several other site specific cancers. This publicity created many community pressures to take action in what was locally being called "cancer county." Industry countered by saying that San Francisco and Alameda counties had cancer rates as high as Contra Costa County. This issue became an emotional and a political battle between various adversaries which prevented the Contra Costa Department of Health from getting started on the study.

As a result of this impasse, the funds were transferred to California State Department of Health Services, Resources for Cancer Epidemiology, where the California Tumor Registry is located.
California State legislature and OSHA funded Dr. Austin's group to extend the cancer study to four additional counties (Figure 1). These are: San Francisco, Alameda, San Mateo, and Marin. San Francisco and Alameda counties have cancer mortality rates comparable to Contra Costa County.

Bear with me a moment please; we were not out of the woods yet. During the summer of 1978, the voters of California passed "Proposition 13", which resulted in the Executive Branch of the State Government freezing all personnel actions, including filling of positions. Thanks to the effort and support of the Department of Health Services, the effort and support of Mr. Paul DeFalco, Jr., Administrator, Region IX and his staff, and the picketing of Dr. Austin's office and the Health Department by various environmental groups, Dr. Austin was given permission in late June to complete the staffing of the project. This was completed by August 1, 1979.

The northern part of Contra Costa County is heavily industrialized with five major petroleum refineries, many petrochemical plants, and was the home of Kaiser shipbuilding during World War II. Many complaints have been raised by individuals and groups about the air quality in certain sections of Contra Costa County.

Population census was collected and data published at the census tract level for both 1970 and 1975 in Contra Costa County. Cancer incidence data has been collected in Contra Costa County since 1969. The Third National Cancer Survey collected incidence data between 1969 and 1971. The California Tumor Registry collected incidence data from 1972 onwards.
This study contains four major tasks. These are:

- Cancer incidence analyses;
- Occupational monitoring;
- Case control studies;
- Industrial emissions analyses

**Cancer Incidence Analyses:**

Preliminary analyses revealed a dramatic difference in lung cancer rates for males between the industrialized and non-industrialized parts of Contra Costa County for years 1972-1975. Years 1967-1971 are being added. The investigators have gone back to the 1970 and 1975 census data to validate the classification of each census tract as industrial or non-industrial. The analysis is being rerun for the extended period on the reclassified (validated) (industrial-non-industrial) census tracts.

The size of the difference between lung cancer incidence rates in the two parts of the county gives the highest priority for further investigation to this cancer site. Current funding for this grant includes the case-control study for lung cancer incidence.

**Occupational Monitoring:**

The purpose of occupational monitoring is to determine whether any labor union or occupational group has a higher incidence rate for any cancer site than other union or occupational groups in Contra Costa County. Names of union members, employed persons, and professional groups are being merged with the cancer incidence files of Resources for Cancer Epidemiology Section (RCE). RCE has collected identifying information on various cohort groups (Table 3). Matching has been completed for these. RCE has contacted many additional groups (Table 4).
Monitoring of occupational groups is an attempt to identify working groups with special cancer risks who, by their residential patterns, may affect the observed cancer rates of the population in a specific geographic area.

Case-Control Study:

The past two months have been spent in planning this study. A questionnaire has been circulated for comment and is being prepared for printing. The study is scheduled to start June 2, 1980.

Approximately 150 cases (lung cancer, men and women, white and black, ages 20-74) and 300 controls will be stratified and matched by age, sex, and race. The primary intent of the case-control study is to identify the major factors associated with the difference in lung cancer rates between the industrialized and non-industrialized parts of Contra Costa County. Some of the factors included are smoking history, occupation history, and geographic location of residence history.

This phase of the study examines the problems of latent period, occupational and environmental exposure history, migration history, smoking history, and socioeconomic class. The investigator may be able to get some suggestion as to the effect of family income below the poverty line since 15% of the workforce in Contra Costa County falls into this socio-economic class (Tables 1 and 2).

Industrial Emission Analyses:

Air emission collection sites were established in November 1978. The 15 station network contains 5 permanent (part of the Bay Area Air Management District) stations and 10 temporary stations (Figure 2). Air samples collected from November 1978 through October 1978 were analyzed.
for total suspended particulates, inorganic substances (lead, nitrates, sulfates), benzene-soluble organics, polycyclic aromatic hydrocarbons, and mutagenicity using the Ames test. Gas and meteorological data were collected during this one-year period.

Data are now being used in a modeling technique to characterize Contra Costa County census tracts by estimating a value for each of the air pollutants measured.

**ACCOMPLISHMENTS**

The first major accomplishment: We have a research effort underway after being caught between the various industrial, environmental, and political adversaries for two years. This project is staffed with a highly motivated and qualified staff who are interested in obtaining sound, valid scientific results.

Established the following Technical Advisory Committee:

- Dr. James Sandberg, Meteorologist;
- Dr. Alice Whitemore, Biostatistician; and
- Dr. Warren Winklestein, Jr., Epidemiologist.

Many staff tasks and activities have been completed during the past six months, for example, review of literature (Attachment A); meetings with Technical Advisory Committee, and the Citizen's Liaison Committee; meeting with persons, agencies and organizations in the effort to develop cooperative program and project relationships; and preparation of numerous in-house reports.

Drs. Austin and Mandel presented a paper at the 72nd Annual Meeting of the American Institute of Chemical Engineers, November 28, 1979, San
Francisco, California.³ They discussed the role and importance of population monitoring as a step to cancer prevention in professional chemists.

Analyses of air samples have been completed for five standard air pollutants in samples collected during July 1979 to October 1979. These samples are currently being analyzed for chemical carcinogens and for mutagenic activity, with a scheduled completion date of July 1980.

The findings and progress in the past six months are described in a report presented at the second symposium and the application of short-term bioassays in the fractionation and analysis of complex environmental mixtures at the Williamsburg, Virginia, March 4-7, 1980.⁴

Dr. Austin discovered, as a collateral development of the Contra Costa County and Bay Area cancer incidence study, what appears to be a sharp increase in incidence of malignant melanoma among employees of the Lawrence Livermore Laboratory. More than 18 months¹ work went into completing the status report,⁵ which is being reanalyzed by a panel of experts assembled by the Department of Energy. Nineteen cases of malignant melanoma occurred among laboratory employees between 1972 and 1977 including three deaths, among them the Laboratory Director. Since then six more cases have been reported, three in 1980.
REFERENCES


Figure 1. Bay area environmental cancer study counties.
Figure 2. Location of sampling stations in Contra Costa county, CA.
Table 1
CONTRA COSTA COUNTY
CIVILIAN LABOR FORCE BY RACE AND SEX
Projected, 1980

<table>
<thead>
<tr>
<th></th>
<th>Contra Costa County (excluding Richmond)</th>
<th>Richmond</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>288,500</td>
<td>36,000</td>
<td>324,500</td>
</tr>
<tr>
<td>Male</td>
<td>170,370</td>
<td>18,790</td>
<td>189,160</td>
</tr>
<tr>
<td>Female</td>
<td>118,130</td>
<td>17,210</td>
<td>135,340</td>
</tr>
<tr>
<td>Total white</td>
<td>273,010</td>
<td>14,570</td>
<td>287,580</td>
</tr>
<tr>
<td>Male</td>
<td>162,120</td>
<td>8,100</td>
<td>170,220</td>
</tr>
<tr>
<td>Female</td>
<td>110,890</td>
<td>6,470</td>
<td>117,360</td>
</tr>
<tr>
<td>Total black</td>
<td>7,880</td>
<td>19,310</td>
<td>27,190</td>
</tr>
<tr>
<td>Male</td>
<td>4,090</td>
<td>9,610</td>
<td>13,700</td>
</tr>
<tr>
<td>Female</td>
<td>3,790</td>
<td>9,700</td>
<td>13,490</td>
</tr>
<tr>
<td>Total others</td>
<td>7,610</td>
<td>2,110</td>
<td>9,720</td>
</tr>
<tr>
<td>Male</td>
<td>4,160</td>
<td>1,080</td>
<td>5,240</td>
</tr>
<tr>
<td>Female</td>
<td>3,450</td>
<td>1,030</td>
<td>4,480</td>
</tr>
<tr>
<td>(Spanish-American)**</td>
<td>(23,810)</td>
<td>(3,860)</td>
<td>(27,680)</td>
</tr>
<tr>
<td>Male</td>
<td>(14,490)</td>
<td>(2,100)</td>
<td>(16,590)</td>
</tr>
<tr>
<td>Female</td>
<td>(9,330)</td>
<td>(1,760)</td>
<td>(11,090)</td>
</tr>
</tbody>
</table>

*6,810 are age 65+ (2.1%)

**Already counted

Source: State of California, Employment Development Department, May 1, 1979.
### Table 2
CONTRA COSTA COUNTY
PERSONS BELOW POVERTY LEVEL, 1980

<table>
<thead>
<tr>
<th></th>
<th>Contra Costa County (excluding Richmond)</th>
<th>Richmond</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>31,970</td>
<td>2,250</td>
<td>34,220</td>
</tr>
<tr>
<td>Black</td>
<td>4,890</td>
<td>8,110</td>
<td>13,000</td>
</tr>
<tr>
<td>Other</td>
<td>1,050</td>
<td>390</td>
<td>1,440</td>
</tr>
<tr>
<td>Total</td>
<td>37,910</td>
<td>10,750</td>
<td>48,660</td>
</tr>
</tbody>
</table>

Table 3  
STATUS OF PAST COHORTS

<table>
<thead>
<tr>
<th>No. groups</th>
<th>No. locals</th>
<th>Approximate No. persons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Unions updated by EHA*</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>2. Unions to be updated**</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>3. Asbestos workers</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
| 4. Dry cleaners  
  Union members | 1 | 1 | 4,000 |
  Fabric care licensees | - | - |  
| 5. Cosmetologists | 1 | - | 20,500 |
| 6. Livermore Radiation Lab | 1 | - | 6,000 |
| Total | 21 | 76 | 60,750 |

*1. Bakers  
2. Painters  
3. Plasterers  
4. Plumbers & Steamfitters  
5. Roofers

**1. Cement Masons/Plasters  
2. Cooks/bartenders  
3. Firefighters  
4. Hod Carriers  
5. Industrial Iron Workers  
6. Lathers & Plasterers  
7. Laborers  
8. Oil, Chemical and Atomic Workers  
9. Painters  
10. Paint Makers  
11. Plumbers  
12. Steamfitters
<table>
<thead>
<tr>
<th>Group</th>
<th>Approximate No. persons</th>
</tr>
</thead>
<tbody>
<tr>
<td>State of California</td>
<td>18,000</td>
</tr>
<tr>
<td>Operating Engineers</td>
<td>6,900</td>
</tr>
<tr>
<td>Teamsters</td>
<td>6,500</td>
</tr>
<tr>
<td>Contra Costa County</td>
<td>6,000</td>
</tr>
<tr>
<td>State University</td>
<td>4,200</td>
</tr>
<tr>
<td>Apprentices</td>
<td>3,900</td>
</tr>
<tr>
<td>American Chemical Society</td>
<td>2,000</td>
</tr>
<tr>
<td>Oil, Chemical, Atomic Workers (1-1978)</td>
<td>1,600</td>
</tr>
<tr>
<td>Int'l Association Flight Attendants</td>
<td>1,000</td>
</tr>
<tr>
<td>City of Hayward</td>
<td>800</td>
</tr>
<tr>
<td>California Veterinarian Association</td>
<td>450</td>
</tr>
<tr>
<td>Oil, Chemical, Atomic Workers (1-326)</td>
<td>350</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>51,700</strong></td>
</tr>
</tbody>
</table>
ATTACHMENT A

SEVEN SELECTED MAJOR REFERENCE SOURCES ON OCCUPATIONAL CARCINOGENESIS
(Listed Chronologically)

I. Compilation of some 28,500 citations, including approximately 5,800 reprints, published during 1960 and 1975.

II. Approximately 1,450 abstracts on occupational and environmental carcinogenic hazards (1969 to 1974).


V. Listing of 584 abstracts of current cancer research on occupational and environmental carcinogenesis.

VI. Abstracts on cancer research epidemiology with many citations on occupations and cancer.

VII. Listing of 148 references in a summary article on occupationally-related carcinogens.

Compilations of 28,498 citations (including approximately 5,784 reprints) on occupational cancer published during 1960 to 1975. The citations were gathered from secondary sources (e.g., Index Medicus and Excerpta Medica). The citations have been classified by subject headings. Selected subject bibliographies have been prepared. This reference source was prepared under contract with the Occupational Cancer Data Bank of the George Washington University.


This compilation of approximately 1,450 abstracts was prepared for a conference on cancer registries and occupational cancers held in 1975. The abstracts were obtained from Volumes 7 through 11 (1969 to 1973) and the first two issues of Volume 12 (1974) of Carcinogenesis Abstracts. Where abstracts were not present, they were taken from Excerpta Medica, Biological Abstracts, Chemical Abstracts or from the article. There are three extensive indexes; namely, agents, sites, and agents-site-tumors.

The pages are not numbered. The references are listed by the volume and reference number in Carcinogenesis Abstracts. The publication brings together many abstracts dealing with occupational and environmental carcinogenic hazards.


This monograph contains the proceedings of a conference held on May 28, 1976. The many articles are grouped into nine sections with references following each article. These references serve as a comprehensive and varied listing of citations in the field of occupational carcinogenesis.


This study, performed at the Roswell Park Memorial Institute contains 233 references from the American and European literature during the years 1963 and 1974. The references cover occupations, industries, hazards, and cancers.

This listing contains 584 abstracts of current research projects in seven major categories; namely, asbestos; metallic salts or oxides; organic chemicals, specific occupations; pollution and other environmental factors; detection and measurement of environmental carcinogens; and other studies on environmental and occupational carcinogenesis.

The only index consists of an alphabetical listing of investigators.


This summary article on occupationally-related carcinogens contains 148 references. These references cover many of the important current topics in the field and can serve as an excellent source for persons interested in the subject matter.

NO DISCUSSION FOLLOWING THIS PAPER
An Etiologic Study of Respiratory Cancer in Coastal Texas
T.J. Mason and L.W. Pickle
Environmental Epidemiology Branch, National Cancer Institute

Since the turn of the century there has been a rapidly rising incidence of lung cancer in the United States and other countries. Recent mappings of U.S. cancer mortality statistics on a county level (Mason, et al, 1975, 1976) revealed elevated lung cancer mortality for white males in the northeast and near large metropolitan areas, with the highest rates clustered in contiguous counties along the Gulf of Mexico and the southeast Atlantic coast. Rates among white women and among blacks were also high in urban areas but did not show the southern coastal excess.

Although smoking accounts for a large fraction of lung cancer, other environmental determinants are involved to some extent, and may act synergistically with tobacco smoke as in the case of asbestos workers and uranium miners (Selikoff and Hammond, 1975). The precise contribution of occupational factors to the overall risk of lung cancer in the U.S. is uncertain, but the hazards may be more conspicuous and easier to identify in high risk areas than elsewhere. As an initial step toward explaining the geographic variation of lung cancer in the United States, a series of correlation studies was conducted (Blot, et al, 1976, 1977, 1979; Hoover and Fraumeni, 1975) linking cancer mortality rates with demographic and environmental data available at the county level. These studies suggested that lung cancer mortality rates were elevated in counties where the petrochemical, paper manufacturing, smelting, and shipbuilding industries are concentrated. Mortality rates for cancer of
the larynx, particularly for white males, were also elevated in counties where the shipbuilding industry had been concentrated during World War II. These excesses at the county level suggest that the environmental hazards associated with certain occupational exposures may have spread beyond the workplace.

More definitive case-control epidemiologic studies have recently been conducted in several high-risk areas of the country, particularly along the seacoast. In these areas, lung cancer patients and controls, or their next-of-kin, were interviewed to determine their lifetime histories of occupation, residence, tobacco consumption, and other environmental exposures. Interview studies of male residents of several counties in Georgia and coastal Virginia have shown an approximate 70% elevation of respiratory cancer risk among men employed in the shipbuilding industry during World War II (Blot, et al, 1978, 1980). Similar studies are now being conducted in Jacksonville, Florida, and in southern Louisiana.

Death certificate studies in Louisiana and Texas have suggested excess mortality from various cancers among former employees of the oil refining and shipbuilding industries (Gottlieb, et al, 1979; Thomas et al, 1980). Elevated lung cancer mortality was also seen among residents of Louisiana towns where the petroleum industry was a major employer, again raising the possibility that the exposures had spread beyond the workplace.

In order to pursue leads generated by these and other studies we have contracted with the University of Texas School of Public Health to conduct a case-control interview study of respiratory cancer in the Texas Gulf Coast area, a major shipbuilding center during World War II and site of a major concentration of petrochemical plants today.
Interviews will be conducted with approximately 2000 respiratory cancer patients and 2000 controls, or their next-of-kin, among white residents of Jefferson, Orange, Chambers, Galveston, Harris, and Brazoria counties in Texas. Cases are all white residents of the study area between the ages of 30 and 79 who were diagnosed as having lung cancer between July 1, 1976, and June 30, 1980, or laryngeal cancer (males only) between July 1, 1975 and June 30, 1980. Male lung cancer cases will not be ascertained from Harris county (Houston) so that the study will include approximately equal numbers of male and female subjects. Principal sources for case ascertainment will be the admissions/discharge lists and medical records of all adult medical/surgical and cancer hospitals in the study area. In addition, pathology reports and state death certificate tapes will be utilized.

Appropriate controls matched by sex, race, age, vital status, and county of residence (Harris vs. other) will be selected from the general population. Probable sources of control selection will be through drivers' license records and Medicare records for those aged 65 and over. Potential comparison subjects with chronic respiratory diseases or other smoking-related diseases will be excluded. Information will be gathered by face-to-face interviews on lifetime histories of residence, occupation, tobacco and alcohol consumption, medical history, and cancer history among the subject's family. A brief dietary supplement will be used to determine the subject's level of micronutrient intake, including vitamin A.

Most of the preliminary work has been completed for this project. Hospitals in the area have been contacted and the staff has been assembled. Interviewer training will be conducted in July, with the actual field work commencing by August. Interviewing is expected to continue for one year. Preliminary results should be available late in 1981.
Acknowledgement

This study is being conducted by the University of Texas School of Public Health, Patricia Buffler, Ph.D., Principal Investigator, under NCI/EPA contract #N01CP91025.
REFERENCES


Discussion

Dr. Bellin, EPA: In terms of history, are you going to question people as to their occupational history and health and how far back do you go?

Dr. Mason, NCI: To age 12. We are taking lifetime occupational histories, lifetime cigarette smoking, pipe, cigar or any other tobacco products. Because it is both lung and larynx in Texas, we will also be taking detailed alcohol consumption histories. We also take lifetime residential histories which get at townships, because there is a growing interest and commitment on our part to utilize information with regard to specific components of municipal water. So we are addressing the issue of when you lived, where you lived, what kind of water did you drink. This will tie in also with the parallel study which we are doing in New Jersey where there is ample opportunity for the same chemical exposure but with slightly different factors related to the exposure, not the least of which is climate.

Dr. Saffiotti, NCI: I was interested in your point about the study related to vitamin A levels. I would like to have some further information on how you are going to approach that.

Dr. Mason, NCI: Carefully.

Dr. Saffiotti, NCI: Particularly in this respect, that there are two possible types of data that you can try to correlate. One is simply vitamin A intake in the diet. The other is to try to get some biological marker data, as more and more information has developed in the last decade on the role of vitamin A in mechanisms of carcinogenesis. That role becomes more and more complex. It may well be linked to the availability of receptor sites and to host characteristics more than the total amount of retinoids taken with the diet. What kind of parameter are you going to relate?

Dr. Mason, NCI: What we are doing is we are concentrating on something like 28 particular foods, looking at usual portions, looking at methods of preparation, getting at estimates of levels of consumption. It will be done with both cases and controls. These are general population controls in both instances. We feel that taking this abbreviated form on what is going to be about 3,000 cases, which gives us a total of 6,000 persons, will improve our understanding with regard to what is happening in this instance. Then we are taking the detailed, approximately 45 minute dietary questionnaire, and interviewing a sample of both cases and controls in New Jersey. After that, I believe, we would then be in the position to consider perhaps some of the other things.

Right now, we are of the opinion that we can prioritize a series of foodstuffs. We can get at the vitamin A, vitamin C, etc. issues. We can do it in identified places with reasonable understanding of other factors as they relate. We obviously have to have all the detail on the person with regard to smoking and occupation and other such factors as they relate to the disease, and then we have an opportunity to follow it up. So it may well happen that next year at this time, when some preliminary things come from this, we could consider some of the subsequent more clinical laboratory types of investigations of some samples of persons in these places. That is how it is currently envisioned.
We have an historic interest in the State of New Jersey. In 1975, when I presented this map of bladder cancer among white males for the first time to the Society of Epidemiologic Research, the State Epidemiologist from New Jersey said, well, I assume we are going to have to follow this up. I said, yes, that seemed prudent. For the past year, we have had a very large commitment by the Environmental Epidemiology Branch to the State Department of Health and now the State Department of Environmental Protection in an attempt to try to characterize exposures and to quantify exposures and to utilize historic information to look for surrogates.

Let me explain. Last year when we were doing the National Survey of Environment and Health, which addressed the saccharin issue, we identified 1,257 newly diagnosed bladder cancer cases in the State of New Jersey, 1/3 of our total case load. This is over 12 months. It is not an insignificant problem. In the lung cancer study which I talked about earlier, we will identify 1,100 a year in five counties.

Cancer is an interesting problem in the State of New Jersey because you have an opportunity to look at some very specific exposures in real time. As we were able to show in the National Survey of Environment and Health, we can get results in humans in a comparable time frame for that which is possible in experimental animals.

Well, the New Jersey Department of Environmental Protection is really the EPA equivalent in New Jersey. The EPA has given over to them a number, if you will, of its responsibilities. They have certified a number of labs. They have historically attempted to characterize ambient exposures. They are in the business of monitoring discharge permits, and things such as that.

In an attempt to better understand and to follow up on some of the things that we had done when we identified associations between bladder cancer and the non-chloroform tri-halomethane complex, we decided to enter into a contract with the Department of Environmental Protection in New Jersey to do the following: (1) To define the service areas and average production, population served and raw water sources for all major water purveyors; (2) to determine historic and current chlorination practices of the major purveyors; (3) to identify those dischargers which may be potential pollution sources to unprotected surface water supplies; (4) to compile the results of all historic routine water quality monitoring for all major purveyors; and (5) to compile the results of all monitoring for toxic
and carcinogenic contaminants conducted by the U.S. EPA in the New Jersey Program on Environmental Carcinogenesis and Toxic Substances on both raw surface and ground water supplies and finished drinking water.

A large portion of these data were available in hard copy in filing cabinets in New Jersey and had not been computerized. We felt it important to fund this particular project. The timing of it got a little messed up in that we were not able to get it through all of its appropriate review on the other side of the fence before the end of the last fiscal year. So, we at the NCI, are funding it in its first year. It is approved for subsequent years of funding jointly.

I would like to share with you the success that we have realized so far. Basically, we have completed a tri-halomethane substudy where the study has examined the seasonal variations in THM levels in raw, treated and delivered water. Split samples have been sent to both EPA and the DEP labs. Initial results indicate excellent correlation between the labs as well as consistent ranking of THM levels with seasonal change. We believe this has some encouraging implications, because we are specifically pursuing some of the comments that have been made and some of the findings that have been reported.

There has been a one year contract executed with Rutgers University for the completion of all data forms in the water sampling of all purveyors. At the present time, all water purveyors serving 2,000 or more people have been sampled for organics and heavy metals. Approximately 40 purveyors serving under 2,000 could not be sampled because of an expiration of one of the contracts that they had. Approximately 1/3 of all the data forms have been completed, and we expect by midsummer to have the balance completed. There is an interagency agreement between the CEQ and the New Jersey DEP in order to make these data, which are collected in this particular manner, available to all individuals who have access to the upgrade system. They are also continuing on with regard to their own in-house computing capabilities.

So, basically, we are in a set of circumstances where we are attempting to facilitate the access to historic data, and to collect new information with regard to water quality. It ties in very nicely and it is one of the reasons that we are doing it in New Jersey because of the historic residence question and water source which was asked of all of these 1,257 bladder cancer cases and their controls last year. We then have an opportunity to say something with regard to their quality, with regard to the level of certain contaminants of interest to us and of interest to a number of other persons.
Discussion

Dr. Bull, EPA: I am a little curious about what other organic parameters you are looking at in the drinking water other than THM.

Dr. Mason, NCI: It is my understanding, and if Ken Cantor were here he could tell you exactly which ones are being looked at, but I think it is a reasonably broad spectrum, as far as characteristics.

Dr. Bull, EPA: Do you mean GC?

Dr. Mason, NCI: I believe, yes. That was my understanding going into this, that this would be the appropriate mechanism to use. We wanted to get at the THM's, but we also wanted to get a reasonable representation of what is there.

Dr. Bull, EPA: The total organic carbon and total organic chlorine.

Dr. Mason, NCI: Yes.

Unidentified Speaker: I may have missed this in the presentation, but how far back does the THM data go?

Dr. Mason, NCI: The THM data are current. It is for the past year or so. What we are doing is we are going back historically. A number of people have argued that you could relate some of the metals, if you will. If you could look at metals and other such things as are currently in the water and develop a reasonably predictive association, you could go back historically and look at chlorination levels and metals, which is all you are going to have historically. See, if that were true, could you say something with regard to potential levels? If you know parallel to that all of the discharges, which they have, could you then say something? They have an idea and they have some estimate, although I will not say exactly with what precision, but at least they can have an estimate there with regard to what could have been. If you recall, what we are trying to do is to complement our earlier study and afford yet another stratified analysis of bladder cancer in New Jersey.

Unidentified Speaker: Is colon turbidity still factored in?

Dr. Mason, NCI: I believe so, yes. That was my understanding.

Dr. Kraybill, NCI: What water sources are you going to be dealing with? Is this going to be ground water or surface water.

Dr. Mason, NCI: All sources. So, basically, what we are interested in is all water sources. There are a number of measurements that have been done with regard to private wells. I do not want to get into that, but we are interested in all of the different ways in which persons have gotten water. You can characterize the purveyors as their usual source and then you have
information with regard to dischargers as well as historic industrial profiles which get at an estimate of potential levels of contaminants and contaminants of a certain kind. But it is an attempt to try to characterize the water as it is being consumed by persons.

Dr. Kraybill, NCI: How do you epidemiologists control for the other variants?

Dr. Mason, NCI: We already have all the details on the persons.

Dr. Kraybill, NCI: I am referring to air pollutants and diet.

Dr. Mason, NCI: We have the history of smoking, occupation and residence histories on large numbers of cases and controls. So this is an attempt now to add an additional component to that, looking at some of the specifics in the water, because there are some certain hypotheses which have been suggested and there are some consistent findings.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Tuesday Afternoon, May 6

EPIDEMIOLOGICALSTATISTICAL SESSION (CONTINUED)

SESSION CHAIRPERSON

Dr. Kenneth Bridbord
National Institute for Occupational Safety and Health
IDENTIFICATION OF HIGH RISK OCCUPATIONAL GROUPS USING NOHS/RTECS

David H. Pedersen
Hazard Section, Surveillance Branch, DSHEFS
NIOSH - Cincinnati, Ohio

The National Institute for Occupational Safety and Health (NIOSH) has developed two data files, the Registry for the Toxic Effects of Chemical Substances (RTECS) and the National Occupational Hazard Survey (NOHS). The data from these two files can be used to assess the relative potential health risk to occupational and industrial groups arising from workplace chemical exposure.

In the course of this research effort, algorithms were developed which can be used to: (1) rank-order chemicals by their relative potential toxicological effect, (2) rank-order chemicals by simultaneous consideration of their relative toxicity and the number of people exposed to each under industrial conditions, (3) rank-order industries and occupations by relative potential health risk due to aggregate chemical exposure.

The algorithms producing these indices have been designed to accommodate a wide variety of research interests. Accordingly, indices of risk can be produced which emphasize such outcomes as dermatitis or cancer or which consider only certain types of animal or exposure route data. The purpose of this presentation is to describe how the indices produced by this modelling procedure have potential application in such disparate activities as prioritization of occupational health research needs, identification of occupations and industries with previously unrecognized hazardous chemical exposure problems and assistance in the allocation of health service resources.
The Surveillance Branch, Division of Surveillance, Hazard Evaluations, and Field Studies (DSHEFS), National Institute for Occupational Safety and Health (NIOSH), has a responsibility for the identification of high risk portions of the work force and for providing comparative assessment of their risk.

Two of the tools available to us in the accomplishment of this task are:

- The National Occupational Hazard Survey (NOHS) data base, which associates potential health hazards with occupational groups, industry types, and occupations within industries, and

- The NIOSH Registry for the Toxic Effects of Chemical Substances (RTECS), which is a compilation of published chemical toxicological data derived from review of the international literature.

Consideration of the data in RTECS and NOHS resulted in the initiation of a NIOSH contract effort, in which I served as project officer. This report is based on the results of that contract, which developed algorithms designed to:

1. Calculate an index number for each chemical found in both RTECS and NOHS which is representative of the relative health risk posed by an individual chemical.

2. Calculate an index number for each of those chemicals which modifies the individual chemical's index number by considering observations of chemical use in the workplace.

3. Calculate an index number which measures the relative health risk posed by the entire range of chemical exposures of workers in specific industries, occupations, and occupations within industries.

DEVELOPMENT OF A CHEMICAL RISK INDEX NUMBER

Development of a system to calculate a value known as the Hazard Risk Index (HRI) was the first task in this project.

The initial step in this task was to find out which chemicals were in both NOHS and RTECS. By matching on chemical abstract system (CAS) numbers, a total of 1,904 chemicals common to both bases was found.

The toxicity data in RTECS are reported by species, route of administration, and test end point, defined as "test classes," shown in Figure 1. Matrices were prepared which show the number of RTECS compounds in each of the test classes, as shown in Table 1.

(1) NIOSH contract #210-78-0076, "Identification of High Risk Occupational Groups and Industrial Processes Using RTECS/NOHS Data"
a. An acronym which stands for "Toxic Dose".
b. This is an abbreviation for the route of administration or entry of this substance.
c. This is an abbreviation for the species.
d. This is the type of dose reported.
e. This is the dose which caused the toxic effect.
f. The first part of this notation, "TFX," is an acronym which stands for "Toxic Effect." The last part of this notation refers to the organ system affected by the dose administered.
g. This is a code denoting the reference from which the toxic data was derived.
h. Volume number of the reference.
i. Page number of the reference.
j. These two digits stand for the year of publication, i.e., 1936.

Figure 1. A Typical Toxic Dose Entry From RTECS
Table 1. An Example RTECS Species/Route/Test End Point Data Distribution Matrix
It became obvious that certain test classes contained a very small number of compounds. A decision was therefore made to eliminate from further consideration those test classes with fewer than 100 chemicals. Almost all test classes with acute toxicity end points met this criteria, but significant portions of the chronic test data were excluded. To compensate for this, chronic test classes excluded by the 100 chemical threshold were combined by grouping species and routes into an "any species, any route," (any-any) test class. Certain similar species were also grouped. The final result of this effort was the 66 test classes shown in Table 2.

Because the RTECS dose data is reported in a number of different units, it was necessary to standardize the dose units by converting inhalation data to parts per million, and all other data to milligrams per kilogram of unit body weight.

Three special treatments of the data were necessary. First, all units were expressed in molar form. Second, the dosage was expressed in log form. Third, the Draize procedure (J. Pharmacol. Exp. Ther., 82:277-419, 1944) was used as a standard for irritation data.

It was necessary to develop a method for comparison of data across test classes as well as from chemical to chemical within a test class since no single test class covered all the chemicals common to RTECS and NOHS. This meant that dose data had to be normalized by expressing each dose in a test class ($d_i$) as a function of the range of doses in that class using the formula:

$$d_n = \frac{d_{\text{max}} - d_i}{d_{\text{max}} - d_{\text{min}}}$$

WHERE: $d_n$ = normalized dose
$d_i$ = observed dose
$d_{\text{max}}$ = maximum observed dose in the test class
$d_{\text{min}}$ = minimum observed dose in the test class

This normalization technique was selected because it results in positive numbers that range from zero to 1.0 within class. The maximum and minimum values for each test class and the number of RTECS records in each class are shown in Table 3.

The final step in manipulation of the dose data was to develop user options for (1) the generation of neoplastic dose data from carcinogesesis dose data, and (2) the use of neoplastic data as estimators of carcinogenic potential when only one of these effects was reported for a chemical.
Table 2. Test Classes* Selected for Use in HRIN Algorithm

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<th>Test Class</th>
<th>Route</th>
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<th>End Point</th>
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<td>orl-mus</td>
<td>CAR</td>
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* Test classes are defined in terms of route, species, and end point. RTECS abbreviations are used.
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<td>ipl-rod LD50</td>
<td>12664115</td>
<td>27741834</td>
<td>000489</td>
<td>orl-rod KEO</td>
<td>12368568</td>
<td>27777573</td>
<td>000195</td>
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<td>03561710</td>
<td>26685097</td>
<td>000507</td>
<td>orl-rod LD50</td>
<td>12368568</td>
<td>27777573</td>
<td>000195</td>
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<tr>
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<td>14087122</td>
<td>27337250</td>
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<td>12368568</td>
<td>27777573</td>
<td>000195</td>
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<td>27173447</td>
<td>000100</td>
<td>orl-rod LD50</td>
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<tr>
<td>ipl-rod LD50</td>
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<td>27332942</td>
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<td>12368568</td>
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<td>ipl-rod LD50</td>
<td>14979315</td>
<td>27959300</td>
<td>000166</td>
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<td>000195</td>
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<td>29163559</td>
<td>001211</td>
<td>orl-rod LD50</td>
<td>12368568</td>
<td>27777573</td>
<td>000195</td>
</tr>
</tbody>
</table>

* Units for the minimum and maximum values are ln millimoles per kilogram body weight, with the decimal point after the second digit.
A preliminary Hazard Risk Index (HRI) algorithm was developed using the normalized dose for each test class reported for a chemical, modified by the weighting factors shown in Table 4. Weights were based on the assumptions that: (1) higher animals most closely duplicate human response, (2) chronic outcomes are of greater importance than acute, (3) a large test class data population gives better comparisons between chemicals, (4) multiple-species testing of a chemical gives a better estimate of overall toxic effect, and (5) those routes most closely duplicating human industrial exposure (i.e. inhalation and skin/eye contact) are more relevant than other experimental modes of administration.

The algorithm multiplied the normalized dose number for each chemical by the relevant weights, summed the resulting figures, and divided the sum by the total of the weight factors to produce a weighted average indicating the relative toxicity of the chemical across all reported toxic effects as shown in Figure 2.

This preliminary model was submitted to a panel of toxicologists from industry, government, and academia. The panel concluded that: (1) the rank-ordering of hazards was not entirely correct, (2) misplacement of chemicals in the ranking resulted in part from combining acute, chronic and irritation data, (3) weighting factors seemed to contribute to improper chemical ranking, (4) the fact that no one test class or small group of test classes provided total coverage of the RTECS data was a significant limitation, (5) the use of normalized dose data appeared reasonable and useful, (6) the expression of dose data in molar form was consistent with the current views of pharmacologists and toxicologists, (7) chronic effects were insufficiently emphasized, (8) the procedure for handling primary irritation data could be improved, (9) the RTECS data may be skewed since only the lowest reported dose at which a particular effect appears is listed and the data is in all cases unevaluated. In recognition of these comments, the second version of the HRI was produced. This version produced a risk index composed of data on acute toxicity, primary irritation, carcinogenicity, neoplasticogenicity, teratogenicity, and other toxic effects categories (sub-HRI's) for each chemical. The HRI for the chemical was calculated by averaging those sub-HRI's for which there was data as the HRI for the chemical (See Figure 3). This averaging procedure was adopted to avoid the assumption that missing data equates to zero effect.

The final output of the algorithm is shown as Figure 4. The final form of the algorithm allows the user considerable flexibility in choosing which toxic effects to emphasize. The impact of any sub-HRI upon the final HRI can be increased or eliminated by using a multiplier (a, c, e, g, i, and k), or the user can cause chemicals with data in an area such as carcinogenesis to be emphasized by using sufficiently large constants (b, d, f, h, j, and l). In addition, the test class weights previously discussed can be used. In short, the "dials" on the model can be set to adapt the algorithm to any desired toxicological priority desired. In the project model discussed here, all multipliers and weights were set at 1.0 and all constants at zero.
Table 4. Weighting Factors Used in First Calculation of Hazard Risk Index Numbers

<table>
<thead>
<tr>
<th>Weight</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>hum</td>
</tr>
<tr>
<td>2</td>
<td>cat, dog</td>
</tr>
<tr>
<td>1</td>
<td>rat, mus, rbt, rod</td>
</tr>
<tr>
<td>0.5</td>
<td>bir, frg</td>
</tr>
</tbody>
</table>

Test Species:

Test End Point:

5        CAR, MUT
4        NEO
2        TER
1        All others

Number of Chemicals in Test Class:

<table>
<thead>
<tr>
<th>Number of Chemicals</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>500-1000</td>
</tr>
<tr>
<td>1</td>
<td>less than 500</td>
</tr>
</tbody>
</table>

Number of Different Species:

<table>
<thead>
<tr>
<th>Number of Species</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>3 or more</td>
</tr>
<tr>
<td>1.1</td>
<td>2</td>
</tr>
<tr>
<td>1.0</td>
<td>1</td>
</tr>
</tbody>
</table>

Route of Administration:

<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHL, eye, skn</td>
<td>4</td>
</tr>
<tr>
<td>All others</td>
<td>1</td>
</tr>
</tbody>
</table>
HRIN CALCULATION BASED ON RIECS DATA AS OF SEPTEMBER 1978

<table>
<thead>
<tr>
<th>HRIH</th>
<th>SPEC</th>
<th>HRIH</th>
</tr>
</thead>
<tbody>
<tr>
<td>AJ9625000</td>
<td>ACETIC ACID, TRIFLUORO-</td>
<td>.521</td>
</tr>
<tr>
<td>ipx-mus LLDo</td>
<td>2323</td>
<td>3.00</td>
</tr>
<tr>
<td>oxl-zat LD50</td>
<td>5064</td>
<td>3.00</td>
</tr>
<tr>
<td>WT = 6.00</td>
<td>N SPEC = 2</td>
<td>HRIN = .479</td>
</tr>
<tr>
<td>KL7525000</td>
<td>ETHANOL, 2,2'-METHYLIMINO)DI-</td>
<td>.406</td>
</tr>
<tr>
<td>eye-rbt SSSS</td>
<td>679</td>
<td>8.00</td>
</tr>
<tr>
<td>ipx-mus LD50</td>
<td>2323</td>
<td>3.00</td>
</tr>
<tr>
<td>oxl-zat LD50</td>
<td>5064</td>
<td>3.00</td>
</tr>
<tr>
<td>skn-rbt SSSS</td>
<td>1009</td>
<td>12.00</td>
</tr>
<tr>
<td>WT = 26.00</td>
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<td>HRIN = .475</td>
</tr>
<tr>
<td>KJ9100000</td>
<td>ETHANOL, 2-(2-BUTOXYETHOXY)-</td>
<td>.445</td>
</tr>
<tr>
<td>eye-rbt SSSS</td>
<td>679</td>
<td>8.00</td>
</tr>
<tr>
<td>ipx-mus LD50</td>
<td>9217</td>
<td>3.00</td>
</tr>
<tr>
<td>oxl-zat LD50</td>
<td>402</td>
<td>1.00</td>
</tr>
<tr>
<td>oxl-zod LD50</td>
<td>930</td>
<td>8.00</td>
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<tr>
<td>skn-rbt LD50</td>
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<td>8.00</td>
</tr>
<tr>
<td>WT = 23.00</td>
<td>N SPEC = 4</td>
<td>HRIN = .472</td>
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<tr>
<td>VB8225000</td>
<td>QUINOLINE, 6-ETHOXY-1,2-DIHYDRO-2,2,4-TRIMETHYL-</td>
<td>.453</td>
</tr>
<tr>
<td>ipx-mus LD50</td>
<td>2323</td>
<td>3.00</td>
</tr>
<tr>
<td>oxl-zat LD50</td>
<td>5064</td>
<td>3.00</td>
</tr>
<tr>
<td>WT = 6.00</td>
<td>N SPEC = 2</td>
<td>HRIN = .466</td>
</tr>
<tr>
<td>TH4330000</td>
<td>PHOSPHORUS SESQUISULFIDE</td>
<td>.461</td>
</tr>
<tr>
<td>oxl-zat LDLo</td>
<td>309</td>
<td>1.00</td>
</tr>
<tr>
<td>WT = 1.00</td>
<td>N SPEC = 1</td>
<td>HRIN = .461</td>
</tr>
<tr>
<td>E02975000</td>
<td>BUTYLAMINE</td>
<td>.471</td>
</tr>
<tr>
<td>inh-rat LDLo</td>
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<td>4.00</td>
</tr>
<tr>
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<tr>
<td>skn-rbt LD50</td>
<td>930</td>
<td>8.00</td>
</tr>
<tr>
<td>skn-rbt SSSS</td>
<td>1009</td>
<td>12.00</td>
</tr>
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<td>TOLUENE, 2,4,6-TRINITRO-</td>
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<tr>
<td>eye-rbt SSSS</td>
<td>679</td>
<td>8.00</td>
</tr>
<tr>
<td>oxl-cat LDLo</td>
<td>100</td>
<td>2.00</td>
</tr>
<tr>
<td>oxl-rat LDLo</td>
<td>891</td>
<td>2.00</td>
</tr>
<tr>
<td>oxl-rbt LDLo</td>
<td>309</td>
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<td>sub-rbt LDLo</td>
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<td>.503</td>
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<tr>
<td>ipx-mus LDLo</td>
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</tr>
<tr>
<td>ipx-rat LD50</td>
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<td>3.00</td>
</tr>
<tr>
<td>ivn-rbt LDLo</td>
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<tr>
<td>WT = 7.00</td>
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</tr>
</tbody>
</table>

Figure 2. Sample Page from First HRI Draft
FIGURE 3 - THE HRIN ALGORITHM

HRIN = \((a \times AT \oplus b) + (c \times PI \oplus d) + (e \times CAR \oplus f) + (g \times NEO \oplus h) + (i \times TER \oplus j) + (k \times TFX \oplus l)\)

\(\frac{N}{N}\)

Where:

- \(AT\) = the weighted average of all acute toxicity normalized doses, termed the acute toxicity sub-HRIN
- \(PI\) = the primary irritation sub-HRIN
- \(CAR\) = the carcinogenic sub-HRIN
- \(NEO\) = the neoplastic sub-HRIN
- \(TER\) = the teratogenic sub-HRIN
- \(TFX\) = the sub-HRIN derived from all other chronic toxic effects

Lower case letter a through l = weighting factors for which values are selected by the user

- \(N\) = the number of sub-HRIN's for which there are data
- \(\oplus\) = an addition that is performed only if the associated sub-HRIN is not equal to zero
<table>
<thead>
<tr>
<th>SEQ</th>
<th>20 H</th>
<th>T12450000</th>
<th>PHYSOSTIGMINE, SALICYLATE (1:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>any-amy TFX</td>
<td>650</td>
<td>1.00</td>
<td>.823</td>
</tr>
<tr>
<td>lpr-mus LD50</td>
<td>9486</td>
<td>1.00</td>
<td>.558</td>
</tr>
<tr>
<td>1yr-rat LD50</td>
<td>7194</td>
<td>1.00</td>
<td>.506</td>
</tr>
<tr>
<td>orl-mus LD50</td>
<td>5022</td>
<td>1.00</td>
<td>.799</td>
</tr>
<tr>
<td>sub-zbt LD50</td>
<td>2426</td>
<td>1.00</td>
<td>.614</td>
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<tr>
<td>sub-zth LD50</td>
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<td>1.00</td>
<td>.698</td>
</tr>
<tr>
<td>sub-zlt LD50</td>
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<td>.651</td>
</tr>
<tr>
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<td>CAR = .000</td>
<td>NEO = .000</td>
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</table>

<table>
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<tr>
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<th>CATION SULFATE (1:1)</th>
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<tr>
<td>any-amy TER</td>
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<td>.524</td>
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<tr>
<td>sub-deg LD50</td>
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<tr>
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<tr>
<td>sub-rat NEO</td>
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<tr>
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</table>

<table>
<thead>
<tr>
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<th>D19175000</th>
<th>BENZODIC ACID, 5-[(ETHYLAMINO)-5-(ETHYLAMINO)-2,7-DIMETHYL-3H-XANTHEN-9-YL]-</th>
</tr>
</thead>
<tbody>
<tr>
<td>lpr-mus LD50</td>
<td>2266</td>
<td>1.00</td>
<td>.899</td>
</tr>
<tr>
<td>sub-rat NEO</td>
<td>442</td>
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<td>.637</td>
</tr>
<tr>
<td>AT = .899</td>
<td>PI = .000</td>
<td>CAR = .637</td>
<td>NEO = .637</td>
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</tbody>
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<table>
<thead>
<tr>
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<th>UX6825000</th>
<th>PYROPHOSPHORIC ACID, TETRAETHYL ESTER</th>
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</thead>
<tbody>
<tr>
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<td>.748</td>
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<tr>
<td>lpr-mus LD50</td>
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<td>1.00</td>
<td>.527</td>
</tr>
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<td>.556</td>
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<tr>
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<tr>
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<td>ori-med LD50</td>
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<tr>
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<td>.997</td>
</tr>
<tr>
<td>sub-zht LD50</td>
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<td>.821</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>CAR = .000</td>
<td>NEO = .000</td>
</tr>
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</table>

<table>
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<th>STRYCHNINE</th>
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<td>.527</td>
</tr>
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<td>.733</td>
</tr>
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<td>.501</td>
</tr>
<tr>
<td>sub-rat LD50</td>
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<td>.494</td>
</tr>
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<td>lip-rat LD10</td>
<td>489</td>
<td>1.00</td>
<td>.817</td>
</tr>
<tr>
<td>orl-hum LD50</td>
<td>323</td>
<td>1.00</td>
<td>.725</td>
</tr>
<tr>
<td>orl-hum LD50</td>
<td>100</td>
<td>1.00</td>
<td>.940</td>
</tr>
</tbody>
</table>

Figure 4. HRI Sample Index Page
It was decided that since the HRI list was in fact a "relative risk" list, we should express exposed workers as a function of observed NOHS worker groups. (A worker group is defined as a population within NOHS with the same occupation, industry, or chemical exposure characteristics. See Table 5.) This was accomplished by expressing people exposed as:

$$\frac{\text{PES}}{\text{PEN}}$$

Where, by specified worker group:

PES is defined as the number of people noted in the NOHS data base as potentially exposed to a specific chemical hazard.

PEN is defined as the number of people noted in the NOHS data base as exposed to any chemical, physical, or biological hazard.

This "worker exposed ratio" allowed comparison of relative worker group risk. For example, we can compare the benzene exposure of one out of the forty (1/40) plumbers observed in SIC "A" to twelve out of the ninety (12/90) plumbers observed in SIC "B".

By assuming that part-time exposures (less than four hours per work day) resulted in half the risk of full-time exposures, exposure duration is represented in the algorithm by the expression $0.5 + 0.5 \times \text{PFT}$, where PFT = the percent of workers exposed to a chemical hazard more than four hours per work day.

To integrate information on the control practices used in conjunction with chemical exposures, "controlled exposures" were expressed as percent of all exposures controlled (PC). Since few control measures are 100% effective, the 90% control achieved by an acceptable respirator is used to express the general effectiveness of control measures. The expression for input of control data therefore became $(1.0 - 0.9 \times \text{PC})$.

WORKER GROUP RTECS/NOHS RISK INDICES

Worker group-specific risk indices are produced in the four general categories described below.

THE ADJUSTED HAZARD RISK INDEX (AHRI)

This index is based on the rationale that assessing the risk posed by a chemical hazard is dependent upon both the chemical's toxicity and the extent of worker exposure. That is, a highly toxic chemical with very restricted use may be of less concern than a widely used chemical which is less toxic.
<table>
<thead>
<tr>
<th>File Number</th>
<th>Number of Records</th>
<th>Worker Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,904</td>
<td>All workers without regard to industry or occupation.</td>
</tr>
<tr>
<td>2</td>
<td>29,548</td>
<td>Workers within an industry at the 2-digit SIC level, without regard to occupation.</td>
</tr>
<tr>
<td>3</td>
<td>74,859</td>
<td>Workers within an industry at the 3-digit SIC level, without regard to occupation.</td>
</tr>
<tr>
<td>4</td>
<td>113,248</td>
<td>Workers within an industry at the 4-digit SIC level, without regard to occupation.</td>
</tr>
<tr>
<td>5</td>
<td>78,078</td>
<td>Workers within an occupation without regard to industry.</td>
</tr>
<tr>
<td>6</td>
<td>305,466</td>
<td>Workers within an occupation within an industry at the 2-digit SIC level.</td>
</tr>
<tr>
<td>7</td>
<td>474,094</td>
<td>Workers within an occupation within an industry at the 3-digit SIC level.</td>
</tr>
<tr>
<td>8</td>
<td>550,020</td>
<td>Workers within an occupation within an industry at the 4-digit SIC level.</td>
</tr>
</tbody>
</table>
The algorithm used for production of this index is expressed as:

\[ AHRIN = \frac{HRIN \cdot PES}{PEN} (1.0 - 0.9 \ PC)(0.5 + 0.5 \ PFT)(K) \]

WHERE:

- **AHRIN** = Adjusted Hazard Risk Index Number
- **HRIN** = Hazard Risk Index Number
- **PES** = Number of people observed exposed to a specific chemical hazard in NOHS
- **PEN** = Number of people observed exposed to any NOHS hazard
- **PC** = Percent of controlled exposures
- **PFT** = Percent of full-time exposures
- **K** = A constant used to remove leading zeroes

An example of the output from the AHRIN algorithm is shown as Figure 5. All input factors are shown in the printout.

**THE INDUSTRIAL RISK INDEX (IRI)**

The rationale of the IRI is that industry worker group risk is a function of the summed risk associated with the chemicals to which the group is exposed.

Examination of the algorithm results indicates two products of this algorithm. This became apparent when considering the algorithm equation, which is expressed as:

\[ IRIN = \frac{HRIN \cdot PES}{PEN} (1.0 - 0.9 \ PC)(0.5 + 0.5 \ PFT)(N) \]

WHERE:

- **IRIN** = Industrial Risk Index Number
- **HRIN** = Hazard Risk Index Number
- **PES** = Number of people (specified SIC) exposed to an NOHS chemical hazard
- **PEN** = Number of people (specified SIC) exposed to any NOHS hazard
- **PC** = Percent of controlled exposures
- **PFT** = Percent of full-time exposures
- **N** = Population (specified SIC) from census data
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<thead>
<tr>
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<th>1</th>
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<th>TITANIUM OXIDE</th>
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<tr>
<td></td>
<td>2</td>
<td>ZE2195000</td>
<td>ETHYLENE (gassed)</td>
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<tr>
<td></td>
<td>3</td>
<td>KO6250000</td>
<td>METHYL ALCOHOL</td>
</tr>
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<td></td>
<td>4</td>
<td>XS5250000</td>
<td>TOLUENE</td>
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<td>5</td>
<td>YY7310000</td>
<td>SILICA, AMORPHOUS FUSED</td>
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<td>6</td>
<td>MT0500000</td>
<td>ISOPROPYL ALCOHOL</td>
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<tr>
<td></td>
<td>7</td>
<td>KJ2975000</td>
<td>ETHYLENE, 1,1,1-TRICHLORO-</td>
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<td></td>
<td>8</td>
<td>RD1200000</td>
<td>ALUMINUM OXIDE (2:3)</td>
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<td></td>
<td>9</td>
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<td>ETHYLENE, TRICHLORO-</td>
</tr>
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<td></td>
<td>10</td>
<td>EL6475000</td>
<td>2-BUTANONE</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>ZI1400000</td>
<td>BENOIC CHLORIDE</td>
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<tr>
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<td>12</td>
<td>VZ4725000</td>
<td>SODIUM CHLORIDE</td>
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<tr>
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<td>13</td>
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<tr>
<td></td>
<td>14</td>
<td>JJ7080000</td>
<td>DIPHENYLAMINE</td>
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<tr>
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<table>
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<th>BTECS DATE 01/79</th>
<th>RUN DATE 11/02/79</th>
<th>ROHS DATE 01/79</th>
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<td></td>
<td></td>
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</tbody>
</table>

**Figure 5.** AHRI Sample Index Page
Removal of the "N" modifier converts this algorithm to an expression of relative individual risk, rather than relative industry risk.

In reviewing NOHS worker group population data, a number of instances were noted where the observed PEN was less than 10. When this occurred, the PES/PEN ratios frequently approached 1.0. It was felt that this was probably an artifact of particular observations rather than a probability sample. It was decided that NOHS worker group data should be used only when the observed PES/PEN proportion was within a selected range of the Gaussian approximation. Using a confidence interval of .95 with an accuracy of ± 0.25, a minimum PEN size of 16 is derived which was used for this project. Based on these calculations, a 95% confidence interval of the IRIN based on the PEN value is provided in the output.

An example of the IRI output is shown as Figure 6.

THE OCCUPATIONAL RISK INDEX (ORI)

The rationale of the ORI is the same as that of the IRI for occupational groups.

The algorithm for production of the ORI is identical to that used for the IRI except that the NOHS data elements are for occupations regardless of industry.

The use of census data as a modifier and the expression of individual versus group relative risk apply equally to the ORI, as do the confidence interval and minimum sample size discussions.

An example of the ORI output is shown as Figure 7.

THE OCCUPATION WITHIN INDUSTRY RISK INDEX (OWIRI)

The rationale of the OWIRI is identical to that of the IRI and ORI for occupations within two-, three-, and four-digit SIC's.

The algorithm for the production of the OWIRI varies from that used for the IRI and ORI in two particulars: (1) the NOHS data elements are occupation within industry specific, and (2) no census data is available at this level of specificity, so a constant to remove leading zeroes is used.

The OWIRI output is shown in Figure 8.

Th sample size and confidence interval provisions are equivalent to those in the IRI and ORI.
**Figure 6. IRI Sample Index Page**

<table>
<thead>
<tr>
<th>SIC</th>
<th>Description</th>
<th>Number of Chemicals</th>
</tr>
</thead>
<tbody>
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<td>806</td>
<td>Hospitals</td>
<td>856</td>
</tr>
<tr>
<td>551</td>
<td>New and Used Car Dealers</td>
<td>293</td>
</tr>
<tr>
<td>580</td>
<td>Eating and Drinking Places</td>
<td>294</td>
</tr>
<tr>
<td>554</td>
<td>Gasoline Service Stations</td>
<td>194</td>
</tr>
<tr>
<td>508</td>
<td>Machinery, Equipment, and Supplies</td>
<td>247</td>
</tr>
<tr>
<td>809</td>
<td>Health and Allied Services, Nec</td>
<td>339</td>
</tr>
<tr>
<td>801</td>
<td>Offices of Physicians and Surgeons</td>
<td>251</td>
</tr>
<tr>
<td>599</td>
<td>Retail Stores, Nec</td>
<td>178</td>
</tr>
<tr>
<td>541</td>
<td>Grocery Stores</td>
<td>225</td>
</tr>
<tr>
<td>739</td>
<td>Miscellaneous Business Services</td>
<td>437</td>
</tr>
<tr>
<td>481</td>
<td>Telephone Communication</td>
<td>417</td>
</tr>
<tr>
<td>509</td>
<td>Miscellaneous Wholesalers</td>
<td>290</td>
</tr>
<tr>
<td>531</td>
<td>Department Stores</td>
<td>511</td>
</tr>
<tr>
<td>371</td>
<td>Motor Vehicles and Equipment</td>
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</tr>
<tr>
<td>651</td>
<td>Real Estate Operators and Lessors</td>
<td>298</td>
</tr>
<tr>
<td>275</td>
<td>Commercial Printing</td>
<td>336</td>
</tr>
<tr>
<td>701</td>
<td>Hotels, Tourist Courts, and Hotels</td>
<td>268</td>
</tr>
</tbody>
</table>
Figure 7. ORI Sample Index Page
Figure 8. OWIRI Sample Index Page
APPLICATION OF THE RISK INDICES

This system is designed to respond to user priorities. For example, user emphasis of potential carcinogens, and examination of the potential exposure patterns of the various NOHS worker groups to chemicals with this potential effect accomplishes several purposes: (1) the user is provided with a listing of all the potential carcinogens in RTECS and a listing of their other toxicological effects, (2) the user is presented a rank-ordered list of chemicals (AHRI) exhibiting carcino­genic potential based on both their toxicologic potential and the number of workers affected, (3) the user is provided a risk-ordered listing of various worker groups to aid in establishing priorities for field investigation or research, (4) using risk indices and chemical lists relevant to specific worker groups, a user could derive an overall risk number for a specified geographical area. Specifically, this concept might be utilized to forecast comparative health system demands in an area where the industrial composition is changing. For example, if a new facility of SIC X will double the employment in SIC X in the area, a rough approximation of the additional health system demand could be made.

CONCLUSION

This system, as presented, is a trial effort. Our intent is to make it useful to the occupational health community. To this end, in-house research is continuing in several areas.

Through manipulation of the algorithm, we are examining the effect of each component on the resulting risk index numbers.

We are also producing risk indices based only on specific outcomes (e.g. skin irritation) in order to identify worker groups at high risk and to examine the contribution of specific outcomes to the overall risk index number for various worker groups.

We intend to compare the results of these efforts with published data from such sources as the Bureau of Labor Statistics, Social Security Administration, and the National Center for Health Statistics using statistical techniques. The results of these conclusions will be used to direct necessary changes in the system.

Finally, we intend to continue evaluating alternate sources of toxicological and chemical exposure data, and to supplement or replace the data used in this project.
Discussion

Unidentified Speaker: Can you explain some of the figures on this slide?

Mr. Pedersen (NIOSH): I will give it a try. Let us take number 4, if you will, Occupation 635, metal platers. They are exposed to 108 of the chemicals from the matched set of 1,904, according to the NOHS database. That occupation within SIC 32 has the risk index number expressed at the right-hand side of the page. In that particular line we are addressing only metal platers employed in SIC 32.
Industrial Hygiene

Study of Workers Exposed

to Nitrosamines

WRITTEN BY:
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Industrial Hygiene Section
Industry-wide Studies Branch
Division of Surveillance, Hazard Evaluations, and Field Studies
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First NCI/EPA/NIOSH Collaborative Workshop
Progress on Joint Environmental and
Occupational Cancer Studies
Rockville, Maryland
May 6-8, 1980

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ABSTRACT

Under the NIOSH-sponsored contract, a total of 45 plant surveys were conducted at 37 separate manufacturing plants. The industries surveyed were the azo dyes, fish processing, fish meal, cutting fluid manufacturers and users, rubber and tanning. Airborne concentrations of Thermal Energy Analyzer (TEA) responsive compounds were found in all the industries except fish processing. The dye industry had airborne TEA responsive material as high as 40 ug/cu m, but they were not identified. Air levels of N-nitrosodiethanolamine were detected at 0.08 ng/cu m in a plant which uses cutting fluids. A fish meal factory was found to contain N-nitrosodimethylamine (NDMA) at 0.06 ug/cu m. In a chrome tannery NDMA was identified at 47 ug/cu m. The rubber industry has airborne levels of N-nitrosomorpholine as high as 250 ug/cu m.

This study has resulted in an increased understanding of man's exposure to performed N-nitrosamines. It is conceivable, from the information that has been generated in this study, that nitrosamine exposure as large as that in the tire and rubber industry exist in other industries not yet surveyed.
INTRODUCTION

The National Institute for Occupational Safety and Health (NIOSH) has conducted, under contract, environmental monitoring in a wide variety of industrial facilities to determine workers' exposure to N-nitrosamines. These compounds have been demonstrated to be highly toxic and potent carcinogens in laboratory animals (Druckrey et al., 1967). N-nitrosamines consist of a large family of compounds of which more than 100 of the 130 different N-nitroso compounds tested have been shown to be carcinogenic in a wide variety of animal species (Druckrey et al., 1969; Magee & Barnes, 1967; Magee & Schoental, 1964; Magee et al., 1976). Some of these compounds have been shown to be carcinogenic in rats with doses as low as 1 to 5 ppm of N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) in the diet.

N-nitrosamines are the N-nitroso derivatives of secondary amines with the general formula \( R_2N\cdotNO \), \( R_1 \) and \( R_2 \) being virtually any organic group. One of the simplest members of this family of compounds is N-nitrosodimethylamine \( CH_3\cdotN\cdotNO \). This compound is also a regulated carcinogen under part 1910.016 of the United States Occupational Safety and Health Standards. N-nitrosamines may be formed by the reaction of secondary amines (Mirvish, 1975; Scanlan, 1975) and nitrogen oxides, however, under appropriate conditions primary and tertiary amines (Smith, 1967; Ohshima & Kawabata, 1977) can also be nitrosated to produce these compounds.

The NO, or nitrosyl part of the compound, can be derived from nitrogen oxides such as NO, NO\(_2\), N\(_2\)O\(_4\), or N\(_2\)O\(_3\) (Challis et al., 1977) or from
nitrous acid or nitrite salts (Mirvish, 1975; Scanlan, 1975). N-nitrosamines can also be formed by transnitrosation whereby other nitro or nitroso compounds serve as the nitrosating agent (Singer et al., 1977; Buglass et al., 1974). N-nitrosamines are commonly made by the reaction of a secondary amine with sodium nitrite at acidic pH, however, depending on the reactant and catalysts that are used, N-nitrosation can also occur at neutral or alkaline pH (Fine, 1979). Compounds known to catalyze N-nitrosation include formaldehyde (Keefer & Roller, 1973), chloral (Keefer & Roller, 1973), ozone (Fine, 1977a), and some metal ions (Keefer, 1976).

The amine fragment of the N-nitroso compounds can be found in a large variety of both man-made and natural products. Secondary amines such as dimethylamine, diethylamine and morpholine are produced in large quantities and are used in both consumer and industrial products. These products are, for example, used in agricultural chemicals, detergents, rust inhibitors, rubber additives, solvents, drugs, plastics, leather tanning, textiles, cosmetics and synthetic cutting and grinding fluids. Given the widespread use of secondary amines and the ever present nitrogen oxides of an industrial society, the likelihood of N-nitrosamines being found in these products or in an industrial situation where these compounds may occur together, is high.

Recent advances in detection have made it possible to examine consumer and industrial products and the environment for N-nitrosamines. It has
been found that substantial numbers of people are indeed exposed. Levels which have been determined in commercial products and environmental samples range from parts per billion to percent amounts. Six human populations have been identified as having a potential exposure to significantly higher than background levels of carcinogenic \(\text{N-nitrosamines}\).

They are, chemical workers at a rocket fuel factory making unsymmetrical dimethylhydrazine (UDMH) from NDMA (Fine et al., 1976a), agricultural workers handling pesticides contaminated with nitrosamines (Fine, et al., 1977b), machinists using synthetic cutting and grinding fluids contaminated with \(\text{N-nitrosodiethanolamine (NDELA)}\) (Fan et al., 1977b), persons using facial cosmetics contaminated with \(\text{N-nitrosodiethanolamine}\) (Fan et al., 1977a), rubber chemical workers exposed to \(\text{N-nitrosomorpholine (NMOR)}\) (Fajen et al., 1979), and leather tanners exposed to \(\text{N-nitrosodimethylamine in tannery air (Rounbehler et al., 1979)}\). The probability that certain other occupations may involve exposure to \(\text{N-nitrosamines}\) is the basis of this NIOSH-sponsored study. While direct evidence for the carcinogenicity of \(\text{N-nitroso compounds in man is presently lacking, it is unlikely that man alone will be uniquely resistant to their carcinogenic action.\n
STUDY DESIGN

During the study, a total of 51 on-site plant visits were conducted at 37 separate manufacturing plants. These plants represented 5 different industries. The study was intended to determine if there was worker exposure to \(\text{N-nitrosamines}\). The basis for selecting the plants that were surveyed included:
- Known or suspected use of N-nitroso compounds
- Known use of products likely to contain N-nitroso compounds as impurities
- Use of chemicals that could give rise to N-nitroso compounds
- Epidemiological data which, along with the possibility of worker exposure to N-nitroso compounds, suggested a higher than usual risk of worker exposure to an environmental carcinogen
- Results of the study as it proceeded

In order to illustrate the level of N-nitrosamines found in the factory environment, the data from the rubber and leather tanning industries will be discussed. Nineteen factories were visited, nine associated with the manufacture of rubber and tire products, and ten associated with the leather trade. The rubber and tire industry was chosen because a variety of amines, nitrosamines, nitroso and nitro compounds are used in various aspects of the manufacturing process, including N-nitroso-diphenylamine (NDPhA), diethanolamine, and morpholine based accelerators (Rubber World, N.Y., 1975). In addition, rubber industry workers have been identified in several epidemiological studies as suffering from excess mortality from cancer of a variety of organs including lung, bladder, stomach, and prostate (McMichael et al., 1976). The leather industry was chosen because the tanning process includes a dimethylamine salt used in the dehairing processes (Walker et al., 1976). What little data is available on leather workers show an increased
tumor incidence of nasopharyngeal and bladder cancers among shoe and bootmakers (Cole, et al., 1972). Another group stated that the increased buccal, larynx, nasopharyngeal and bladder cancer incidence they observed among "leather industry operatives" (job sites not specified) might be related to the tanning process (Viadana, et al., 1976). In both cases the specific agents responsible for the excess cancer deaths have not been clearly identified.

DATA COLLECTION

The laboratory apparatus, including a Shimadzu programmable gas chromatograph, a high pressure liquid chromatograph, fume hood, and two Thermal Energy Analysers (TEA™) are located inside a fully equipped, self-contained mobile laboratory (Krull, et al., 1978), which was parked nearby each site.

The majority of the air samples were collected by two methods. The first method pulled air at a flow rate of between 1 and 2 L/min through a glass impinger containing 45 ml of 1N KOH. The second method used a Thermosorb/NR tube which consisted of 15 mm ID x 20 mm length tubes containing a mixture of magnesium silicate and an amine trapping (complexing) agent and a nitrosating inhibitor. The air samples were collected by drawing air through the traps at a constant rate of from 1.5 to 6 L/min for 5 to 200 minutes using a Bendix C115 pump or a 10 L/min metal bellows air pump. The Thermosorb/NR tubes were used to absorb N-nitroso compounds and to assess the presence of nitrosating agents in the sampled air (Rounbehler, et al., 1979). The nitrosating capacity
of the sampled air was estimated by measuring the amount of N-nitrosomor-
pholine formed from the reaction of morpholine, which was spiked on the
Thermosorb/N adsorbant, and whatever nitrosating agent may have
been present in the air. The other trapping methods used in collecting
samples were dry cellulose fiber traps, alkaline cellulose fiber and
Tenax GC cartridges.

Analytical techniques for the quantitative analysis of NDMA in air at
levels down to 0.001 µg/m$^3$ are available in the literature (Fine, et
al., 1976b; Fine, et al., 1977c). Analysis of nitrosamines has been
greatly simplified by the availability of the TEA (Fine, et al., 1975b)
designed to be nitroso specific. The TEA is used as a detector for both
gas chromatography (GC-TEA) (Fine, et al., 1976a) and high pressure
liquid chromatography (HPLC-TEA) (Fine, et al., 1976c). The TEA sim-
plifies the analysis because virtually no cleanup of the air sample is
required.

RESULTS

The industries surveyed by the NIOSH sponsored study were the azo dyes,
fish processing, fish meal, cutting fluid manufacturers and users, rubber
and tanning. Airborne concentrations of TEA responsive compounds were
found in all the industries except fish processing. The dye industry
had airborne TEA responsive material as high as 40 µg/m$^3$, but they were
not identified. Air levels of NDELA were detected at 0.08 ng/m$^3$ in a
plant that used cutting fluids. A fish meal factory was found to contain

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0.06 µg/m$^3$ of NDMA. The data generated on the leather and rubber industries will be discussed in further detail.

In an aircraft tire factory, NMOR was found to be present at levels between 0.6 and 27 µg/m$^3$. All 16 air samples which were collected inside the factory were positive for NMOR, with the average NMOR level being 4.85 µg/m$^3$. The highest NMOR levels were found where rubber was being cured and extruded. Further evidence as to the identity of NMOR was obtained by combining the samples and identifying the NMOR by GC-high resolution mass spectrometry (GC-MS).

In a chemical plant which was manufacturing rubber chemicals three N-nitrosamines were found: NMOR, NDMA and NDPhA. The NMOR levels varied from 0.07 µg/m$^3$ in the lunchroom to 4.6 µg/m$^3$ near the NDPhA reactor. The highest level of NDMA detected was 0.3 µg/m$^3$. NDPhA, which was being produced in the factory, was found to be present at most of the sites sampled. The highest NDPhA level found was 47 µg/m$^3$. A dirt sample, scraped from a staircase in the factory, contained 731 µg/g of NMOR and 15,000 µg/g of NDPhA. The presence of NMOR in the dirt sample was confirmed by GC-MS.

Further investigations were made at five tire manufacturing plants to determine if the levels in the aircraft tire plant were unique or if indeed nitrosamines were ubiquitous in the rubber industry. Each plant had NMOR levels similar to the aircraft tire plant (0.6 to 27 µg/m$^3$), however, one plant had 248 µg/m$^3$ of NMOR. Table I summarizes the N-nitroso compounds found in the rubber industry survey.
At the leather tannery, NDMA was found to be present in all the air samples which were taken inside the factory. The NDMA level varied from 0.1 µg/m³ in the lunchroom, to 47 µg/m³ inside the tannery, adjacent to the coloring and fat liquoring process. The average NDMA level in the 19 air samples which were collected was 17 µg/m³. Further evidence as to the identity of the NDMA was obtained by combining the samples and identifying the NDMA by GC-MS. Air samples taken at the doping area were also found to contain NMOR at the 2 µg/m³ level, plus smaller amounts of two unidentified TEA responsive compounds.

Further investigation of the leather industry was also made to determine if N-nitrosodimethylamine was unique to this plant. Nine other tanneries were surveyed for N-nitrosamines. Four tanneries had levels ranging from 0.03 – 10.8 µg/m³; N-nitrosamines were not detected in the other five plants. Table II summarizes the levels of nitrosamines found in the first tannery which was surveyed.

**DISCUSSION**

The question arises as to the source of the airborne N-nitrosamines in these two industries.

N-nitrosomorpholine was found in both the chemical factory and the factory where tires were being produced. In the chemical factory, N-nitrosomorpholine was found as an impurity in the morpholine (0.8 µg/g) and in the product, bismorpholinecarbamylsulfonamide (.4 to .7 µg/g),

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which is used as an accelerator. Also, the steam condensate contained 0.002 μg/g, possibly from the use of morpholine as a corrosion inhibitor in the steam process equipment.

We believe this to be the first report of N-nitrosomorpholine as an air pollutant. While the effects of NMOR by the inhalation route have not been tested, and its actions in humans are unknown, studies in animals by both oral and parenteral dosing have shown it to be carcinogenic to a variety of species. Shank and Newberne (1976) have reported increased incidence of liver angiosarcomas (15%), and lung angiosarcomas (9%) in rats fed a diet containing 5 μg/g (5ppm) NMOR.

In the leather tannery, NDMA was found at 0.5 μg/kg in an aqueous solution of dimethylamine sulfate which is a depilatory agent in the unhairing step. However, the NDMA impurity in the dimethylamine sulfate is insufficient, in this plant, to account for the level of NDMA in the plant environment. The causitive agent or agents responsible for the total environmental load of NDMA in the tannery has not been found; however, it can be speculated that the source of the airborne NDMA may be due to gas phase nitrosation of airborne amines by nitrogen oxides. It can be further speculated that the source of the airborne amines in these plants may be the dimethylamine sulfate and the dimethylamine produced or released during the unhairing process.
The significance of the tannery findings may be inferred from the results of a recent study by Moiseev and Benemansky (1975). They reported that 30 male Wistar rats breathing air containing 220 µg/m³ NDMA 24 hours/day for 25 months showed an incidence of malignant tumors of 83% in the exposed as compared to 13% in the control animals. These tumors were mainly of the liver and kidney. However, it is not possible to extrapolate the animal data on NMOR or NDMA, as these compounds have not been identified as human carcinogens.

**SUMMARY**

Under the NIOSH-sponsored contract, a total of 51 plant surveys were conducted at 37 separate manufacturing plants. The industries investigated were the fish processing, fish meal, manufacturers and users of cutting fluids, azo dye, leather and rubber.

NDELA, NMOR, NDMA, NDPhA were found in the environmental air of several factories. In a chrome tannery, NDMA was identified as high as 47 µg/m³ and NMOR at 248 µg/m³ in a rubber tire plant.

This study has resulted in an increased understanding of man's exposure to preformed N-nitrosamines. It is conceivable, from the information that has been generated in this study, that nitrosamine exposure as large as that in the tire and rubber industry exists in other industries not yet surveyed.

NIOSH is continuing its research on nitrosamines in the industrial environment.
REFERENCES


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<table>
<thead>
<tr>
<th>Location</th>
<th>NMOR$_3$ µg/m$^3$</th>
<th>NDMA$_3$ µg/m$^3$</th>
<th>NDPhA$_3$ µg/m$^3$</th>
<th>NYPR*$_3$ µg/m$^3$</th>
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</thead>
<tbody>
<tr>
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<td></td>
<td></td>
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</tr>
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<td>0.9, 4.6</td>
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<td>0.8, 0.6</td>
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<td>4.6, 3.9</td>
<td>0.05, 0.07</td>
<td>12, 25</td>
<td></td>
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<tr>
<td>Lunch room</td>
<td>0.07</td>
<td>0</td>
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<td></td>
</tr>
<tr>
<td>Outdoors</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>2. Industrial Rubber Products Factory</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Solution area</td>
<td>0</td>
<td>0.14</td>
<td>0</td>
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<td>Banbury machining</td>
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<td>0.14</td>
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<tr>
<td>Batch off mill area</td>
<td>0</td>
<td>0.09</td>
<td>0</td>
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<tr>
<td>Office</td>
<td>0</td>
<td>0.07</td>
<td>0</td>
<td></td>
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<tr>
<td><strong>3. Aircraft Tire Factory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curing press</td>
<td>2.2, 4.9</td>
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<tr>
<td>Extruder</td>
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<td>Extruder</td>
<td>27, 12</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td>Warm-up and mixing</td>
<td>2.2, 1.3</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Cooling pool</td>
<td>3.3</td>
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<tr>
<td>Cutting area</td>
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<td>0</td>
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<td>Large tire curing</td>
<td>7.1, 2.6</td>
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<td>Small tire curing</td>
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<td>Batchstock storage</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Finishing and inspection</td>
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<tr>
<td>Office</td>
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<td>0</td>
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<td>Outdoors</td>
<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td><strong>4. Synthetic Rubber and Latex Factory</strong></td>
<td></td>
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<tr>
<td>Four areas</td>
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<td><strong>5. Other Tire Plants</strong></td>
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<tr>
<td>Curing</td>
<td>1.5, 1.3, .03</td>
<td>.24</td>
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<tr>
<td>Extruder</td>
<td>22.0, 9.2, 8.5</td>
<td>2.0, .03</td>
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<tr>
<td>Warm-up mill</td>
<td>2.8, .73</td>
<td>0</td>
<td>0</td>
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<td>Calendar</td>
<td>248</td>
<td>2.9</td>
<td>12.4</td>
<td>2.6</td>
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<td>Warehouse</td>
<td>3.7</td>
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<td>Outdoors</td>
<td>0</td>
<td>0</td>
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<td></td>
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</tbody>
</table>

*N-nitrosopyrrolidine*
Table 2: N-Nitrosodimethylamine in Air Samples Collected in a Chrome Tannery

<table>
<thead>
<tr>
<th>Leather Process</th>
<th>NDMA (Area Air Samples)</th>
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</thead>
<tbody>
<tr>
<td>Hide Restoring</td>
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<tr>
<td>Soaking</td>
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<tr>
<td>Fleshing</td>
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</tr>
<tr>
<td>Unhairing</td>
<td></td>
</tr>
<tr>
<td>Bating</td>
<td></td>
</tr>
<tr>
<td>Pickling</td>
<td></td>
</tr>
<tr>
<td>Chrom Tanning</td>
<td></td>
</tr>
<tr>
<td>Splitting</td>
<td></td>
</tr>
<tr>
<td>Re-Tanning, Coloring, and Fat-Liquoring</td>
<td>N.S.</td>
</tr>
<tr>
<td>Color Set-Out</td>
<td></td>
</tr>
<tr>
<td>Drying</td>
<td></td>
</tr>
<tr>
<td>Conditioning</td>
<td></td>
</tr>
<tr>
<td>Staking</td>
<td>N.S.</td>
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<tr>
<td>Buffing</td>
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<tr>
<td>Doping Area</td>
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<tr>
<td>Seasoning</td>
<td></td>
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<tr>
<td>Spray Finish</td>
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<tr>
<td>Grading</td>
<td>N.S.</td>
</tr>
<tr>
<td>Shipping</td>
<td></td>
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<tr>
<td>Lunch Room</td>
<td></td>
</tr>
<tr>
<td>Outside Plant</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

μg NDMA/m³
Dr. Kraybill (NCI): I was intrigued when you mentioned fish processing. What was your rationale there?

Mr. Fajen (NIOSH): The natural amines that are in the fish. That is the one industry that did not contain any airborne nitrosamines.

Dr. Kraybill (NCI): That is correct.

Mr. Fajen (NIOSH): But your common fish sandwich that you can buy commercially does contain measurable levels of dimethyl-nitrosamine. The reason we went there was because of your natural amines that are in fish.

Dr. Kraybill (NCI): From where do you get your nitrite source?

Mr. Fajen (NIOSH): In the environment that you are working in, in the plant itself, one of the sources of nitrosation that we are interested in is your propane-powered forklifts. Especially in the tanning industry, they use an awful lot of propane-powered forklifts.

Unidentified Speaker: How specific was your method for detecting other nitroso compounds, other than the ones you may have been looking for initially? Did you detect other peaks and attempt to identify the substances that appeared?

Mr. Fajen (NIOSH): The mobile laboratory contains a library of standards. We can only detect those that we have standards on board for. We carry about ten different standards on each survey. When I say we found nitrosomorpholine, we would do a screening for many different nitrosamines and if one comes out at a specific retention time, we would try to match it with the standard. We also have GC and HPLC capabilities in both these industries, rubber and leather, the samples were confirmed by GC mass spectrometry. So we are not riding on the Thermal Energy Analyzer alone.
MORTALITY AND INDUSTRIAL HYGIENE STUDY OF WORKERS EXPOSED TO POLYCHLORINATED BIPHENYLS

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ACKNOWLEDGEMENTS

The authors would like to express their appreciation for the work of many individuals who helped to successfully complete this study including the guidance of Joseph Wagoner, Richard Lemen and Richard Waxweiler; the assistance of the clerical and secretarial staff in the Biometry Section; the data entry and data analysis provided by the Southwest Ohio Regional Computer Center; the cooperation of the companies and labor unions chosen for the study; and for the information provided by the New York State Department of Health.
Due to the demonstrated toxic effects from polychlorinated biphenyls (PCB's) on exposed animals, the National Institute for Occupational Safety and Health (NIOSH) conducted a retrospective cohort mortality study of two worker populations who manufactured electrical capacitors. The two study cohorts included 968 workers from Plant 1 and 1599 from Plant 2 who were employed for at least three months in areas of the plants where PCB's were used.

The vital status of over 97 percent of the two cohorts was determined as of January 1, 1976 and 38,890 person-years were accumulated. All-cause mortality was lower than expected (163 obs. vs. 174 exp.) as well as all cancer mortality (39 obs. vs. 40.6 exp.). Rectal cancer (4 obs. vs. 1.07 exp.) and liver cancer (3 obs. vs. 0.88 exp.) excesses were noteworthy although not statistically significant. In one of the plants, the observed mortality due to cirrhosis of the liver was also elevated. The results are discussed with regard to the detailed industrial hygiene surveys conducted in each plant.
Polychlorinated biphenyls (PCB's) are a class of compounds composed of biphenyl molecules with a varying number of substituted chlorine atoms. In commercially prepared PCB's, the weight-percent of chlorine has varied between 21 and 68 percent. In some preparations, there has also been some degree of contamination by chlorodibenzofurans. (1)

The primary use of PCB's has been as a liquid insulating material in electrical capacitors and transformers, and the greatest potential for occupational exposure has been in the manufacturing and repair of these components. PCB's have also been used in heat exchange units, hydraulic systems, vacuum pumps, gas transmission turbines, plasticizers, adhesives, pesticide extenders, paints, and carbonless copying papers.

As of 1971, PCB's were sold only for use in closed systems. According to the Toxic Substances Control Act of 1976, specific rules and regulations were promulgated to limit the manufacture and use of PCB's. This Act stipulated that all U.S. production of PCB's end January 1, 1979 and that all U.S. sale and distribution of PCB's end July 1, 1979. However, continual exposure to PCB's will occur among workers who maintain transformers and capacitors, and among the general population mainly through contaminated food.
During the past few years, there has been a great deal of interest in studying the health effects among individuals exposed to PCB's. This interest has been stimulated by: (a) the tendency for PCB's to accumulate in tissues and in certain organs, (2, 3) (b) the stability of PCB's and their persistence in the environment, (4, 5) and (c) the demonstrated long term effects in exposed laboratory animals (6-13) - including liver tumors and other liver diseases. Much of this interest was expressed at the National Conference on Polychlorinated Biphenyls in November, 1975 (14) and the toxicity of PCB's has been extensively reviewed in the NTOSH Criteria Document on PCB's. (15)

In order to determine whether or not past occupational exposure to commercially produced PCB's has caused any long term health effects, NIOSH initiated an epidemiologic study among workers in two capacitor manufacturing plants. In conjunction with this study, detailed industrial hygiene surveys were also conducted by NIOSH.

DESCRIPTION OF FACILITIES

The two facilities chosen for the study were selected after preliminary walk-through surveys were conducted at numerous types of plants where PCB's were used. Both of the plants manufacture electrical capacitors. These plants were selected because of their large workforce, the early dates (1938 & 1946) at which PCB's were
first used at these plants, the potential for exposure to PCB's with little potential for exposure to other known toxic contaminants, and the availability of records necessary to identify individuals to be included in the study population. At the time the study was initiated both plants were still using PCB's. Plant 1 is located in New York State and is divided into two manufacturing facilities within close proximity. One facility produced small industrial capacitors using PCB's since 1946 and the other produced large PCB filled power capacitors since 1951. The type of PCB's used has varied over the years from "Aroclor" (Aroclor is a Monsanto tradename) 1254 (54 percent chlorine) to 1242 (42 percent chlorine) to 1016 (41 percent chlorine). Several other kinds of oils were used, but in a small number of capacitors. These oils included castor oil, dibutyl sebaceate, diotylthalate and mineral oil.

Plant 2 is located in Massachusetts where the use of PCB's to manufacture capacitors started in 1938. This plant also changed the type of PCB's used from "Aroclor" 1254 to 1242 to 1016. Until 1972, other types of capacitors which did not contain PCB's were made at this plant including mica, electrolytic and tubular. Castor oil was used in lieu of PCB's to produce the large power capacitors at this plant.
Both plants assemble the capacitors using the same general techniques, whether they are the small or large types. The following is a brief description of the assembly process:

A. **Winding and Pre-assembly** - The inner components of the capacitor are made of paper, foil and sometimes plastic film, wound together, which are subsequently loaded into metal casings. This job is done in an enclosed dust-free room where there is minimal exposure to PCB's.

B. **Impregnation** - The pre-assembled capacitors are filled or impregnated with the PCB's. Within this area there is potential for exposure to PCB's.

C. **Final Assembly** - The tops of the capacitors are closed using various techniques – crimpping (rubber stoppers) or soldering, which involves some exposure to PCB's. The capacitors are washed to remove excess PCB's by running them through a detergent wash or a degreaser such as trichloroethylene. Finally, they are sent through the final operations involving drying, testing, and painting.

Other areas of importance where there is potential exposure to PCB's in the plants, include the laboratory and the area where rejected
capacitors are rebuilt. Approximately 10 percent of the two workforces have been employed in jobs where there has been potential exposure to PCB's.

Historically, the workforce at Plant 1 has been approximately 50 percent white males and 50 percent white females. Plant 2 has had a less homogeneous workforce with two-thirds being female and reflects the general ethnic make-up of the area, which is largely Cape Verdean and Portuguese.

METHODS

A retrospective cohort study of mortality was conducted to determine whether or not individuals occupationally exposed to PCB's have experienced any increase in cause-specific mortality. The study cohorts were defined as all workers who accumulated at least three months of employment any time between January 1, 1946 and January 1, 1976 for Plant 1 and January 1, 1940 and January 1, 1976 for Plant 2, in areas of the plants where there was a potential for exposure to PCB's. These exposed jobs were designated by the companies and verified by the labor unions, and through the industrial hygiene surveys.
An effort was made to determine the vital status (alive or deceased) of each individual in the cohorts as of January 1, 1976. Vital status was determined through records maintained by Federal and State agencies, including the Social Security Administration, state motor vehicle registration, and state vital statistics offices. For those individuals who could not be located through these sources, U.S. Postal Mail Correction Services and other follow-up searches were used. For all those who were known to be deceased, death certificates were requested and causes of death were interpreted by a qualified nosologist according to the International Classification of Diseases (ICDA) in effect at the time of death and then converted to the 7th Revision of the ICDA. Those who had an unknown vital status were assumed to be alive as of January 1, 1976 so that the true risk of mortality was not overestimated. Those who died after January 1, 1976 were considered alive for purposes of analysis.

Person-years were accumulated for each worker starting at the point in time when three months of employment in exposed jobs was completed and ending at the date of death or the study end date (1/1/76), whichever occurred first. Using a modified life table computer program similar to that described by Cutler, (16) the person-years for each cohort were combined into five-year calendar and five-year age time periods and multiplied by the corresponding U.S. white male (for male cohort members) and U.S. white female (for female cohort members)
cause-specific mortality rates to yield the expected number of deaths. Person-years were additionally distributed by five-year exposure and five-year latency (number of years from date first employed in exposed jobs to date of death or study end date) categories. Observed and expected cause-specific deaths were compared and differences were tested using the Poisson distribution.

The detailed industrial hygiene surveys included personal time-weighted air samples of employees from selected job titles, as well as area air samples. In both plants, samples were taken for PCB's (Aroclor 1016), trichloroethylene, lead, tin, and zinc. In addition, samples for toluene, methyl isobutyl ketone (MIBK), aluminum and iron were taken at Plant 1. These surveys were designed to characterize the exposures occurring at the time of the survey and may not represent exposures of previous years, especially those of Plant 1 where exposures may have been reduced because of new production techniques which had recently been initiated.

RESULTS

There were a total of 2,567 workers who met the definition of the study cohort. Table 1 gives a breakdown of vital status ascertainment and the number of person-years within each sub-cohort. The vital status ascertainment was more than 97 percent complete.
The possibility of missing records from the personnel files that were used to assemble the Plant 1 cohort was questioned at the initiation of the study. In an effort to determine whether or not eligible workers were missing from the plant 1 cohort, a validity check was conducted by the New York State Department of Health (personal communication from Phil Taylor, NYSDH, April, 1980), similar to that described by Marsh et al. (17). Social Security Administration (SSA) quarterly earning statements (SSA form 941) from 1945-1965 were obtained and compared to the names appearing on the microfilmed personnel records which were used to assemble the cohort. The results of this comparison yielded 35 additional workers (3.5 percent of cohort) who should have been included in the plant 1 study cohort. The vital status of these missing workers is not known at this time, however, the NYSDH is currently trying to ascertain this information. Nevertheless, the validity check confirmed that only a small portion of the total population at risk was missing from the study cohort and the results should not be biased. According to plant officials, there was no reason to believe that the personnel file system at Plant 2 was missing records, and it appeared from our inspection that the personnel file system had been maintained intact.

Tables 2 and 3 summarize the number of deaths observed from the study cohorts and the number of deaths expected. The all-cause mortality is lower than expected in each cohort, with an SMR (SMR = observed
deaths/expected deaths x 100) of 99 (73 obs. vs. 73.2 exp.) for Plant 1 and an SMR of 89 (90 obs. vs. 100.84 exp.) for Plant 2. These SMR's may be affected by the "healthy worker effect". (18) There was no increase in observed mortality for any of the major causes of death listed in Table 2.

Table 3 lists the observed and expected number of deaths by specific cancer cause and for cirrhosis of the liver. When both cohorts were combined, the observed number of deaths was more than that expected for cancer of the rectum (4 obs. vs. 1.07 exp.) and liver cancer – ICD=155, 156A (3 obs. vs. 0.88 exp.). The only statistically significant difference (at p<0.05) in observed versus expected deaths occurred in females from Plant 2 for cancer of the rectum (3 obs. vs. 0.46 exp.; p<0.05). For both cohorts combined, there were 6 deaths due to cirrhosis of the liver, while 5.47 were expected. Five of these cases were from the Plant 2 cohort while 3.1 were expected. According to hospital reports, at least three of the six persons who died of cirrhosis of the liver were known to have consumed alcohol on a regular basis.

The relationship between latency and the mortality from all cancer, cancer of the rectum, liver cancer, and cirrhosis of the liver is shown in Table 4. For "all cancer" there is no apparent pattern in either cohort. However, for cancer of the rectum, there is a slight
increase with an increase in the latency period. The risk of mortality due to cirrhosis of the liver does not show a consistent increase with an increase in the latency periods, however there is a greater risk after a 20 year period.

The relationship between these same causes of mortality and length of employment in PCB exposed areas of the plants is given in Table 5. As indicated in the table there is no increase in mortality with increasing lengths of exposure, except for cirrhosis of the liver, however; the numbers in this comparison are small.

The industrial hygiene survey results of area and personal sampling for PCB's (Aroclor 1016) are summarized in Tables 6 and 7. Due to differences in the production processes, the results by specific jobs or work areas are not comparable between the two plants. However, relative comparisons can be made and the range of concentrations observed in Plant 1 were lower than those in Plant 2. In Plant 1, the time weighted average (TWA) personal air samples ranged from 24 µg/m³ to 393 µg/m³ and the TWA area air samples ranged from 3 µg/m³ to 476 µg/m³. In Plant 2, the TWA personal air samples ranged from 170 µg/m³ to 1260 µg/m³ and the TWA area air samples ranged from 50 µg/m³ to 810 µg/m³. The current OSHA standard and ACGIH TLV for chlorodiphenyl (42 percent chlorine) is 1000 µg/m³. There is no current OSHA standard of ACGIH TLV for Aroclor 1016.
Trichloroethylene was measured near the degreasers in both plants. Out of eleven area air samples from Plant 1, all were below 35 ppm except for two which measured 195 ppm and 321 ppm. At Plant 2, three area air samples were taken which ranged from 53.4 ppm to 77.5 ppm. The OSHA standard for trichloroethylene is 100 ppm based on a 8 hour time weighted average.

Even though most exposures to trichloroethylene were usually below the TLV, an attempt was made to exclude workers from the study who were employed around the trichloroethylene degreasers.

Area air samples were measured for tin, lead and zinc near the soldering operations. There were no detectable levels for tin at either plant. Out of four samples collected for lead and zinc at Plant 1, lead was detected in one sample at a level of 12 µg/m³, zinc was detected on two samples at levels of 8 and 24 µg/m³. At Plant 2, fifteen samples were collected for lead and zinc. All but one of these samples showed no detectable levels for lead, the one detectable sample was 41.2 µg/m³. Six of the fifteen samples found concentrations of zinc ranging from 2.3 to 94.1 µg/m³. The current OSHA standard for lead and zinc oxide (reported as zinc) are 50 µg/m³ and 5 mg/m³ respectively.
Both personal and area samples were taken around the welding operations for measuring aluminum and iron at Plant 1. The aluminum samples ranged from non-detectable to 233 µg/m³ and the iron samples from 47 µg/m³ to 123 µg/m³. The ACGIH TLV for aluminum (Al₂O₃) is 10 mg/m³ and the current OSHA standard for iron oxide (measured as iron) is 10 mg/m³.

Twelve personal samples were collected for toluene and MTBK during painting operations at Plant 1. Toluene concentrations ranged from 0.48 to 22 ppm and MTBK ranged from 2 to 5 ppm. The current OSHA standard for toluene is 200 ppm and 100 ppm for MTBK.

Although the exposures to PCB's at the time of the surveys (Plant 1 - April, 1977; Plant 2 - March, 1977), were relatively higher in Plant 2, the, historic levels of exposure may have been more equivalent. It is these exposures that occurred 20 to 30 years ago that are more relevant when considering the occupational cancer risk among the study cohorts. The PCB mixtures used during these time periods were Aroclor 1254 and 1242, whereas, Aroclor 1016 was first used in 1971. In addition, several different stabilizers have been added to the PCB's (1 percent or less by weight) used at Plant 1 since the early 1960's. These include potential carcinogens such as diglyceride ether-disphenol-a and more recently, vinyl cyclohexene dioxide. It is not known which stabilizers have been used at Plant 2.
DISCUSSION

There are few previous epidemiologic studies that have examined the long term health effects of humans exposed to PCB's. Individuals poisoned by rice oil heavily contaminated with PCB's (Yusho Disease) have been studied extensively years after the incident took place in Japan in 1968. (19, 20) However, the rice oil contaminant also contained polychlorinated dibenzofurans, and quarter phenyls in higher concentrations than that found in commercially prepared PCB's. A high prevalence of skin and eye conditions were noted in the Yusho patients. In addition, there were clinical and laboratory findings that included changes in the microanatomy of liver cells and a decreased concentration of bilirubin in the serum of these individuals. (21, 22)

Early reports regarding the health effects from occupational exposure to PCB's include chloracne (23), digestive disturbances, eye irritation, liver injury and impotence. (24, 25) Most of these findings have been reported as case histories.

In a recent study of volunteers conducted by the Mount Sinai School of Medicine (26), 326 workers who were employed at Plant 1 were examined. The most prevalent symptoms noted were dermatological, and those of the central nervous system. There was a low prevalence of
abnormal liver findings on physical examination. However, a subgroup exposed to PCB's were found to have liver enzyme changes different from those of a normal, non-exposed group. In addition, abnormal SGOT levels were associated with plasma levels of PCB's. There was a relatively high prevalence of decreased lung capacity among a subgroup of 243 workers tested. (27)

In a preliminary report, Bahn (28) reported an increase in deaths due to malignant melanoma (2 obs. vs. 0.04 exp.) and cancer of the pancreas among 51 research and development employees and 41 refinery plant employees at a New Jersey petrochemical facility. These individuals were considered to have had some exposure to Aroclor 1254 during various periods between 1949 and 1957, along with exposure to other toxic and potentially carcinogenic compounds.

In a summary of case histories (G. Roush. Written communication to NIOSH, September, 1976) among approximately 300 workers employed in the manufacturing of PCB's, no malignant melanomas or pancreatic cancers were observed. However, among the death certificates of 50 former workers at this manufacturing facility, seven cases of lung cancer were observed whereas 2.7 cases were expected. The findings were preliminary and were not adjusted for age or smoking.
The previously reported findings of an increased risk for mortality due to malignant melanoma, cancer of the pancreas, and lung cancer among workers exposed to PCB's were not corroborated in the present study. There were no observed deaths due to malignant melanoma and only 1 observed death from pancreatic cancer while 1.77 were expected. There were 7 observed deaths from respiratory system cancer, whereas 7.69 were expected. The only categories of cancer in which the number of observed deaths were greater than expected were for cancer of the rectum and cancer of the liver and only slight increases for cancer of intestine except rectum, and breast cancer. When both cohorts and sex groups were combined none of the excesses were statistically significant at $P<0.05$. However, the excess in liver cancer is noteworthy because it is consistent with the toxicology data observed in laboratory animals exposed to PCB's, where effects have been noted in the liver (6-13). The slight increase in deaths due to cirrhosis of the liver in the Plant 2 cohort is also consistent with the notion that PCB's have a toxic effect on the liver.

In most occupational health studies where cancer mortality is being assessed, latency is an important variable; the hypothesis being that there is an increased risk of mortality once a certain time period after initial exposure has elapsed. In this study, this hypothesis is difficult to examine due to the small number of deaths. None of the causes of death analyzed according to latency clearly demonstrated
this association. Rectal cancer showed a slight increase with an increase in latency and cirrhosis of the liver showed an increase in risk with an increase in latency after 20 years.

There was no relationship between increasing lengths of employment in PCB exposed jobs and the risk of mortality due to cancer or cirrhosis of the liver.

When the cancer mortality is examined by plant, it is evident that most of the excesses occur in plant 2, especially among the female group. This finding may be related to heavier exposures to PCB's at plant 2 as indicated by the industrial hygiene results. In addition, there was an opportunity for earlier exposures at plant 2, potentially allowing for a longer latency period. However, this difference in mortality may be a function of the size of the cohorts (plant 1 only has half the number of person-years as plant 2) and thus simply be a statistical quirk.

A potential confounding variable or interaction variable in this study is the possible effect of alcohol ingestion on the observed increase (at Plant 2) in mortality from cirrhosis of the liver. However, this cannot be properly assessed in the present study since not enough is known about the ingestion of alcohol among the entire study cohort.
CONCLUSIONS

Due to a relatively small number of deaths, conclusions drawn from the results of this study are tentative.

Despite these study limitations, observed excesses for liver cancer and cirrhosis of the liver are consistent with previously reported findings on experimental animals exposed to PCB's, and suggest that there may be an association between these causes of death and occupational exposure to PCB's (Aroclor 1254 and 1242). The observed excess in cancer of the rectum related to PCB workers was unexpected and needs further investigation.
REFERENCES


15. NIOSH, CDC, PHS, DHEW. 1977. Criteria for a recommended standard...Occupational Exposure to polychlorinated biphenyls (PCB's).


Table 1

Vital Status of PCB Workers

<table>
<thead>
<tr>
<th></th>
<th>Plant 1</th>
<th></th>
<th>Plant 2</th>
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<tr>
<td></td>
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<td>Females</td>
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<tr>
<td>Known to be alive</td>
<td>520</td>
<td>360</td>
<td>880</td>
<td>633</td>
<td>836</td>
</tr>
<tr>
<td>Known to be deceased</td>
<td>55</td>
<td>18</td>
<td>73</td>
<td>28</td>
<td>62</td>
</tr>
<tr>
<td>Unknown vital status</td>
<td>8</td>
<td>7</td>
<td>15</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>583</td>
<td>385</td>
<td>968</td>
<td>675</td>
<td>924</td>
</tr>
<tr>
<td>Person-Years</td>
<td>7,800</td>
<td>5,181</td>
<td>12,981</td>
<td>9,191</td>
<td>16,718</td>
</tr>
</tbody>
</table>

55(2%)
### Table 2

**Observed and Expected Deaths (O/E) According to Major Causes Among PCB Workers**

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Plant 1</th>
<th>Plant 2</th>
<th>Total (SMR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>Nervous System (330-334, 345)</td>
<td>3/3.08</td>
<td>1/1.92</td>
<td>2/1.78</td>
</tr>
<tr>
<td>Circulatory System (400-468)</td>
<td>26/22.05</td>
<td>7/6.64</td>
<td>14/13.87</td>
</tr>
<tr>
<td>Accidents (800-962)</td>
<td>7/5.86</td>
<td>1/1.06</td>
<td>3/7.26</td>
</tr>
<tr>
<td>All Other Causes</td>
<td>10/12.06</td>
<td>5/4.94</td>
<td>6/9.53</td>
</tr>
<tr>
<td>All Causes</td>
<td>55/52.02</td>
<td>18/21.18</td>
<td>28/38.57</td>
</tr>
</tbody>
</table>
Table 3

Oberved and Expected Deaths (O/E) According to Specific Cancer Causes and Cirrhosis of the Liver Among PCB Workers

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Plant 1</th>
<th>Plant 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>(7th Revision ICD No.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Malignant Neoplasms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach (151)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine exp. Rectum (152, 153)</td>
<td>1/0.79</td>
<td>0/0.67</td>
<td>0/0.51</td>
</tr>
<tr>
<td>Rectum (154)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biliary Pass Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver not specified (155, 156A)</td>
<td>1/0.19</td>
<td>0/0.14</td>
<td>0/0.11</td>
</tr>
<tr>
<td>Pancreas (157)</td>
<td>0/0.50</td>
<td>1/0.24</td>
<td>0/0.32</td>
</tr>
<tr>
<td>Respiratory System (160-164)</td>
<td>5/3.15</td>
<td>1/0.66</td>
<td>0/2.14</td>
</tr>
<tr>
<td>Breast (170)</td>
<td>------</td>
<td>1/1.83</td>
<td>------</td>
</tr>
<tr>
<td>Lymphatic &amp; Hematopoietic</td>
<td>0/1.00</td>
<td>0/0.50</td>
<td>0/0.83</td>
</tr>
<tr>
<td>(200-205)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1/3.58</td>
<td>1/2.22</td>
<td>2/1.77</td>
</tr>
<tr>
<td>Cirrhosis of Liver (581)</td>
<td>1/1.66</td>
<td>0/0.71</td>
<td>2/1.22</td>
</tr>
</tbody>
</table>

* p<0.05
Table 4

Observed and Expected Deaths According to Latency\textsuperscript{1}

Among PCB Workers

I. All Cancers

<table>
<thead>
<tr>
<th>Latency (years)</th>
<th>Plant 1</th>
<th></th>
<th>Plant 2</th>
<th></th>
<th>Plants 1 &amp; 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>E</td>
<td>SMR</td>
<td>O</td>
<td>E</td>
</tr>
<tr>
<td>&lt; 10 yrs.</td>
<td>6</td>
<td>4.83</td>
<td>124</td>
<td>6</td>
<td>7.29</td>
</tr>
<tr>
<td>10-&lt;20 yrs.</td>
<td>3</td>
<td>6.07</td>
<td>49</td>
<td>16</td>
<td>10.24</td>
</tr>
<tr>
<td>&gt; 20 yrs.</td>
<td>4</td>
<td>4.65</td>
<td>86</td>
<td>4</td>
<td>7.50</td>
</tr>
</tbody>
</table>

II. Cancer of Rectum (ICD = 154)

<table>
<thead>
<tr>
<th>Latency (years)</th>
<th>Plant 1</th>
<th></th>
<th>Plant 2</th>
<th></th>
<th>Plants 1 &amp; 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>E</td>
<td>SMR</td>
<td>O</td>
<td>E</td>
</tr>
<tr>
<td>&lt; 10 yrs.</td>
<td>0</td>
<td>0.13</td>
<td>---</td>
<td>0</td>
<td>0.19</td>
</tr>
<tr>
<td>10-&lt;20 yrs.</td>
<td>0</td>
<td>0.17</td>
<td>---</td>
<td>2</td>
<td>0.26</td>
</tr>
<tr>
<td>&gt; 20 yrs.</td>
<td>1 *</td>
<td>0.13</td>
<td>769</td>
<td>1</td>
<td>0.18</td>
</tr>
</tbody>
</table>

III. Liver Cancer (ICD = 155, 156A)

<table>
<thead>
<tr>
<th>Latency (years)</th>
<th>Plant 1</th>
<th></th>
<th>Plant 2</th>
<th></th>
<th>Plants 1 &amp; 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>E</td>
<td>SMR</td>
<td>O</td>
<td>E</td>
</tr>
<tr>
<td>&lt; 10 yrs.</td>
<td>1</td>
<td>0.09</td>
<td>1111</td>
<td>1</td>
<td>0.15</td>
</tr>
<tr>
<td>10-&lt;20 yrs.</td>
<td>0</td>
<td>0.13</td>
<td>---</td>
<td>1</td>
<td>0.23</td>
</tr>
<tr>
<td>&gt; 20 yrs.</td>
<td>0</td>
<td>0.11</td>
<td>---</td>
<td>0</td>
<td>0.16</td>
</tr>
</tbody>
</table>

IV. Cirrhosis of Liver (ICD = 581)

<table>
<thead>
<tr>
<th>Latency (years)</th>
<th>Plant 1</th>
<th></th>
<th>Plant 2</th>
<th></th>
<th>Plants 1 &amp; 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>E</td>
<td>SMR</td>
<td>O</td>
<td>E</td>
</tr>
<tr>
<td>&lt; 10 yrs.</td>
<td>1</td>
<td>0.79</td>
<td>127</td>
<td>1</td>
<td>0.95</td>
</tr>
<tr>
<td>10-&lt;20 yrs.</td>
<td>0</td>
<td>0.98</td>
<td>---</td>
<td>1</td>
<td>1.32</td>
</tr>
<tr>
<td>&gt; 20 yrs.</td>
<td>0</td>
<td>0.59</td>
<td>---</td>
<td>3</td>
<td>0.85</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Latency = number of years from date first employed in exposed job.

* p<0.05
### Table 5

**Observed and Expected Deaths According to Length of Exposure Among PCB Workers**

#### I. All Cancers (ICD = 140-205)

<table>
<thead>
<tr>
<th>Length of Employment</th>
<th>Plant 1</th>
<th>Plant 2</th>
<th>Plants 1 &amp; 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E SMR</td>
<td>E SMR</td>
<td>E SMR</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&lt; 5 yrs.</td>
<td>11.49</td>
<td>96</td>
<td>17.97</td>
</tr>
<tr>
<td>5-9 yrs.</td>
<td>2.59</td>
<td>39</td>
<td>3.65</td>
</tr>
<tr>
<td>10-14 yrs.</td>
<td>0.84</td>
<td>--</td>
<td>2.01</td>
</tr>
<tr>
<td>15-19 yrs.</td>
<td>0.59</td>
<td>169</td>
<td>0.89</td>
</tr>
<tr>
<td>≥ 20 yrs.</td>
<td>0.08</td>
<td>--</td>
<td>0.51</td>
</tr>
</tbody>
</table>

#### II. Cancer of Rectum (ICD = 154)

<table>
<thead>
<tr>
<th>Length of Employment</th>
<th>Plant 1</th>
<th>Plant 2</th>
<th>Plants 1 &amp; 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E SMR</td>
<td>E SMR</td>
<td>E SMR</td>
</tr>
<tr>
<td>&lt; 5 yrs.</td>
<td>0.32</td>
<td>313</td>
<td>0.45</td>
</tr>
<tr>
<td>5-9 yrs.</td>
<td>0.08</td>
<td>--</td>
<td>0.10</td>
</tr>
<tr>
<td>10-14 yrs.</td>
<td>0.03</td>
<td>--</td>
<td>0.05</td>
</tr>
<tr>
<td>15-19 yrs.</td>
<td>0.02</td>
<td>--</td>
<td>0.02</td>
</tr>
<tr>
<td>≥ 20 yrs.</td>
<td>0.001</td>
<td>--</td>
<td>0.01</td>
</tr>
</tbody>
</table>

#### III. Liver Cancer (ICD = 155, 156A)

<table>
<thead>
<tr>
<th>Length of Employment</th>
<th>Plant 1</th>
<th>Plant 2</th>
<th>Plants 1 &amp; 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E SMR</td>
<td>E SMR</td>
<td>E SMR</td>
</tr>
<tr>
<td>&lt; 5 yrs.</td>
<td>0.24</td>
<td>417</td>
<td>0.40</td>
</tr>
<tr>
<td>5-9 yrs.</td>
<td>0.06</td>
<td>--</td>
<td>0.08</td>
</tr>
<tr>
<td>10-14 yrs.</td>
<td>0.02</td>
<td>--</td>
<td>0.05</td>
</tr>
<tr>
<td>15-19 yrs.</td>
<td>0.01</td>
<td>--</td>
<td>0.01</td>
</tr>
<tr>
<td>≥ 20 yrs.</td>
<td>0.001</td>
<td>--</td>
<td>0.01</td>
</tr>
</tbody>
</table>

#### IV. Cirrhosis of the Liver (ICD = 581)

<table>
<thead>
<tr>
<th>Length of Employment</th>
<th>Plant 1</th>
<th>Plant 2</th>
<th>Plants 1 &amp; 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E SMR</td>
<td>E SMR</td>
<td>E SMR</td>
</tr>
<tr>
<td>&lt; 5 yrs.</td>
<td>1.76</td>
<td>57</td>
<td>2.22</td>
</tr>
<tr>
<td>5-9 yrs.</td>
<td>0.38</td>
<td>--</td>
<td>0.46</td>
</tr>
<tr>
<td>10-14 yrs.</td>
<td>0.11</td>
<td>--</td>
<td>0.22</td>
</tr>
<tr>
<td>15-19 yrs.</td>
<td>0.09</td>
<td>--</td>
<td>0.12</td>
</tr>
<tr>
<td>≥ 20 yrs.</td>
<td>0.02</td>
<td>--</td>
<td>0.07</td>
</tr>
</tbody>
</table>
### Table 6

Concentrations of PCB's (Aroclor 1016) at Plant 1 - Taken April, 1977

#### A. Power Capacitor Manufacturing Facility

<table>
<thead>
<tr>
<th>Job Titles</th>
<th>No. of Samples</th>
<th>Time (minutes)</th>
<th>TWA* (g/m)</th>
<th>Location</th>
<th>No. of Samples</th>
<th>Time (minutes)</th>
<th>TWA* (g/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery Repair</td>
<td>2</td>
<td>840</td>
<td>298</td>
<td>Test &amp; Paint</td>
<td>2</td>
<td>840</td>
<td>41</td>
</tr>
<tr>
<td>Salvage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operator</td>
<td>1</td>
<td>426</td>
<td>155</td>
<td>Assembly</td>
<td>2</td>
<td>851</td>
<td>29</td>
</tr>
<tr>
<td>EMF Operator</td>
<td>1</td>
<td>431</td>
<td>115</td>
<td>Shipping</td>
<td>1</td>
<td>426</td>
<td>16</td>
</tr>
<tr>
<td>Treat Helper</td>
<td>2</td>
<td>867</td>
<td>80</td>
<td>Storage</td>
<td>1</td>
<td>427</td>
<td>14</td>
</tr>
<tr>
<td>Treat Operator</td>
<td>2</td>
<td>731</td>
<td>66</td>
<td>Winding</td>
<td>1</td>
<td>420</td>
<td>3</td>
</tr>
<tr>
<td>Repair</td>
<td>1</td>
<td>422</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### B. Small Capacitor Manufacturing Facility

<table>
<thead>
<tr>
<th>Moveman (Sealing Area)</th>
<th>No. of Samples</th>
<th>Time (minutes)</th>
<th>TWA* (g/m)</th>
<th>Location</th>
<th>No. of Samples</th>
<th>Time (minutes)</th>
<th>TWA* (g/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>689</td>
<td>393</td>
<td>Soldering</td>
<td>2</td>
<td>782</td>
<td>476</td>
<td></td>
</tr>
<tr>
<td>Moveman (Testing Soldering Area)</td>
<td>3</td>
<td>1306</td>
<td>220</td>
<td>Assembly</td>
<td>2</td>
<td>834</td>
<td>45</td>
</tr>
<tr>
<td>827</td>
<td>115</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testing</td>
<td>3</td>
<td>1290</td>
<td>218</td>
<td>Shipping</td>
<td>2</td>
<td>838</td>
<td>56</td>
</tr>
<tr>
<td>Packer</td>
<td>3</td>
<td>1287</td>
<td>199</td>
<td>Winding</td>
<td>2</td>
<td>828</td>
<td>54</td>
</tr>
<tr>
<td>Treat Operator</td>
<td>2</td>
<td>845</td>
<td>160</td>
<td>Can</td>
<td>2</td>
<td>836</td>
<td>51</td>
</tr>
<tr>
<td>Rework &amp; Final Assembly</td>
<td>2</td>
<td>824</td>
<td>152</td>
<td>Manufacturing</td>
<td>2</td>
<td>834</td>
<td>45</td>
</tr>
<tr>
<td>Maintenance</td>
<td>1</td>
<td>404</td>
<td>150</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rework Tester</td>
<td>1</td>
<td>433</td>
<td>140</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rework Packer</td>
<td>1</td>
<td>435</td>
<td>132</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rework Tester Solder</td>
<td>1</td>
<td>271</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* TWA is calculated over the total sampling time period.
Table 7
Concentrations of PCB's (Aroclor 1016) at Plant 2 - Taken March, 1977

<table>
<thead>
<tr>
<th>Job Titles</th>
<th>No. of Samples</th>
<th>Total Sampling Time (minutes)</th>
<th>TWA* (g/m)</th>
<th>Location</th>
<th>No. of Samples</th>
<th>Total Sampling Time (minutes)</th>
<th>TWA* (g/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degreaser</td>
<td>1</td>
<td>381</td>
<td>1,260</td>
<td>Impregnation</td>
<td>2</td>
<td>176</td>
<td>810</td>
</tr>
<tr>
<td>Solder</td>
<td>3</td>
<td>884</td>
<td>1,060</td>
<td>Pump Room</td>
<td>3</td>
<td>1079</td>
<td>490</td>
</tr>
<tr>
<td>Tanker</td>
<td>9</td>
<td>2120</td>
<td>850</td>
<td>Testing</td>
<td>5</td>
<td>1424</td>
<td>320</td>
</tr>
<tr>
<td>Moveman (Soldering Area)</td>
<td>3</td>
<td>752</td>
<td>720</td>
<td>Pre-assembly</td>
<td>4</td>
<td>1213</td>
<td>140</td>
</tr>
<tr>
<td>Heat Soak Operator</td>
<td>3</td>
<td>872</td>
<td>630</td>
<td>Shipping</td>
<td>2</td>
<td>741</td>
<td>90</td>
</tr>
<tr>
<td>Tester</td>
<td>3</td>
<td>917</td>
<td>290</td>
<td>Winding</td>
<td>4</td>
<td>637</td>
<td>70</td>
</tr>
<tr>
<td>Pump Mechanic</td>
<td>1</td>
<td>377</td>
<td>280</td>
<td>Cover Manufacturing</td>
<td>3</td>
<td>1089</td>
<td>60</td>
</tr>
<tr>
<td>Floorman (pre-assembly)</td>
<td>6</td>
<td>1683</td>
<td>170</td>
<td>Office</td>
<td>2</td>
<td>741</td>
<td>50</td>
</tr>
</tbody>
</table>

* The TWA is calculated over the total sampling time period.
Discussion

Unidentified Speaker: I know your numbers are small, but were you able to do separate analyses by sex? In particular, were there liver cancers in women?

Mr. Brown (NIOSH): I did separate analyses by sex. I will have to go back to check to see about the liver cancers in the women. I am not sure.

Unidentified Speaker: In both plants, TCE was used as a degreaser and it is known that TCE has induced liver cancer in laboratory animals. How did you separate those exposed to TCE from those exposed to PCB's to determine whether or not some of the excess is attributable to TCE?

Mr. Brown (NIOSH): Based on the work histories that we got from the plant, we could tell who worked around the TCE degreaser and we eliminated them from our study.
Painting Trades Study
Progress Report

Dennis Zaebst
Marie Haring
Shiro Tanaka

NCI/EPA/NIOSH Collaborative Workshop
May, 1980

Industry-wide Studies Branch
Division of Surveillance, Hazard Evaluations and Field Studies
National Institute for Occupational Safety and Health
Cincinnati, Ohio
A NIOSH study of health hazards of workers applying paints and coatings was begun in 1978. The study was started because of reports of adverse health effects including bronchitis and chest x-ray changes in an earlier NIOSH pilot study.

A literature review was conducted and nine industries that have painting operations were selected for study: construction/maintenance, auto manufacturing, shipbuilding, and furniture manufacturing, appliance manufacturing, railroad car manufacturing, aircraft manufacturing and maintenance, bus, truck, farm, and construction machinery manufacturing, metal furniture manufacturing. Fifty-one walk through surveys to obtain information on paint usage and exposure, completeness of personnel records and other basic information were conducted in the nine industries.

The information obtained in the walk through surveys is being analyzed in order to decide which industries and specific sites are most suitable for in-depth industrial hygiene and/or retrospective cohort mortality studies. Cross-sectional medical studies may be conducted if warranted by early results of the mortality or industrial hygiene studies.
INTRODUCTION

Purposes of the Painting Contract

A NIOSH study of health hazards of workers applying paints and coatings was begun in 1978. The purpose of this study is to evaluate, on an industrywide basis, possible health effects resulting from worker exposures to a variety of coating processes. It is a three phase study, including industrial hygiene, epidemiological, and cross-sectional medical surveys.

Background

The study was initiated for several reasons:

1. Only a limited amount of significant research has been conducted to estimate acute and chronic effects caused by exposure to paints and coatings.

2. During the past decade, there has been a growing concern that the materials in coatings may present potential health hazards to workers applying the coatings on a regular basis, for example, i.e. metallic pigments and additives, certain catalysts and activators, and solvents.

3. Several recent but limited occupational studies have suggested that painters experience an increased risk of developing certain diseases, including among others, central nervous system and respiratory effects, and cancer (1-8).

The motivating force behind the project was a pilot study conducted under a NIOSH contract in 1975 by Mt. Sinai (1) which clinically surveyed 1000
members of the International Brotherhood of Painters and Allied Trades (I.B.P.A.T.). The study results indicated that among the various trades included in the union (e.g. general painter, dry wall construction workers, wood finishers, paint factory workers and many others, Table 1), anesthetic effects on the central nervous system were more frequently reported among general painters, particularly those who had reported working with epoxy paints, and among those classified as metal painters and sandblasters.

The study also reported symptoms of respiratory irritation, such as increased frequency of bronchitis as seen among sandblasters and paint factory workers. Chest x-ray changes such as plural thickening or the occurrence of small rounded opacities were more frequent among general painters, paint manufacturers, and sandblasters.

Although this study had many inherent limitations - since it focused largely on construction painting and related trades - it did indicate a need to look more carefully at the potential hazards of paints, paint application processes, and the mortality and morbidity experience of involved workers.

However, it should be said again that at least part of the impetus for the present study was the lack of well defined and controlled studies relating to application of paint.

Scope and Limitations

For the purpose of this study, we have defined the "painting" industry to include those establishments that use coating products in the finishing of manufactured products and in new construction, building maintenance, and rehabilitation, specifically excluding paint manufacturing. We have also
defined paint as "a mixture of pigment or vehicle (as oil or water) applied to a surface to form a thin film adhering to a substrate". The group of finishes which are considered treatments because they penetrate the surface, and a variety of other specialized finishing processes (e.g. metalizing) were excluded from the study.

While attempting to define the areas of study or scope of the project, we became aware of the immense variety of coatings used in construction/maintenance or for product finishing, the number of raw materials used in paint manufacture, the variety of application methods, and the variety of settings in which paint is applied. Although we have tried to maintain a broad perspective on these aspects during this study, we have necessarily had to pre-select at several stages, the coating types to study, the environments and settings in which to study the associated hazards, and the specific sites and populations to be included. Although this may have caused us to overlook significant aspects of paint application which deserved study, given the complexity of the subject and funding limitations, this was unavoidable.

ACCOMPLISHMENTS

Selection of Study Sites

The initial task was to select groups of painters which would be reasonably representative of all painters in the United States, or failing that, to select groups to study which would be representative of the largest number of painters, or those with the most hazardous exposures.
The criteria developed to facilitate group selection followed a three point decision scheme based on resin type, industrial use and the availability of a study population:

(1) **Resins** - paints are frequently classified by resin type. We concentrated mainly on those (5-7) resin types which were in widespread and increasing use, or those which exhibited unique toxicity in other studies or case reports.

Table 2 shows the estimated total 1974 consumption of resins in paints and coatings (9). The three resin types in largest use were alkyd, vinyl, and acrylic. Although of substantially lower volume usage, epoxy and urethane paints were also selected because of their reported toxicity and potential hazards in use, as well as their projected increasing use in industry. Cellulosic based paints were also selected because it was known that these were used primarily, and in fact nearly exclusively, in the wood furniture industry.

2. **Industrial Use** - the selection of industries to study was then based on the type and volume of resin used, and the extent of exposure based on the method of application, number of workers exposed, and the projected usage.

3. **Population at Risk** - additional criteria for the final selection of industries were those related to selecting populations for mortality studies, based on the size and stability of the population, the length of use of the selected paints (or age of the industry), and information obtained prior to actual site visits.
on the condition and availability of records.

After examining approximately 18-20 different industrial categories, including architectural and maintenance painting, and eliminating several due to such factors as having unusual or unique coating processes, or difficulties in identifying suitable sites or populations, nine possible areas were chosen for study (Table 3).

In summary, each of the illustrated industrial areas were selected for study based on their high volume use of the selected paint resin types, the number of painters in the industry, application methods, and potential exposure. Also, it became obvious that by working within these chosen industrial categories, we could assess effects of exposure to many other paint resin types not originally selected for study.

Groups 9 and 10 - Construction and Maintenance, and the Mortality Study of the IBPAT Membership, are included as separate treatments of the same "category". Because of the apparent lack of large, stable workforce or adequate records documenting worker exposure at work sites or contractor offices, we investigated whether the mortality studies could be conducted using available records of the international painter's union. In conjunction with this study, industrial hygiene studies were to be conducted to explore potential exposures at a number of construction/maintenance worksites identified by the union.

Subsequently, thirty-nine walk through surveys were conducted in the eight manufacturing industries, five in each industry (4 in ship building), and surveys were conducted at all eleven construction/maintenance sites identified, for a total of 50 walk through surveys conducted. These sites,
including plant and construction locations, were selected in general to include (to the extent possible) the multiple environments present in each industry, and to include where possible large, medium, and some small establishments. However, this was not possible in all categories due to the limited amount of painting being done and the small number of workers at some of the smallest sites.

Walk Through Survey Results

Table 4 presents an overall summary of the findings in the nine areas examined in the walk through surveys. This is presented primarily to show examples of some of the data and criteria we used in selecting industries for in-depth epidemiology studies. These include: the number of painters and "halo" workers potentially exposed (as of measure of total U.S. impact) and the percent of total production workers who are painters, the degree of potential exposure, and an assessment of the workforce and records available for study. Not shown but additional important criteria used were the types of coatings used, the kinds of potential hazards seen, and the use of the newer kinds of coatings in increasing use.

A brief look at this summary indicates the following:

1. The three categories with the largest number of painters are in order: construction and maintenance, automobile manufacturing, and wood furniture. This, among other factors, led to the selection of construction and wood furniture for in-depth studies. Automobile OEM was a viable potential study, but exposures were subjectively low, and records would be difficult to search.

2. Metal furniture and appliances were ruled out because the
number of painters was low, hazardous exposures were (subjectively)
low, and records were marginal.

3. Railroad equipment manufacturing was ruled out because of the
small population available for study, and poor to marginal
records. Worker exposures, however, were judged to be relatively
high and potentially hazardous.

4. This left large transportation equipment, aircraft, and ship
building. Although all exhibited variable and potentially
hazardous exposures, aircraft was selected because of the availabili-
ity of records at one site, and in particular because of the
extensive use of the urethane paints, which are in increasing
use in several other industries.

The characteristics of painting in each of the three selected industries
(wood furniture, aircraft, construction/maintenance) and additional reasons
for the selection of each, are discussed below.

Wood Furniture Finishing

Table 5 presents some of the characteristics of finishing processes in the
Wood Furniture industry. Previous research in this industry does not
address the problem of the finisher. Most work concentrates on the hazards
and effects of wood dust exposure rather than those caused by finishing
products. Our planned studies concentrating in this area of the industry
will complement the research already investigating wood and wood dust
exposures.

Nitrocellulose lacquers are traditionally utilized in the furniture
finishing process. Although other finish types may be adopted, nitrocellulose is the finish of choice. In many operations, the finish is applied by hand or using a hand-held conventional air or airless spray technique.

Environmental controls are usually limited to back draft booths, with minimal use of personal protective equipment despite the fact that workers often conduct sanding and wiping operations outside the booths. This may cause worker exposure to evaporating solvent vapors.

The primary hazards, based on subjective observations and material safety data sheets on the products in use, were exposure to complex mixtures of aliphatic and aromatic hydrocarbons, ketones, plus other solvents and chemicals. It was our impression that although local exhaust was extensively used, overall control of solvent vapors in many instances was marginal. Confirmation of this, of course, would require extensive environmental surveys of several of these sites.

Finally, a few of the sites were found to contain data suitable for a retrospective mortality study of wood furniture finishers. This part of the furniture industry is composed of a large number of highly skilled workers who remain classified as a finisher once the person is identified as such. Therefore, it is felt that long term finishers will have been exposed to the nitrocellulose lacquers despite the rather high mobility of the workforce. The cohort is anticipated to include approximately 4500-5000 workers, working over an estimated 30 years.
Aircraft Manufacturing

Comprehensive surveys in the aircraft industry (Table 6) should provide data on the chronic and acute health effects of urethane-type coatings, as well as zinc chromate and epoxy constituents. Although used as top coatings on aircraft for approximately 20 years, other industries are just beginning to use urethanes with any frequency. Because of their durability and fast-curing nature, urethanes are coming into increasing use and should be more extensively investigated to ensure proper worker protection in the future. Several health hazard evaluations conducted by NIOSH in the past few years, and other reports, have indicated that exposure to free isocyanate may occur in the use and application of these paints (10-13). Also, a recently published PMR study of zinc chromate exposed painters in the aircraft overhaul industry has indicated an increased risk of lung cancer in these workers (16).

Urethane (usually 2-part applied by handheld air spray) are the most frequently used topcoats; primers vary depending on the geographical area and type of aircraft service intended but often include zinc chromate and/or epoxy primers. Small parts painting is usually done in ventilated booths but whole aircraft (particularly large aircraft) are done in hangars with variable ventilation. Personal protection is extensively used due to the difficulties in using engineering controls. Principal potential exposures include solvents, lead and/or zinc chromate, methylene chloride (frequent in paint stripping formulations), and isocyanates (frequently in pre-polymer form).

Currently, a study to assess the feasibility of conducting a mortality investigation in the aircraft manufacturing industry is underway. Results
of the inquiry will be released within the next few weeks.

Construction/Maintenance Painting Studies of construction and maintenance painting would help to characterize exposures and disease processes in the single largest group of painters in the United States, and would include situations (along with wood finishing) similar to those in which non-professional painters may engage. Previous research is limited to the Mt. Sinai pilot study cited earlier, and several reports from Sweden which have indicated psychiatric and neurological changes among house painters (14-15).

Table 7 summarizes environmental findings from nine of the walk-through surveys conducted at the eleven construction/maintenance sites. The 11 sites included visits to two contractor's offices to examine feasibility of using contractor records for a potential epidemiology study; actual working sites were not visited.

The remaining nine sites can be classified as new construction painting only, or maintenance painting only. Maintenance painting sites included four sites under five contractors, one of which involved a complex of commercial buildings, and three involved industrial maintenance painting. Maintenance painting of building complexes uses 70%-80% flat water based paints applied with roller or brush, and lesser quantities of solvent based enamels are used. The painting is often done in unventilated areas with inadequate or no respiratory protection and solvent exposures potentially could be quite high; it should be noted that some so-called "water base" paints do contain volatile toxics such as glycol ethers.

Maintenance painting in industry requires a large variety of paint types depending on the application, geographical area, etc., but typical were 2
system epoxy, latex, alkyd enamels, epoxy, polyamides, and (at chemical/refinery locations) additional types such inorganic zinc primers, vinyls, and rarely, urethanes. They are usually applied by brush, or handheld air or airless spray, often in open areas (outdoors) or in closed rooms of spaces with variable ventilation and degree of respiratory protection. Potential exposure include solvents (MEK, Toluene, MIBK, Xylene, Methylene Chloride), inorganic zinc, chromates and (since these painters are often involved in surface preparation) silica from abrasive blasting. In general, respiratory protection and work practices were better controlled at the industrial sites.

Four sites of construction painting were also visited; two were power plants, (one coal, one nuclear), one was a nuclear waste treatment facility, and one was a large home/apartment complex. In the home/apartment painting, latex and alkyd enamel were applied by brush and roller in closed, unventilated spaces, and with little or no respiratory protection. At the power plant, 2 part epoxies are extensively used (particularly at the nuclear facilities) and are applied in rooms or spaces with variable ventilation by brush, roller, or spray. At the coal powered plant, polyurethane coatings and zinc chromate primers are also used. In all of the industrial construction sites, respirators of various types are available on demand, but are only occasionally required for specific jobs (e.g. tank lining, sandblasting). Potential exposures in home/apartment painting are primarily volatile organics, but in the case of the industrial construction painting, also include various solvents and zinc chromate and rarely NCO from polyurethanes, sensitizing agents (amines, glycidyl ethers) present in epoxies and free silica.
The mortality study of the membership of the International Brotherhood of Painters and Allied Trades (IBPAT) includes a cohort size of approximately 300,000. Deaths are identified through the death benefit and disability records available at the union. The anticipated number of deaths are estimated at 12,000 for the cohort. This population size should yield stable mortality rates even for rare causes of death.

IN-DEPTH STUDIES

In summary, three industrial categories were chosen for in-depth epidemiological study; namely wood furniture, aircraft, and construction/maintenance. The mortality studies will utilize the records of the IBPAT (the international painters union), the records of one large site in the aircraft industry, and those of possibly two or more sites in the wood furniture industry. In each of these categories, three plants or construction locations will be selected for participation in the comprehensive industrial hygiene surveys. In addition, industrial hygiene surveys will be conducted at approximately one representative site in each of the remaining industries, for a total of 15 surveys. Cross-sectional medical studies will be conducted as warranted following completion of the industrial hygiene surveys and the release of at least the preliminary findings of the mortality studies.

The planned studies should increase the available information concerning both the potential and existing hazards of paint application in the workplace. However, the studies will necessarily fall short of achieving an in-depth evaluation of all the hazards involved, given the inherent variability of conditions and processes between industries and even between
plants in the same industry.

Also, environmental conditions and paint processes have changed, and will continue to change, in many of the industries surveyed, making it difficult to correlate specific causes of death or illness with specific exposures. Evaluation of past exposures in many cases will rely on historical data obtained and kept by individual companies (sparse or nonexistent in many cases), and available histories of process changes, engineering controls, work practices, and use of protective equipment.

At the present time, the NIOSH contractor (Johns Hopkins University) is preparing a detailed protocol for the total in-depth study. This protocol will undergo extensive internal and external technical and statistical review.

We anticipate beginning the in-depth industrial hygiene and epidemiological studies in the fall of 1980. Cross-sectional medical surveys, if warranted, will begin approximately one year later and will be based on the findings of the industrial hygiene studies, and possibly on the basis of early results of the mortality studies. Given these parameters we estimate that the final report of the study will be released in mid-1982.
REFERENCES


INVESTIGATION OF HEALTH HAZARDS
IN THE PAINTING TRADES

NIOSH CONTRACT 210-77-0096

THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF HYGIENE AND PUBLIC HEALTH
1978 - 1982
SELECTED INDUSTRIES

1. Wood Furniture Finishing
2. Aircraft
3. Construction/Maintenance
IMPETUS FOR STUDY

1. Limited Research
2. Growing Concern About Hazards
3. Recent Studies
4. NIOSH Pilot Study (1975)
SCOPE

PAINTING INDUSTRY: "Those establishments which use coating products in the finishing of manufactured products and in new construction, building maintenance, and rehabilitation".

PAINT: "A mixture of pigment or vehicle (as oil or water) which can be applied or spread over a surface to form a thin film or coating which adheres to the substrate (base material)".
SITE SELECTION

DECISION SCHEME:

1. Select Coatings for Study by Resin Type
2. Survey Industrial Use
3. Survey Populations Available
SITE SELECTION

INDUSTRIAL USE:

1. Resin/Paint Type Used
2. Volume of Use
3. Extent of Exposure
   a. Application Method
   b. No. Current Painters
   c. Projected Usage
SITE SELECTION

POPULATION AT RISK

1. Population Size and Stability
2. No. of Years of Painting (Plant Age)
3. Records
<table>
<thead>
<tr>
<th>Trade</th>
<th>Reported Symptoms</th>
<th>Trade</th>
<th>Reported Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. General Painter</td>
<td>CNS, X-ray changes</td>
<td>6. Sandblasters</td>
<td>CNS, respiratory, X-ray changes</td>
</tr>
<tr>
<td>2. Drywall Construction Workers</td>
<td></td>
<td>7. Glaziers</td>
<td></td>
</tr>
<tr>
<td>3. Wood Finishers</td>
<td></td>
<td>8. Sign Painters</td>
<td></td>
</tr>
<tr>
<td>5. Paint Factory Workers</td>
<td></td>
<td>10. Carpet Layers</td>
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</tr>
</tbody>
</table>

### TABLE 2

**ESTIMATED TOTAL CONSUMPTION OF RESINS IN PAINTS AND COATINGS - 1974**

(Millions of Pounds)

<table>
<thead>
<tr>
<th>Resin Type</th>
<th>Consumption</th>
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</thead>
<tbody>
<tr>
<td>Alkyd</td>
<td>750.0</td>
</tr>
<tr>
<td>Vinyl (Water-based)</td>
<td>292.0</td>
</tr>
<tr>
<td>Acrylic (Water-based)</td>
<td>228.9</td>
</tr>
<tr>
<td>Acrylic (Powder)</td>
<td>90.0</td>
</tr>
<tr>
<td>Epoxy (Reactive)</td>
<td>76.6</td>
</tr>
<tr>
<td>Amino</td>
<td>73.7</td>
</tr>
<tr>
<td>Vinyl (Solvent-based)</td>
<td>64.1</td>
</tr>
<tr>
<td>Acrylic (T/P Solvent)</td>
<td>62.0</td>
</tr>
<tr>
<td>Linseed Oil</td>
<td>57.6</td>
</tr>
<tr>
<td>Cellulosic (Solvent-based)</td>
<td>55.1</td>
</tr>
<tr>
<td>Urethane (Reactive)</td>
<td>48.4</td>
</tr>
<tr>
<td>Industry</td>
<td>No. of Sites</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Aircraft Manufacturing</td>
<td>5</td>
</tr>
<tr>
<td>Wood Furniture</td>
<td>5</td>
</tr>
<tr>
<td>Major Appliance Manufacturing</td>
<td>5</td>
</tr>
<tr>
<td>Metal Furniture and Fixtures</td>
<td>5</td>
</tr>
<tr>
<td>Large Transportation Equipment</td>
<td>5</td>
</tr>
<tr>
<td>Shipbuilding</td>
<td>4</td>
</tr>
<tr>
<td>Automobile O.E.M.</td>
<td>5</td>
</tr>
<tr>
<td>Construction/Maintenance</td>
<td>11</td>
</tr>
<tr>
<td>Mortality Study - IBPAT</td>
<td>--</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50</strong></td>
</tr>
<tr>
<td>Industry</td>
<td>Total Painters and Halo %</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>2. Large Transport Equipment</td>
<td>5,020 2</td>
</tr>
<tr>
<td>3. Aircraft</td>
<td>3,780 3.5</td>
</tr>
<tr>
<td>4. Shipbuilding</td>
<td>4,260 3</td>
</tr>
<tr>
<td>5. Railroad Equip.</td>
<td>1,000 2.7</td>
</tr>
<tr>
<td>6. Wood Furn.</td>
<td>21,632 16.9</td>
</tr>
<tr>
<td>7. Metal Furn.</td>
<td>8,213 4.3</td>
</tr>
<tr>
<td>8. Appliances</td>
<td>4,740 3.0</td>
</tr>
<tr>
<td>9. Construction/Maintenance</td>
<td>195,000 N.A.</td>
</tr>
</tbody>
</table>
TABLE 5

WOOD FURNITURE FINISHING

Previous Research - Limited

Resin Type - Nitrocellulose Lacquers (20 + years use)

Application Method - *Handheld Conventional, air or airless Spray Techniques;
*Back Draft Booths

Personal Protection - *Minimal, Heavy Reliance on Engineering Control

Hazards - *Aliphatic, Aromatic Hydrocarbons
*Ketones
*Other Solvents

Cohort Availability - *Relatively Skilled Worker Stays in Same Type Job;
*Many Halo Exposures
*Followup to 30 Years
*Estimated 4500-5000 Persons
TABLE 6

**AIRCRAFT MANUFACTURING**

**Previous Research** - *Health Hazard Evaluations
   *PMR Study of Zinc Chromate Exposed Workers (16).

**Resin Type** - Urethane Topcoats (20 + years use)
   Zinc Chromate, Epoxy Primers

**Application Methods** - *Handheld, Conventional Air Spray
   *Booths or Large Hangars

**Personal Protection** - Used Extensively

**Hazards** - *Urethanes (NCO, frequently pre-polymer)
   *Lead
   *Zinc Chromate
   *Solvents
   *Methylene Chloride

**Cohort Availability** - Feasibility Assessment
Table 7

Construction-Maintenance

<table>
<thead>
<tr>
<th>Maintenance</th>
<th>Construction</th>
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</thead>
<tbody>
<tr>
<td>Resin Type</td>
<td>Resin Type</td>
</tr>
<tr>
<td>*Alkyds</td>
<td>*Alkyd</td>
</tr>
<tr>
<td>*Acrylics</td>
<td>*Acrylic</td>
</tr>
<tr>
<td>*Vinyls</td>
<td>*Vinyl</td>
</tr>
<tr>
<td>*Epoxy; Epoxy Polyamide</td>
<td>*Epoxy (Reactive)</td>
</tr>
<tr>
<td>*Urethane</td>
<td>*Urethane</td>
</tr>
<tr>
<td>*Inorganic Zinc Primers</td>
<td>*Zinc Chromate Primers</td>
</tr>
</tbody>
</table>

Application - Varies with Job Requirements

Personal Protection - Variable, Available but not Required

Hazards -
<table>
<thead>
<tr>
<th>Hazards</th>
<th>Hazards</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Solvents</td>
<td>*Solvents</td>
</tr>
<tr>
<td>*Inorganic Zinc</td>
<td>*Zinc Chromate</td>
</tr>
<tr>
<td>*Chromate</td>
<td>*NCO (rare)</td>
</tr>
<tr>
<td>*Silica</td>
<td>*Silica</td>
</tr>
<tr>
<td>*NCO</td>
<td>Sensitizing Agents</td>
</tr>
<tr>
<td>*Sensitizing Agents (Amines, Gycidyl Ethers)</td>
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Cohort Availability - 300,000 Current and Previous Union Members; Potentially 12,000 Deaths
Dr. Keefer (NCI): On one of your slides, you singled out methylene chloride which is used so much in stripping operations. It was listed together with several other chemicals that I thought were known to be particularly hazardous. Do you have any comments on the toxicity and hazards associated with methylene chloride itself? I am back two or three years ago when some of the people from Dow and elsewhere, I think, cleared the material as a carcinogen. I just wondered if you have any special reasons for indicting it in this way.

Mr. Zaebst (NIOSH): The list of chemicals shown in the slide just gave some examples of some of the hazards seen. Methylene chloride has been studied in several NIOSH health hazard evaluations involving several different aircraft overhaul and construction facilities. Overexposures were found. As far as the toxicology, I believe that it should be looked at in more detail.
General Discussion

Dr. Bridbord (NIOSH): We do have some time for discussion, if anyone would like to comment on any of the four papers or ask additional questions. Please come to the microphone, if you do.

Dr. Kraybill (NCI): I would like to ask a question of Larry Keefer. Methylene chloride is now being tested and I was not familiar with the Dow statement. Did they clear methylene chloride? They did a study?

Dr. Keefer (NCI): All I recall is that Chemical Engineering News a few years ago had a small column which said that Dow's recent studies show that methylene chloride is not carcinogenic. Other than that, I really cannot say. I have not seen any original data.

Dr. Cantor (EPA): I do not think that they ever published that information I think they made it available through a testing program. It was a small local inhalation study. It was more pharmacokinetics than anything else. I think it was a simple bioassay. The NCI testing program had methylene chloride on test halfway through a two year gavage study. I think they were right in the process of starting a new study.

Dr. Keefer (NCI): I have a news clipping. You can read it if you would like. But that is all I have.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Tuesday Afternoon, May 6

EPIDEMIOLOGICAL/STATISTICAL SESSION (CONTINUED)

SESSION CHAIRPERSON

Dr. Joseph Fraumeni
National Cancer Institute
Mortality Study of Workers Employed at Organochlorine Pesticide Manufacturing Plants

David P. Brown
David Ditraglia
Tsukasa Namekata
Norman Iverson

1 U.S. Department of Health, Education and Welfare
Public Health Service
Center for Disease Control
National Institute for Occupational Safety and Health
Division of Surveillance, Hazard Evaluations and Field Studies
Cincinnati, Ohio

2 University of Illinois
School of Public Health

May, 1980
A retrospective cohort study was conducted to examine the mortality of workers employed in the manufacture of the chlorinated hydrocarbon pesticides, chlordane, heptachlor, DDT and aldrin/dieldrin/endrin. Four manufacturing plants were selected for study, and each cohort included all workers employed for at least 6 months prior to January, 1964. The entire study group totalled approximately 2100 individuals. Vital status ascertainment for these cohorts ranged from 90% to 97% complete, the cut-off date for follow-up was December 31, 1976.

In general, there are too few deaths in this study to make any meaningful conclusions. The standardized mortality ratio (SMR) for all causes in each cohort is below expected (100), ranging from 66 to 82, probably reflecting the "healthy worker effect". For "all malignant neoplasms", the SMR's range from 68 to 91 and for respiratory cancer from 55 to 132. In the aldrin/dieldrin/endrin cohort, pneumonia and "other respiratory diseases" were significantly above that expected. These causes of death need to be examined in more detail.

It is recommended that these cohorts be followed for several more years and the mortality patterns re-examined.
Introduction

The organochlorine (OC) pesticides such as DDT, Aldrin, Dieldrin, Lindane, Chlordane, Heptachlor, Toxaphene and Mirex have been an important class of compounds in terms of production volume and use. Due to the widespread use of these pesticides during the past 30 years, there has been an opportunity for widespread exposure to workers who manufacture, formulate and apply these compounds, and ultimately to those in the general population through ingestion of contaminated food and general environmental pollution.

There has been a great deal of concern about the long term, latent health effects from exposure to OC pesticides. These chemicals have a tendency to penetrate cell membranes and to be stored in the body fat.1 Some of these chemicals have been shown to be toxic to the liver and kidney in exposed humans2-3 and benign and malignant tumors in the liver have been induced in experimental animals chronically exposed to several of the OC compounds4-9. There are also reports of effects on the hematopoietic system among individuals exposed to Chlordane10, DDT11, Dieldrin11 and Lindane12-14.

In order to determine whether or not exposure to certain OC pesticides is associated with an increased risk of mortality due to chronic diseases an epidemiologic mortality study was initiated by the National Institute for Occupational Safety and Health (NIOSH). The
study was carried out under contract by the University of Illinois, School of Public Health. The contract stipulated that the study design be a retrospective cohort mortality study. The study cohort was defined as all workers at selected pesticide plants who were employed for at least six months, either continuously or intermittently between January 1, 1940 and December 31, 1964.

The original intent of this study was to examine the mortality of workers employed in OC pesticide formulating plants. However, after visits had been made to numerous formulating plants around the country it was determined that this part of the pesticide industry was not suitable for an epidemiologic study. The formulating plants are usually small operations, where work is seasonal, the turnover rate is high, exposures are multiple and records needed to conduct an epidemiologic study are not generally kept. Therefore, the emphasis of the study was shifted to OC pesticide manufacturing plants. These plants offered a more suitable population for the investigation. However, the exposures at these plants are probably lower than at formulating plants and also include the chemical precursors of the final technical grade pesticide.

An attempt was made to include OC pesticide manufacturing plants in the U.S. which began operations at least 25-30 years ago, which had relatively large workforces and had records available to identify a study cohort. A list of the major OC pesticide manufacturing plants
was assembled from sources such as the Farm Chemical Handbook, trade commission reports, and EPA. Based on accumulated information, a number of potential facilities were contacted and walk through surveys were conducted to make the final selection for the study.

Four facilities were eventually chosen for the study. Table 1 gives a description of the four plants. Plant No. 1 is located in Illinois and has manufactured chlordane since 1946. Plant No. 2 located in Tennessee, has produced Heptachlor since 1951. Also, in 1953 Endrin was manufactured in a pilot operation at this plant and by 1955 full scale commercial production of Endrin began. Other products at plant No. 2 have included hydrogen gas, chlorine, and chlorendic anhydride. Plant No. 3 located in Colorado, has manufactured a variety of pesticides. In 1946 production of Aldrin and Dieldrin began and continued until the 1970's. Endrin production began in 1953 and continued until 1965. In 1955 this plant started manufacturing an organobromine pesticide and in 1956 the production of organophosphates was started. Plant No. 4 is located in California and DDT has been its sole product since 1947.

Methods

The study population consisted of four separate cohorts from the four pesticide plants. For purposes of future analysis some of these cohorts may be combined. However, in this presentation the results of
each cohort will be examined separately. The cohorts were defined as all workers at each plant who had achieved at least 6 months employment prior to December 31, 1964. This cutoff date was selected to allow for accrual of sufficient time or latency for manifestation of disease.

An effort was made to determine the vital status (alive or deceased) of each member in the study cohorts as of December 31, 1976. Vital status was ascertained through records maintained by federal and state agencies, including the Social Security Administration and state motor vehicle offices. For those individuals whose vital status could not be determined through those sources U.S. Postal Mail Correction Services and other follow-up searches were used. For all those known to be deceased, death certificates were obtained and causes of death were coded by a nosologist according to the International Classification of Diseases (ICDA) in effect at the time of death. Those who had an unknown vital status were assumed to be alive as of December 31, 1976 so that the true risk of mortality was not overestimated. Those who died after December 31, 1976 were considered alive for purposes of this analysis.

In each cohort, person-years at risk of dying were accumulated for each worker starting when six months of employment were completed and ending either at the date of death or at the study end date of 12/31/76 whichever occurred first. Using a modified life table
analysis program, similar to that described by Cutler\textsuperscript{15}, the person-years for each cohort were combined into five-year calendar and five-year age time periods and multiplied by the corresponding U.S. white male cause-specific mortality rate to yield the expected number of deaths. Person-years were additionally distributed by five-year exposure and five-year latency (number of years from date first employed) categories. The observed and corresponding expected deaths were compared and differences were tested using the Poisson Distribution.

**Results**

The results of the vital status ascertainment and the total number of person-years for each cohort are given in Table 2. Even with the efforts previously described several of the cohorts have an unknown vital status of 10 percent.

Table 3 summarizes the observed and expected deaths by specific cause. For the category of "all causes", the SMR's (observed deaths/expected deaths x 100) range from 66 to 86. Assuming the record systems used to identify the cohorts were complete, these low SMR's probably reflect the healthy worker effect which has been noted in other studies of occupational groups\textsuperscript{16}, and possibly the lack of complete vital status ascertainment. Mortality due to all malignant neoplasms was also lower than expected, with SMR's ranging from 68 to
91. Other major causes of death in the cohorts including diseases of the circulatory system are also generally lower than expected. The only major category where there was a significant increase in observed deaths over expected was for pneumonia (11 observed vs. 4.3 expected, SMR = 255, p<0.01) and for "other respiratory diseases" (11 observed vs. 5.2 expected deaths, SMR = 213, p<0.05), at plant 3.

Table 4 summarizes observed and expected deaths by specific type of cancer. As stated previously, there is a deficit in observed deaths due to "all malignant neoplasms" in each plant studied. Although there are no statistically significant excesses or deficits in mortality among any specific cancer site, several sites are of note. In plant 1 there are 3 observed deaths due to stomach cancer when 0.99 were expected. In plant 3 there are slight excesses in cancer of the esophagus (2 observed vs. 0.85 expected), cancer of the rectum (3 observed vs. 1.24 expected), liver cancer (2 observed vs. 0.57 expected), and cancer of the lymphatic and hematopoietic system (6 obs. vs. 4.09 exp.). In addition there is a deficit in respiratory cancer (7 observed vs. 12.64 expected).

An analysis by latency is presented in Table 5 for "all malignant neoplasms". In this type of analysis, one looks for trends to examine whether or not the risk of mortality as measured by the SMR is associated with any particular latency period. Plants 2 and 4 show a consistent increase in the risk of cancer mortality with an increase
in the latency period, however the numbers involved in this analysis are small. Since respiratory disease was increased in plant 3, latency was also examined for this cause of death. There was a statistically significant increase in mortality due to respiratory disease during the 10-20 year latency period (12 obs. vs. 4.51 exp.; p<0.05), which decreased during the greater than 20 years latency period (8 obs. vs. 3.96 exp.; 0.05<p<0.1).

Discussion

Plants 1 and 2 have been previously studied by Wang\textsuperscript{17}. In her study, the definition of the cohort included workers from both plants who were employed for at least 3 months between 1946 and 1975. Therefore, although there is overlap between this cohort and plants 1 and 2 included in the present study, the cohorts are not exactly alike. In Wang's study, there was no observed excess in mortality due to specific cancer sites except for a small increase in respiratory cancer. The only cause of death where there was a significant excess was for cerebrovascular disease. Neither of these findings were seen in the present study - cerebrovascular disease in plants 1 and 2 combined was 8 obs. vs. 7.88 exp. and respiratory cancer was 9 obs. vs. 7.87 exp. As noted previously there was an increase in stomach cancer observed in plant 1, however, the small numbers involved in this study preclude any clear association.
In the plant 3 cohort there was a significant increase in deaths due to non-malignant respiratory disease especially among those with at least 10 years of latency. In contrast to this finding there was a deficit in deaths due to respiratory cancer. These findings need to be examined further to determine whether there is a true association between respiratory disease and employment at this plant.

There was no excess in cause specific deaths in the plant 4 cohort. However, when the deaths from malignant neoplasms are examined by latency, there is an increase in risk with an increase in the length of the latent period. The numbers in this analysis are small and this trend could be due to chance alone.

Due to the small number of workers included in this study the statistical power does not enable one to conclude that there is no association between cause-specific mortality and employment at the study plants. The primary reason for these small numbers is due to the rapid turnover at these plants, and thus most workers who were hired left before they achieved 6 months of employment. This was especially true at plant 4 where approximately seventy percent of the employees worked less than 6 months.

Although this study has not identified a specific cancer risk associated with employment at certain types of OC pesticide manufacturers, it points to several causes (stomach cancer in plant 1,
esophagus, rectum, liver and lymphatic/hematopoietic cancer in plant 3) that should be examined further. An attempt should be made to determine if there are any common exposures among those who died from these causes of death. Additional analyses are also necessary to determine, if possible, whether or not the excess in respiratory disease is associated with specific occupational exposure at plant 3. Finally, the mortality experience in each of these cohorts should be followed for several more years with a better ascertainment of vital status to increase the statistical power of the analysis so that more confident conclusions can be made.
REFERENCES


13. Samuels AJ, Milby TH. Human exposure to Lindane – Clinical 
   hematological and biochemical effects. J. Occup. Med. 13:147-51, 
   1971.


15. Cutler SJ, Ederer F. Maximum Utilization of the life table 


17. Wang HA, MacMahon B. Mortality of Workers employed in the 
<table>
<thead>
<tr>
<th></th>
<th>PLANT 1</th>
<th>PLANT 2</th>
<th>PLANT 3</th>
<th>PLANT 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date began OC pesticide production</td>
<td>1946</td>
<td>1951</td>
<td>1946</td>
<td>1947</td>
</tr>
<tr>
<td>OC pesticides produced</td>
<td>- Chlordane</td>
<td>- Heptachlor</td>
<td>- Aldrin</td>
<td>- DDT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Endrin</td>
<td>- Dieldrin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Endrin</td>
<td></td>
</tr>
<tr>
<td>Other pesticides produced</td>
<td>none</td>
<td>none</td>
<td>- organobromines</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- organophosphates</td>
<td></td>
</tr>
<tr>
<td>Other chemicals at plant</td>
<td>- Chlorine</td>
<td>- Chlorine</td>
<td>Numerous precursors</td>
<td>- tri-chloroacetaldehyde</td>
</tr>
<tr>
<td></td>
<td>- Dicyclopentadiene</td>
<td>- Chlorendic anhydride</td>
<td></td>
<td>- sulfuric acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hexachlorocyclopentadiene</td>
<td></td>
<td>- Monochlorobenzene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- vinyl chloride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td>Illinois</td>
<td>Tennessee</td>
<td>Colorado</td>
<td>California</td>
</tr>
</tbody>
</table>
Table 2

Vital Status Follow-up of Workers in Study of Organochlorine Pesticide Manufacturers

<table>
<thead>
<tr>
<th></th>
<th>PLANT 1</th>
<th>PLANT 2</th>
<th>PLANT 3</th>
<th>PLANT 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known to be alive</td>
<td>259 (79%)</td>
<td>265 (87%)</td>
<td>870 (75%)</td>
<td>278 (79%)</td>
</tr>
<tr>
<td>Known to be deceased</td>
<td>59 (18%)</td>
<td>24 (8%)</td>
<td>173 (15%)</td>
<td>42 (11%)</td>
</tr>
<tr>
<td>Unknown vital status</td>
<td>9 (3%)</td>
<td>16 (5%)</td>
<td>112 (10%)</td>
<td>34 (10%)</td>
</tr>
<tr>
<td>Total</td>
<td>327</td>
<td>305</td>
<td>1,155</td>
<td>354</td>
</tr>
<tr>
<td>Person-years of observation</td>
<td>8,354</td>
<td>5,672</td>
<td>24,939</td>
<td>7,601</td>
</tr>
</tbody>
</table>
### Table 3

**Observed/Expected Deaths According to Major Cause Among Workers in Study of Organochlorine Pesticide Manufacturers**

<table>
<thead>
<tr>
<th>CAUSE OF DEATH (7th Revision ICD No.)</th>
<th>PLANT 1</th>
<th>PLANT 2</th>
<th>PLANT 3</th>
<th>PLANT 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Malignant Neoplasms (140-205)</td>
<td>11/15.89 (69)</td>
<td>6/6.60 (91)</td>
<td>31/37.79 (82)</td>
<td>6/8.86 (68)</td>
</tr>
<tr>
<td>Nervous System Diseases (330-334)</td>
<td>7/6.03 (116)</td>
<td>1/1.85 (54)</td>
<td>9/13.32 (68)</td>
<td>-</td>
</tr>
<tr>
<td>Circulatory System Disease (400-468)</td>
<td>28/40.25 (70)</td>
<td>12/15.35 (78)</td>
<td>69/90.17 (77)</td>
<td>17/20.61 (82)</td>
</tr>
<tr>
<td>Non-malignant Respiratory System Disease (470-527)</td>
<td>1/4.58 (22)</td>
<td>22/10.40 (212)*</td>
<td>1/2.28 (44)</td>
<td></td>
</tr>
<tr>
<td>Accidents (800-962)</td>
<td>6/6.28 (96)</td>
<td>1/3.96 (25)</td>
<td>11/18.45 (60)</td>
<td>4/5.46 (73)</td>
</tr>
<tr>
<td>Suicide (963, 970-979)</td>
<td>1/2.12 (47)</td>
<td>-</td>
<td>10/5.99 (167)</td>
<td>-</td>
</tr>
<tr>
<td>All Other Causes</td>
<td>5/12.01 (42)</td>
<td>4/8.57 (47)</td>
<td>21/29.74 (71)</td>
<td>14/11.79 (119)</td>
</tr>
<tr>
<td>All Causes</td>
<td>59/87.16 (68)</td>
<td>24/36.33 (66)</td>
<td>173/205.86 (84)</td>
<td>42/49.00 (86)</td>
</tr>
</tbody>
</table>

1 SMR = Observed Deaths x 100. Expected Deaths

* p<0.01

Blanks represent no observed deaths.
Table 4

Observed/Expected Deaths According to Specific Cancer Causes
Among Workers in Organochlorine Pesticide Manufacturing

<table>
<thead>
<tr>
<th>CAUSE OF DEATH (Seventh Revision ICD No.)</th>
<th>PLANT 1</th>
<th>PLANT 2</th>
<th>PLANT 3</th>
<th>PLANT 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Malignant Neoplasms (140-205)</td>
<td>11/15.89 (69)</td>
<td>6/6.60 (91)</td>
<td>31/37.79 (82)</td>
<td>6/8.86 (68)</td>
</tr>
<tr>
<td>Esophagus (150)</td>
<td>-</td>
<td>-</td>
<td>2/ 0.85 (235)</td>
<td>-</td>
</tr>
<tr>
<td>Stomach (152,153)</td>
<td>3/ 0.99 (303)</td>
<td>-</td>
<td>1/ 2.09 (48)</td>
<td>1/0.44 (227)</td>
</tr>
<tr>
<td>Intestine (152,153)</td>
<td>-</td>
<td>1/0.57 (175)</td>
<td>1/ 3.35 (30)</td>
<td>-</td>
</tr>
<tr>
<td>Rectum (154)</td>
<td>1/ 0.56 (178)</td>
<td>-</td>
<td>3/ 1.24 (242)</td>
<td>-</td>
</tr>
<tr>
<td>Liver (155,156A)</td>
<td>-</td>
<td>-</td>
<td>2/ 0.89 (225)</td>
<td>-</td>
</tr>
<tr>
<td>Pancreas (157)</td>
<td>1/ 0.91 (110)</td>
<td>-</td>
<td>1/ 2.10 (48)</td>
<td>1/0.49 (204)</td>
</tr>
<tr>
<td>Respiratory (160-164)</td>
<td>6/ 5.43 (110)</td>
<td>3/2.45 (122)</td>
<td>7/12.64 (55)</td>
<td>4/3.19 (125)</td>
</tr>
<tr>
<td>Bladder and Urinary (180-181)</td>
<td>-</td>
<td>1/0.15 (666)</td>
<td>1/ 1.10 (91)</td>
<td>-</td>
</tr>
<tr>
<td>Other and Unspecified (1568,165,190-199)</td>
<td>-</td>
<td>1/0.88 (114)</td>
<td>6/ 4.80 (125)</td>
<td>-</td>
</tr>
<tr>
<td>Lymphatic and Hematopoietic (200-205)</td>
<td>-</td>
<td>-</td>
<td>6/ 4.09 (147)</td>
<td>-</td>
</tr>
<tr>
<td>Others</td>
<td>-</td>
<td>-</td>
<td>1/-</td>
<td>-</td>
</tr>
</tbody>
</table>

1 SMR = Observed Deaths x 100 / Expected Deaths

Blanks represent no observed deaths.

224
Table 5
Observed and Expected Deaths Due to Malignant Neoplasms According to Latency\(^1\) Among Workers in Study of Organochlorine Pesticide Manufacturers

<table>
<thead>
<tr>
<th>Years Since First Employed</th>
<th>Observed</th>
<th>Expected</th>
<th>SMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10 yrs.</td>
<td>1</td>
<td>1.52</td>
<td>66</td>
</tr>
<tr>
<td>PLANT 1</td>
<td>4</td>
<td>4.43</td>
<td>90</td>
</tr>
<tr>
<td>&gt; 20 yrs.</td>
<td>6</td>
<td>9.94</td>
<td>60</td>
</tr>
<tr>
<td>&lt; 10 yrs.</td>
<td>0</td>
<td>1.46</td>
<td>-</td>
</tr>
<tr>
<td>PLANT 2</td>
<td>3</td>
<td>3.30</td>
<td>91</td>
</tr>
<tr>
<td>&gt; 20 yrs.</td>
<td>3</td>
<td>1.85</td>
<td>162</td>
</tr>
<tr>
<td>&lt; 10 yrs.</td>
<td>4</td>
<td>7.55</td>
<td>53</td>
</tr>
<tr>
<td>PLANT 3</td>
<td>18</td>
<td>16.25</td>
<td>111</td>
</tr>
<tr>
<td>&gt; 20 yrs.</td>
<td>9</td>
<td>14.00</td>
<td>64</td>
</tr>
<tr>
<td>&lt; 10 yrs.</td>
<td>0</td>
<td>1.40</td>
<td>-</td>
</tr>
<tr>
<td>PLANT 4</td>
<td>1</td>
<td>3.68</td>
<td>27</td>
</tr>
<tr>
<td>&gt; 20 yrs.</td>
<td>5</td>
<td>3.78</td>
<td>132</td>
</tr>
</tbody>
</table>

\(^1\) Latency = number of years from date first employed.
Table 6

Observed and Expected Deaths from Respiratory Disease According to Latency Among Workers at Plant 3 in Study of Organochlorine Pesticide Manufacturers

<table>
<thead>
<tr>
<th>Years Since First Employed</th>
<th>Observed</th>
<th>Expected</th>
<th>SMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10 yrs.</td>
<td>2</td>
<td>1.07</td>
<td>187</td>
</tr>
<tr>
<td>10 - &lt; 20 yrs.</td>
<td>12</td>
<td>4.51</td>
<td>266*</td>
</tr>
<tr>
<td>&gt; 20 yrs.</td>
<td>8</td>
<td>3.96</td>
<td>202</td>
</tr>
</tbody>
</table>

* p<0.05
Discussion

Dr. Kraybill (NCI): This paper interested me very much, because of the selection of the chemicals, these pesticides, particularly DDT. We have been waiting for many years for the story about DDT. Correct me if I am wrong, but we have no human data yet to show that DDT or DDE has been carcinogenic in man. People opine that maybe if you did, then formulators, since they are getting a good exposure, probably more so than in the industrial plant, could show a causation. I am wondering if you could combine a population. Is that plant still producing DDT in California?

Mr. Brown (NIOSH): It is.

Dr. Kraybill (NCI): Are there formulators for DDT?

Mr. Brown (NIOSH): I am sure there are. Most of the formulators formulate thousands of different chemicals depending on the season and on the day.

Dr. Kraybill (NCI): Well, I am not an epidemiologist and that is why I am asking stupid questions. If you could combine them, maybe the numbers would be sufficient to draw a conclusion. But if you could do this on DDT and put that issue to bed once and for all, that would be a great contribution.

Mr. Brown (NIOSH): One thing that we may do is look at these plants and have a shorter cut-off period for length of employment and we would get more people in the study that way. I do not know if the short-term employment people are as important though.

Dr. Keefer (NCI): I was wondering also about the agricultural workers themselves who might be using these chemicals. I do not suppose you would have any better luck with them, but I wanted to ask.

I also wondered about other types of pesticides. I do not know about the usage patterns at all, but some of the dinitrobenzene types of pesticides and herbicides have been shown to contain relatively large amounts of nitrosamine contaminants. These are several orders of magnitude higher concentrations in the commodity than some of the things that we worry about like beer and scotch. I was wondering whether it would be possible to follow the experience of people with those kinds of pesticides as opposed to the chlorinated hydrocarbons or in addition to the chlorinated hydrocarbons.

Mr. Brown (NIOSH): On your first question, agricultural workers are a very difficult group to follow for epidemiological work. They are migratory. There are usually no records of these people or who they are. Many of them are illegally in this country. It is a very difficult study to accomplish. Plus, it is out of NIOSH's purview; I think it is EPA's.
As far as doing work on other compounds, I think NIOSH is looking at other pesticides. In fact, they are looking at some other formulators. I am not sure if they are concentrating on looking at nitrosamines. I think John, who presented a paper on nitrosamines before, may be looking at some of those in his survey. As far as looking at the mortality outcomes, I do not think there are any plans to do that right now.

Dr. Caldwell: Is there any reason that you can think of as to why the people leave in such a short period of time? Did any of the health hazard walk-throughs maybe indicate that people are leaving in six months there because they are developing acute illness or acute hypersensitivity, so that there is a reason why they are gone.

Mr. Brown (NIOSH): I do not really know. A lot of this is seasonal work and a lot of people come in, especially in the DDT plant in Los Angeles, for the summertime and they produce tremendous quantities of DDT and then there is a slowdown and so they leave. A lot of people are there for summer jobs.

The other thing about studying other pesticides is we are trying to look at people who were employed by the government in some of these pest eradication programs. A lot of those people used DDT years back, such as in the fringe beetle eradication program and some other things through the Agriculture Department.

Dr. Galbraith (EPA): Was there any difference in the inert ingredients in the pesticide formulations in the four different plants?

Mr. Brown (NIOSH): Well, since these were not formulators, these plants only made the technical grade product. So there was not any addition of inert ingredients at these plants. They sold these concentrated technical grades to a formulator and the formulators are the ones who add the inert ingredients and mix them up.

Dr. Fraumeni (NCI): Thank you very much.
PRELIMINARY FINDINGS OF AN EPIDEMIOLOGIC STUDY OF TALC WORKERS
(INDUSTRIAL HYGIENE PORTION)

by

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Appalachian Laboratory for Occupational Safety and Health
Morgantown, West Virginia 26505
INTRODUCTION

Talc, a magnesium silicate mineral, is mined in several geographic areas in the United States. The ore bodies examined in this study were Montana, Texas, and North Carolina. We examined seven (7) mines and eight (8) mills.

The purpose of the study was to characterize the talc, evaluate the workers' exposure, and ascertain the chronic effects of exposure (Table A). The environment of each facility was characterized as to total and respirable dust concentrations, percent (%) free silica, trace element concentrations, percent (%) fibrous minerals, calcite, and dolomite. Individual exposure was determined by personal respirable breathing zone samples on all participating employees. Estimates of exposure for each job were obtained from the personal samples.

The mines in Montana and Texas are typical open-pit operations, while the mine examined in North Carolina is underground employing square set timbers and stopes.

RESULTS

Personal respirable breathing zone samples (PRBZ) were collected from each participating employee and Time Weighted Averages (TWA) were obtained. The TWA's were utilized to derive geometric mean values for each job examined. These mean values were then used to develop cumulative exposures.

The time weighted averages for each employee were grouped together according to the actual job performed on the day of the study. The geo-
The geometric mean for each job classification was then calculated from the grouped TWA's.

The geometric mean for the dust levels in the mine and mill are presented by region (Table B & C).

Each ore body was analyzed for the following trace elements: Iron, Manganese, Calcium, Aluminum, Zinc, and Nickle (Table D). These trace elements were selected to compare the talc examined in this study with the talc examined in New York and Vermont.

Montana talc had the lowest concentrations of trace elements of the three regions examined. The trace element concentrations were slightly higher in North Carolina. Texas talc differed most significantly from the other regions by its extremely large concentration of calcium.

The mineral composition of bulk samples also indicated higher calcium value in the Texas. This talc had a much larger percentage of dolomite \( \text{(CaMg(C)\(_3\))} \) and a slightly larger percentage of calcite \( \text{(CaCO}_3\text{)} \) than the other two regions (Table E).

Examination of bulk samples of talc from each region for free silica demonstrated the same trend as other contaminants (Table F). Montana talc had < .8\% which was the limit of detection. North Carolina had a slightly higher percentage, while Texas had the highest observed silica content.

Respirable dust samples revealed the silica content in Montana and North Carolina to be generally below the limit of detection. The Texas talc had slightly higher levels of respirable silica.

Analysis for the presence of fibrous minerals was two-fold. The first analysis was with light microscopy utilizing phase contrast techniques.
Light microscopy was used as a screening tool to detect the presence of fibers. Further analysis of samples from each region was performed utilizing analytical transmission electron microscopy (Table G). Fibrous minerals were not detected in any samples of Montana talc.

There were two fibrous minerals identified in the Texas talc: tremolite and antigorite.

Antigorite, a serpentine mineral was the major constituent. The fibers of both minerals ranged from 0.5 to 3.0 µm in diameter and 4 to 30 µm in length.

The morphology of the North Carolina talc was identified as acicular. The acicular particles had aspect ratios ranging from 5 to 1 to 100 to 1 with some diameters ≤1 µm. These acicular particles may have resulted from mechanical destruction of plates.
TABLE A

INDUSTRIAL HYGIENE CHARACTERIZATION OF TALC

I. Personal Respirable Breathing Zone Samples
II. Trace Element Concentration
III. Mineral Composition
IV. Fibrous Minerals
V. Free Silica
<table>
<thead>
<tr>
<th>Job</th>
<th>Avg (mg/m³)</th>
<th>Variance</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montana</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bagger</td>
<td>2.8</td>
<td>1.9</td>
<td>29</td>
</tr>
<tr>
<td>Labman</td>
<td>.3</td>
<td>2.5</td>
<td>4</td>
</tr>
<tr>
<td>Fork Lift Op.</td>
<td>.5</td>
<td>1.8</td>
<td>5</td>
</tr>
<tr>
<td>Mill Operator</td>
<td>1.0</td>
<td>2.5</td>
<td>7</td>
</tr>
<tr>
<td>Laborer</td>
<td>1.4</td>
<td>2.0</td>
<td>6</td>
</tr>
<tr>
<td>Foreman</td>
<td>.6</td>
<td>2.8</td>
<td>14</td>
</tr>
<tr>
<td>Boiler Operator</td>
<td>.1</td>
<td>4.9</td>
<td>2</td>
</tr>
<tr>
<td>Front-End Op.</td>
<td>.8</td>
<td>6.1</td>
<td>14</td>
</tr>
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<td>Maintenance</td>
<td>.4</td>
<td>4.3</td>
<td>16</td>
</tr>
<tr>
<td>Welder</td>
<td>6.3</td>
<td>---</td>
<td>1</td>
</tr>
<tr>
<td>Wash Plant Op.</td>
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<td>8.7</td>
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<tr>
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<td>Truck Driver</td>
<td>.3</td>
<td>1.8</td>
<td>15</td>
</tr>
<tr>
<td>Miner</td>
<td>.4</td>
<td>1.9</td>
<td>2</td>
</tr>
<tr>
<td>Shovel Operator</td>
<td>.2</td>
<td>3.6</td>
<td>5</td>
</tr>
<tr>
<td>Calciner Operator</td>
<td>.6</td>
<td>---</td>
<td>1</td>
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<tr>
<td></td>
<td>.86</td>
<td>.59</td>
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<td></td>
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<tr>
<td>Bagger</td>
<td>3.1</td>
<td>2.9</td>
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</tr>
<tr>
<td>Stacker</td>
<td>1.6</td>
<td>---</td>
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</tr>
<tr>
<td>Forklift Op.</td>
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<td>Mill Operator</td>
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<td>10</td>
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<td>Sorter</td>
<td>.6</td>
<td>1.8</td>
<td>2</td>
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<tr>
<td>Driller</td>
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Table B (continued)

PERSONAL RESPIRABLE BREATHING ZONE SAMPLES
(AVG = GEOMETRIC MEAN)

<table>
<thead>
<tr>
<th>Job</th>
<th>Avg  (\text{mg/m}^3)</th>
<th>Variance</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Texas</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Truck Driver</td>
<td>.9</td>
<td>1.5</td>
<td>5</td>
</tr>
<tr>
<td>Miner</td>
<td>.1</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>Shovel Operator</td>
<td>.3</td>
<td>1.2</td>
<td>2</td>
</tr>
<tr>
<td>Calciner Operator</td>
<td>1.1</td>
<td>---</td>
<td>1</td>
</tr>
<tr>
<td>Crusher Operator</td>
<td>1.7</td>
<td>---</td>
<td>1</td>
</tr>
<tr>
<td>Welder</td>
<td>8.5</td>
<td>---</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><strong>1.08</strong></td>
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<td><strong>54</strong></td>
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<td><strong>North Carolina</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bagger</td>
<td>.9</td>
<td>---</td>
<td>1</td>
</tr>
<tr>
<td>Mill Operator</td>
<td>.9</td>
<td>---</td>
<td>1</td>
</tr>
<tr>
<td>Laborer</td>
<td>.2</td>
<td>5.8</td>
<td>9</td>
</tr>
<tr>
<td>Foreman</td>
<td>.9</td>
<td>---</td>
<td>1</td>
</tr>
<tr>
<td>Maintenance</td>
<td>.03</td>
<td>5.8</td>
<td>2</td>
</tr>
<tr>
<td>Driller</td>
<td>.1</td>
<td>1.4</td>
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<td>Hoist Operator</td>
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<td>Grader</td>
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<td>Packer</td>
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<td>Cutter</td>
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<td>Rounder</td>
<td>.9</td>
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<td>Officer Personnel</td>
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<td>16.5</td>
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<td></td>
<td><strong>.21</strong></td>
<td><strong>.74</strong></td>
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<td><strong>ALL REGIONS</strong></td>
<td>.72</td>
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### TABLE C

**SUMMARY OF RESPIRABLE DUST SAMPLES**  
**(AVG = GEOMETRIC MEAN)**

<table>
<thead>
<tr>
<th>Region</th>
<th>Avg (mg/m$^3$)</th>
<th>95% Confidence Range of Mean</th>
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</thead>
<tbody>
<tr>
<td>Montana</td>
<td></td>
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<tr>
<td>Mill</td>
<td>1.1</td>
<td>.85 - 1.41</td>
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<td>Mine</td>
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<td>.47 - .92</td>
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<td>Texas</td>
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<tr>
<td>Mill</td>
<td>1.56</td>
<td>2.54 - .96</td>
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<td>Mine</td>
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<td>.18 - .71</td>
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<td>North Carolina</td>
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<tr>
<td>Mill</td>
<td>.26</td>
<td>.13 - .51</td>
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<tr>
<td>Mine</td>
<td>.14</td>
<td>.07 - .31</td>
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</tbody>
</table>

**CONCLUSIONS**

Mill - Baggers and Mill Operators had highest exposures.

Mine - Truck Drivers and Front-end Loader Operators had highest exposure.
### TABLE D

**TRACE METALS**

(\(\text{mg/m}^3\))

<table>
<thead>
<tr>
<th></th>
<th>Iron</th>
<th>Manganese</th>
<th>Calcium</th>
<th>Aluminum</th>
<th>Zinc</th>
<th>Nickel</th>
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<td>.05</td>
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<td>.01</td>
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<td>.03</td>
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<td>(7-20)</td>
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<td>Montana</td>
<td>4.8% (Limit of Detection)</td>
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<tr>
<td>Texas</td>
<td>2.23%</td>
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<tr>
<td>North Carolina</td>
<td>1.45%</td>
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<tr>
<td>Location</td>
<td>Fibrous Minerals</td>
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<tr>
<td>---------------</td>
<td>------------------------</td>
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<tr>
<td>Montana</td>
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<tr>
<td>Texas</td>
<td>Tremolite, Antigorite</td>
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<td>North Carolina</td>
<td>Acicular Particles</td>
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</table>
PRELIMINARY FINDINGS OF AN EPIDEMIOLOGIC STUDY OF TALC WORKERS

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Alice Greife
John Hancock

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INTRODUCTION

Talc is a mineral with a wide variety of uses in paint, paper, ceramics, cosmetics, plastics, roofing products, textile material, rubber, lubricants, corrosion proofing composition fire extinguishing powders, cereal polishing, water filtration, insecticides to name a few. Pure talc is a hydrated magnesium silicate, but the talc found in nature has a quite variable chemical composition. The mineral contaminant in talc of most concern is asbestos, which can produce a clinical condition resembling that seen on exposure to asbestos per se. The possible hazards from exposure to talc free of asbestos contamination is less well documented. The purpose of this study was to ascertain the effects on the respiratory system (symptoms, lung function, radiographic) of exposure to talc dust thought prior to the study to contain no asbestos. Two hundred and ninety-nine talc workers mining and milling talc from Montana, Texas, and North Carolina were studied in this cross-sectional prevalence study. The mineralogy of the talc and exposure of the workers were just discussed by Ms. Greife. In this paper we will report on the chronic or long-term effects of exposure.

The specific questions addressed in this paper are: What is the prevalence of respiratory symptoms, radiographic changes and reduced lung function among these talc workers? What are the dose-response relations? How does "morbidity" of the study populations compare to that of other mining populations?
METHODS

The study population consisted of workers mining and milling talc from three regions of the United States: Montana, Texas, and North Carolina. Although several different companies may be involved, the results for each region are combined, as the characteristics of the talc in each region are similar. Over 90% of the workers participated in the study.

All workers were administered a British Medical Research Council respiratory questionnaire by trained interviewers. Most of the interviews in Texas were conducted in Spanish. Non-talc work history was obtained in the interview; work experience at the talc facility was obtained from company records. Standard posteroanterior chest radiograms were read by three "B" readers using the ILO U/C 1971 scheme. The films were read independently without knowledge of age, occupation, or smoking history. The median of the three readings was used for analysis. Flow volume curves from a minimum of 5 forced maneuvers were obtained and recorded on magnetic tape using an Ohio 800 rolling seal spirometer. Values from the maximum envelope were used for analysis. Before and after shift spirometry was administered to workers on the day shift, and personal environmental samples were also collected on these workers. The results of the personal environmental sampling were used to evaluate (1) dose-response relations of talc dust with acute changes in pulmonary function over the shift (ΔPFT = after shift PFT minus before shift PFT); and (2) estimate talc dust exposure for each job. This estimate was then used to calculate cumulative talc dust exposure by multiplying job exposure by job years to nearest month, and adding the results of each multiplication. The units for cumulative exposure are mg/m³ x years (mg/m³-years). Sputums were collected on workers 35 years or older.
The prevalence of symptoms and pleural thickening were compared to 3 mining populations, after indirect adjustment for smoking, and using the age distribution of all populations.

Internal comparisons of prevalence and dose-response relationships will be examined first (Tables 5-14). Then comparisons with external control populations will be made (Tables 16-18). Dose-response relationships and external comparisons for lung function are in Tables 19-21.

RESULTS

All of the Texas talc workers were male, while about 20% of the Montana and North Carolina talc workers were female. The North Carolina population had the highest proportion of smokers (62%) and lowest proportion of ex-smokers (17%). The highest proportion of nonsmokers (33%) and lowest proportion of smokers (46%) were in Montana. Cigarettes smoked per day was similar among smokers and ex-smokers in North Carolina and Montana (approximately a pack a day), but was 1/3 a pack a day less in Texas. The North Carolina population had worked on average about 3 1/2 years longer (10 years) than the workers in Montana (7 years) and Texas (6 years), but cumulative exposure in North Carolina was one-half (3 mg/m³- years) that of Montana workers (6 mg/m³- years) and about 1/4 that of Texas workers (11 mg/m³- years) (Table 1). Average exposure (cumulative exposure divided by years worked) showed the same ranking.

Only 11% of the workers in Montana and Texas had worked 10 or more years compared to 38% in North Carolina. Most of the study population in Montana and Texas had worked less than 5 years (66% and 73% respectively).
About 20% in all regions had worked from 5-9 years. North Carolina had the lowest cumulative exposure in each years worked category, especially in the less than 10 years category. The men working 5-9 years in Texas had the highest mean cumulative exposure of any group (25 mg/m$^3$-years); twice that of the next highest group of Montana workers with 10 or more years tenure (12 mg/m$^3$-years). Age, years worked, and cumulative exposure were correlated enough to potentially confound any dose-response association.

Table 3 summarizes the characteristics of the low, medium, and high cumulative exposure groups by region. North Carolina had a higher proportion of workers in the low exposure group and a lower proportion in the medium and high exposure groups. The North Carolina population was older and had more years exposure in each cumulative exposure group. Mean cumulative exposure was also lowest in the low and high exposure group from North Carolina. The Montana and Texas populations were generally similar except for the very high mean cumulative exposure (40 mg/m$^3$-years) in the Texas high exposure group. Smokers comprised about 50% of all exposure groups except for the medium exposure group from North Carolina where 90% were smokers.

Table 4 summarizes by region the frequency of working in non-talc jobs where exposure to respiratory irritants was possible. The frequency was generally low and similar in each region.

There were only 2 cases of pneumoconiosis, both grade 1 small rounded opacities in Texas and Montana. This number is too small to analyze further.
Cytology on sputums collected from workers greater than or equal to 35 years of age revealed no cytology suggestive of malignancy.

Tables 5-14 summarize the prevalence of cough, phlegm, shortness of breath, pleural thickening, and obstruction by region, smoking, and exposure. The overall prevalence of cough was 18%, 17%, and 27% in Montana, Texas, and North Carolina. Cough increased with age in all smoking groups in Montana and in the nonsmoking and ex-smoking category in Texas. No increase with age was observed in North Carolina. Smokers had a higher prevalence of cough than nonsmokers and ex-smokers in Montana, and a higher prevalence than nonsmokers in North Carolina (Table 5). The only statistically significant difference was between smokers and ex-smokers in Montana. Cough showed no apparent association with either years worked or with cumulative exposure, although the medium exposure group had a higher prevalence than the low exposure group (Table 6).

The overall prevalence of phlegm was 18%, 17%, and 25% in Montana, Texas, and North Carolina. Phlegm did not increase with age in Montana (all smoking categories), Texas (nonsmokers and smokers), or nonsmokers in North Carolina. Among smokers in North Carolina and ex-smokers in Texas, phlegm increased with age. Smokers had a higher prevalence of phlegm than nonsmokers in Montana and North Carolina; ex-smokers were intermediate. Ex-smokers had the highest prevalence in Texas, and nonsmokers were intermediate (Table 7). None of these differences were statistically significant. There was no consistent tendency for the prevalence of phlegm to increase with years worked. In Texas and North
Carolina (but not Montana), the prevalence of phlegm increased with increasing cumulative exposure (but was not statistically significant) (Table 8).

The prevalence of dyspnea was low compared to cough and phlegm with 4%, 9%, and 6% in Montana, Texas, and North Carolina complaining of shortness of breath when walking on level ground with people their own age. The rates increased with age in all smoking groups and regions (except smokers in North Carolina where prevalence was zero). There was no apparent association of dyspnea with smoking, however. Smokers often had the lowest prevalence of dyspnea (Table 9). No differences were statistically significant. There was no consistent increase with increasing years worked (there was no dyspnea in any region among the 5–9 year tenure group). In North Carolina the prevalence was elevated in the high cumulative exposure group compared to the low cumulative exposure, but the difference was not statistically significant (Table 10).

The overall prevalence of pleural thickening was 4%, 13%, and 18% in Montana, Texas, and North Carolina. Pleural thickening increased with increasing age in all regions but was significant only in Texas. Nonsmokers had the lowest prevalence (only one nonsmoker had pleural thickening), while the prevalence among smokers and ex-smokers was similar (Table 11). Prevalence of pleural thickening increased slightly with increasing years worked, but did not increase with increased cumulative exposure (Table 12).

The overall prevalence of obstruction (FEV₁/FVC < .70) was 18%, 13%, and 23% in Montana, Texas, and North Carolina. The prevalence of obstruction
increased with age in all regions and smoking categories, but was significant only among nonsmokers, smokers, and total in Montana and total in North Carolina. Smokers had a higher prevalence than nonsmokers, but only in Montana, and the differences were not statistically significant (Table 13). There was no apparent association with cumulative exposure or years worked (Table 14).

Tables 16 through 18 compare adjusted prevalence of symptoms, pleural thickening, and obstruction of the talc population with 3 mining populations: 878 potash miners, 503 coal workers who had never worked underground, and 7942 coal miners who had worked only underground. The potash miners were part of a MSHA/NIOSH study. The coal workers are from the second round of the National Coal Workers Study. Demographic characteristics of these populations are summarized in Table 15.

The adjusted rate of cough among underground coal miners was higher overall and in the 40 year or older age group compared to potash, aboveground coal and talc workers. There was little difference in the prevalence rates among these latter groups and no detectable difference among the talc regions in both age categories. Phlegm showed a somewhat similar pattern except the underground coal miners had more phlegm in both age groups. Again the talc groups showed no detectable difference from each other. Montana had a lower prevalence than aboveground coal workers in the older age category (Table 16).

The overall prevalence of dyspnea was significantly higher in the under­ground coal miners (24%) than all other populations. All the other
populations had rates ranging from 5% (Montana) to 14% (aboveground coal). Rates in the 40 or more year group were 7-15% compared to 28% and 41% in the coal populations. In the less than 40 year old group underground coal was again high with 10%, while all other population rates were 5% or less (Table 17).

Pleural thickening was elevated in the talc populations compared to the comparison populations. Prevalence was elevated in the younger talc workers, but only the increase in North Carolina was statistically significant. Montana had the lowest prevalence of pleural thickening among the talc populations, but the differences were not significant (Table 17).

There were no detectable differences in the prevalence of obstruction among any of the populations (Table 18).

Table 19 summarizes the results of multiple regression models of pulmonary function ($FEV_1$, $FVC$, Peak Flow, $FEF_{50}$, $FEF_{75}$) with the predictor variables sex, mine, age, height, pack years and cumulative exposure. Sex, age, and height were significant variables for $FEV_1$, $FVC$, and peak flow. Of these 3 variables, only age significantly reduced the variability in flow rates ($FEF_{50}$, $FEF_{75}$). Pack years was generally significant for $FEV_1$ and flow rates, but not for $FVC$. Mine, region, and cumulative exposure were not significant.

Essentially the same results occur when the class variables smoking status and cumulative exposure group replace the continuous variables pack years and cumulative exposure. Adjusted mean values by smoking status, region, and cumulative exposure group are summarized in Table 20.
In Table 21, pulmonary function of the combined male talc populations is compared to the control populations (after adjustments for age, height, and smoking). \( \text{FEV}_1 \), \( \text{FVC} \), and flow rates at low lung volumes (\( \text{FEF}_{50} \), \( \text{FEF}_{75} \)) were reduced compared to the coal populations. Peak flow was about the same as underground coal, and elevated compared to aboveground coal. When compared to potash miners, flow rates were reduced, but there was no detectable difference in \( \text{FEV}_1 \) and \( \text{FVC} \).

**DISCUSSION**

Interpretation of the data from this study has the inherent problems of all cross-sectional prevalence studies. These include the lack of any past environmental measures so cumulative exposure is based on current exposure levels only. In addition the numbers of workers in North Carolina and Texas was small, especially after stratification. The small numbers result in very wide confidence intervals. Exposure time (years worked) was short compared to other mining populations. The mean years worked for the potash and coal populations was 16, 18, and 15 years compared to 6, 7, and 10 years for the talc populations. This is a short time for the development of chronic symptoms, pneumoconiosis and impaired pulmonary function caused by work exposure.

None of the health variables were consistently or strongly related to the exposure variables (years worked, cumulative exposure). Cumulative exposure is only a crude estimate of past exposures, based on current levels taken over a short time period. Since past environmental measures
were not taken, past exposures for each job were assumed (for the purposes of calculation) to be the same as current exposures. Thus cumulative exposure is only an estimate, and how much season of the year, changes in the talc composition, humidity, and other factors affect this estimate are not known.

Age was consistently associated with increased prevalence of cough, dyspnea, pleural thickening, and decreased pulmonary function. The calculated loss of pulmonary function with age was comparable to values from other cross-sectional studies, (3-5) and there was little difference in the age coefficients among the regions. Except for FVC and FEF_{50}, mean adjusted pulmonary function values were not statistically different among the regions. Mean FVC was largest in the Montana population and least in Texas. The rank order for FEF_{50} was reversed. FVC was reduced in the high exposure group, but the reduction was not large. The high exposure group did not have the lowest value for any of the other lung function parameters. The reductions in lung function compared to the coal populations (particularly FEV₁ and FVC) were not large. It is interesting that lung function was reduced compared to the coal populations, despite their higher prevalence of respiratory symptoms. The relationship between smoking and pulmonary function was as might be expected. Smokers generally had the poorest values, and nonsmokers the best.

Thus there were no apparent dose-response relationships with symptoms or lung function, and no apparent excess symptoms or large reductions in lung function compared to the control populations. This does not mean that
talc may not have an effect on these health parameters. A dose-response relation can be obscured by an inaccurate estimate of exposure. And the comparison populations used in this report were exposed to respiratory irritants (e.g., coal dust, diesel fumes, sylvite or KCl and NaCl), thereby possibly increasing the prevalence of symptoms and reducing lung function. Comparison with a blue collar "nonexposed" population is now underway and will be reported elsewhere.

The same criticism regarding the comparison populations may not be valid for pleural thickening, which was quite low in all the non-talc populations. The pleural thickening was associated with years worked (somewhat confounded with age). But the increased prevalence occurred in all 3 regions, despite the difference in exposure, and difference in talc composition.

In this study age and years worked were associated with an increase of pleural thickening, although one worker with pleural thickening was in the 20-29 year age category, and 4 in the 30-39 year age group. Three of these had worked 5 years or less. While pleural calcification is rare in individuals under 40, uncalcified plaques are "quite often" seen in individuals less than 40. Ochs and Smith report on several cases where as little as a years time interval was necessary for the appearance of pleural thickening.

Asbestos (particularly anthophyllite) from either occupational or community exposure is believed to cause an increased prevalence of pleural thickening. Talc contaminated with asbestos (tremolite and anthophyllite) as seen under the light microscope and EM has also been associated
REFERENCES


TABLE 1
DEMOGRAPHIC CHARACTERISTICS OF THE TALC WORKER POPULATIONS BY REGION

<table>
<thead>
<tr>
<th></th>
<th>MONTANA</th>
<th>TEXAS</th>
<th>NORTH CAROLINA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>177</td>
<td>71</td>
<td>51</td>
</tr>
<tr>
<td>AGE (S.D.)</td>
<td>34.9 (11.5)</td>
<td>38.0 (13.7)</td>
<td>43.1 (12.6)</td>
</tr>
<tr>
<td>HEIGHT (S.D.)</td>
<td>175.5 (8.8)</td>
<td>173.0 (6.9)</td>
<td>172.5 (8.3)</td>
</tr>
<tr>
<td>WEIGHT (S.D.)</td>
<td>77.8 (13.5)</td>
<td>78.3 (15.1)</td>
<td>78.2 (16.3)</td>
</tr>
<tr>
<td>YEARS WORKED (S.D.)</td>
<td>6.6 (6.3)</td>
<td>5.5 (5.7)</td>
<td>10.1 (8.6)</td>
</tr>
<tr>
<td>CUMULATIVE EXPOSURE (S.D.)</td>
<td>5.9 (7.6)</td>
<td>11.3 (45.1)</td>
<td>3.0 (4.8)</td>
</tr>
<tr>
<td>AVERAGE EXPOSURE (mg/m³) (S.D.)</td>
<td>1.21 (.94)</td>
<td>2.64 (7.12)</td>
<td>0.28 (0.33)</td>
</tr>
<tr>
<td>NONSMOKERS (%)</td>
<td>33</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>EX-SMOKERS (%)</td>
<td>21</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td>PACK YEARS (S.D.)</td>
<td>15.7 (17.9)</td>
<td>13.3 (20.7)</td>
<td>18.2 (16.5)</td>
</tr>
<tr>
<td>CIGARETTES/DAY (S.D.)</td>
<td>23 (15)</td>
<td>12 (14)</td>
<td>21.4 (15.7)</td>
</tr>
<tr>
<td>SMOKERS (%)</td>
<td>45</td>
<td>54</td>
<td>62</td>
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<tr>
<td>PACK YEARS (S.D.)</td>
<td>17.9 (16.9)</td>
<td>14.3 (19.7)</td>
<td>23.7 (21.8)</td>
</tr>
<tr>
<td>CIGARETTES/DAY (S.D.)</td>
<td>20.4 (11.0)</td>
<td>14.5 (11.1)</td>
<td>20.4 (10.0)</td>
</tr>
</tbody>
</table>
### A. Characteristics of Talc Workers by Region and Years Worked Categories

<table>
<thead>
<tr>
<th>Region</th>
<th>5</th>
<th>5-9</th>
<th>≥10</th>
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<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>5</strong></td>
<td>92</td>
<td>36.5 (10.4)</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>36.5 (10.4)</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>46.2 (7.8)</td>
<td>46</td>
</tr>
<tr>
<td><strong>Texas</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>5</strong></td>
<td>39</td>
<td>34.8 (14.1)</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>39.6 (13.3)</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>46.5 (9.2)</td>
<td>21</td>
</tr>
<tr>
<td><strong>North Carolina</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>5</strong></td>
<td>19</td>
<td>35.3 (13.8)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>43.8 (12.0)</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>48.6 (8.6)</td>
<td>26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Years Worked</th>
<th>Cumulative Exposure</th>
<th>Average Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (S.D.)</td>
<td>Mean (S.D.)</td>
<td>Mean (S.D.)</td>
</tr>
</tbody>
</table>

### B. Correlation of Age, Years Worked, and Cumulative Exposure by Region

<table>
<thead>
<tr>
<th></th>
<th>Montana</th>
<th>Texas</th>
<th>North Carolina</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age by Years Exposed</strong></td>
<td>.63 (.48 to .78)</td>
<td>.36 (.12 to .48)</td>
<td>.51 (.23 to .79)</td>
</tr>
<tr>
<td><strong>Age by Cumulative Exposure</strong></td>
<td>.41 (.26 to .56)</td>
<td>.12 (-.12 to .36)</td>
<td>.33 (.05 to .61)</td>
</tr>
<tr>
<td><strong>Years Exposure by Cumulative Exposure</strong></td>
<td>.48 (.33 to .63)</td>
<td>.12 (-.12 to .36)</td>
<td>.44 (.16 to .72)</td>
</tr>
</tbody>
</table>

r (95% C.I.)
### TABLE 3

**CHARACTERISTICS OF TALC WORKERS EXPOSURE GROUPS BY REGION**

<table>
<thead>
<tr>
<th>Region</th>
<th>Low Exposure</th>
<th>Medium Exposure</th>
<th>High Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Mean (S.D.)</td>
<td>Mean (S.D.)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Age (Years)</td>
<td>Years Worked</td>
</tr>
<tr>
<td>MONTANA</td>
<td>54 (31)</td>
<td>33.4 (10.5)</td>
<td>5.6 (7.4)</td>
</tr>
<tr>
<td></td>
<td>64 (36)</td>
<td>32.0 (11.4)</td>
<td>4.0 (4.3)</td>
</tr>
<tr>
<td></td>
<td>59 (33)</td>
<td>39.3 (11.5)</td>
<td>10.3 (5.2)</td>
</tr>
<tr>
<td>TEXAS</td>
<td>27 (38)</td>
<td>33.3 (13.1)</td>
<td>4.9 (7.0)</td>
</tr>
<tr>
<td></td>
<td>26 (37)</td>
<td>40.2 (13.6)</td>
<td>4.2 (3.8)</td>
</tr>
<tr>
<td></td>
<td>18 (25)</td>
<td>42.1 (13.4)</td>
<td>8.1 (5.4)</td>
</tr>
<tr>
<td>NORTH CAROLINA</td>
<td>32 (63)</td>
<td>40.6 (13.7)</td>
<td>7.5 (8.0)</td>
</tr>
<tr>
<td></td>
<td>10 (20)</td>
<td>44.3 (8.5)</td>
<td>13.5 (9.7)</td>
</tr>
<tr>
<td></td>
<td>9 (18)</td>
<td>50.6 (9.4)</td>
<td>15.9 (4.9)</td>
</tr>
</tbody>
</table>

LOW CUMULATIVE EXPOSURE = <2 mg/m³-years
MEDIUM CUMULATIVE EXPOSURE = 2 - 5.9 mg/m³-years
HIGH CUMULATIVE EXPOSURE = ≥6 mg/m³-years

AVERAGE EXPOSURE = Σ(cumulative exposure/years worked)
TABLE 4

PREVALENCE (%) BY REGION OF OTHER OCCUPATIONS

<table>
<thead>
<tr>
<th>HAVE YOU EVER WORKED . . . .</th>
<th>MONTANA n (%)</th>
<th>TEXAS n (%)</th>
<th>NORTH CAROLINA n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN A QUARRY?</td>
<td>3 (1.7)</td>
<td>2 (2.8)</td>
<td>6 (11.8)</td>
</tr>
<tr>
<td>IN A FOUNDRY?</td>
<td>2 (1.1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IN A POTTERY?</td>
<td>2 (1.1)</td>
<td>1 (1.4)</td>
<td>0</td>
</tr>
<tr>
<td>IN A COTTON, FLAX, OR HEMP MILL?</td>
<td>1 (0.6)</td>
<td>13 (18.3)</td>
<td>4 (7.8)*</td>
</tr>
<tr>
<td>WITH ASBESTOS?</td>
<td>4 (2.3)</td>
<td>1 (1.4)</td>
<td>0</td>
</tr>
</tbody>
</table>

*95% confidence intervals do not overlap.
<table>
<thead>
<tr>
<th>AGE</th>
<th>40 % (95% C.I.)</th>
<th>&gt;40 % (95% C.I.)</th>
<th>TOTAL % (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MONTANA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NONSMOKER</td>
<td>7 (6 - 19)</td>
<td>19 (5 - 42)</td>
<td>10 (4 - 21)</td>
</tr>
<tr>
<td>EX-SMOKER</td>
<td>0 (0 - 16)</td>
<td>10 (2 - 28)</td>
<td>5 (1 - 17)*</td>
</tr>
<tr>
<td>SMOKER</td>
<td>27 (16 - 40)</td>
<td>38 (19 - 58)</td>
<td>29 (19 - 40)*</td>
</tr>
<tr>
<td>TOTAL</td>
<td>16 (10 - 24)</td>
<td>23 (13 - 35)</td>
<td>18</td>
</tr>
<tr>
<td>TEXAS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NONSMOKER</td>
<td>10 (1 - 40)</td>
<td>25 (1 - 75)</td>
<td>14 (3 - 39)</td>
</tr>
<tr>
<td>EX-SMOKER</td>
<td>11 (1 - 44)</td>
<td>40 (15 - 73)</td>
<td>26 (11 - 50)</td>
</tr>
<tr>
<td>SMOKER</td>
<td>15 (6 - 34)</td>
<td>8 (0 - 35)</td>
<td>13 (5 - 28)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>13 (5 - 28)</td>
<td>23 (11 - 42)</td>
<td>17 (9 - 28)</td>
</tr>
<tr>
<td>NORTH CAROLINA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NONSMOKER</td>
<td>0 (0 - 50)</td>
<td>0 (0 - 40)</td>
<td>0 (0 - 25)</td>
</tr>
<tr>
<td>EX-SMOKER</td>
<td>33 (2 - 87)</td>
<td>17 (1 - 60)</td>
<td>22 (4 - 56)</td>
</tr>
<tr>
<td>SMOKER</td>
<td>38 (17 - 67)</td>
<td>37 (15 - 64)</td>
<td>38 (21 - 58)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>29 (13 - 51)</td>
<td>26 (12 - 45)</td>
<td>27</td>
</tr>
</tbody>
</table>

COUGH = Answering yes to the question: "Do you usually cough on most days for as much as three months each year?"

SUMMARY: Increase with age except in North Carolina and among smokers in Texas.

Smokers had highest prevalence except among 40 or more year old smokers in Texas. Association with ex-smokers variable. Only significant differences was between smokers and ex-smokers in Montana.

* 95% confidence intervals do not overlap.


TABLE 6

PREVALENCE OF COUGH BY EXPOSURE AND REGION

<table>
<thead>
<tr>
<th>YEARS WORKED</th>
<th>MONTANA % (95% C.I.)</th>
<th>TEXAS % (95% C.I.)</th>
<th>NORTH CAROLINA % (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>17 (11 - 25)</td>
<td>20 (10 - 35)</td>
<td>30 (14 - 53)</td>
</tr>
<tr>
<td>5-9</td>
<td>14 (6 - 30)</td>
<td>18 (3 - 50)</td>
<td>33 (12 - 65)</td>
</tr>
<tr>
<td>≥10</td>
<td>30 (9 - 36)</td>
<td>0 (0 - 32)</td>
<td>20 (7 - 41)</td>
</tr>
<tr>
<td>CUMULATIVE EXPOSURE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOW (2)</td>
<td>15 (6 - 28)</td>
<td>7 (1 - 22)</td>
<td>19 (9 - 36)</td>
</tr>
<tr>
<td>MEDIUM (2-6)</td>
<td>17 (9 - 28)</td>
<td>31 (15 - 51)</td>
<td>50 (22 - 78)</td>
</tr>
<tr>
<td>HIGH (_6)</td>
<td>17 (9 - 29)</td>
<td>11 (2 - 33)</td>
<td>22 (4 - 56)</td>
</tr>
</tbody>
</table>

COUGH = Answering yes to the question: "Do you usually cough on most days for as much as three months each year?"

SUMMARY: No consistent tendency to increase with increasing years worked.

No tendency for prevalence to increase with increasing cumulative exposure.

No statistically significant association with either exposure variable.
### TABLE 7

**Prevalence of Phlegm among Talc Workers by Age, Smoking, and Region**

<table>
<thead>
<tr>
<th></th>
<th>40 % (95% C.I.)</th>
<th>≥40 % (95% C.I.)</th>
<th>TOTAL % (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MONTANA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NONSMOKER</td>
<td>12 (4 - 27)</td>
<td>6 (0 - 25)</td>
<td>10 (4 - 21)</td>
</tr>
<tr>
<td>EX-SMOKER</td>
<td>17 (5 - 38)</td>
<td>14 (4 - 34)</td>
<td>10 (6 - 30)</td>
</tr>
<tr>
<td>SMOKER</td>
<td>27 (17 - 40)</td>
<td>23 (13 - 50)</td>
<td>26 (16 - 36)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>20 (13 - 29)</td>
<td>17 (8 - 29)</td>
<td>18</td>
</tr>
<tr>
<td><strong>TEXAS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NONSMOKER</td>
<td>20 (6 - 50)</td>
<td>25 (1 - 75)</td>
<td>21 (6 - 50)</td>
</tr>
<tr>
<td>EX-SMOKER</td>
<td>22 (4 - 56)</td>
<td>40 (15 - 73)</td>
<td>32 (15 - 57)</td>
</tr>
<tr>
<td>SMOKER</td>
<td>8 (1 - 24)</td>
<td>8 (0 - 35)</td>
<td>8 (2 - 21)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>14 (5 - 29)</td>
<td>23 (11 - 39)</td>
<td>17</td>
</tr>
<tr>
<td><strong>NORTH CAROLINA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NONSMOKER</td>
<td>0 (0 - 50)</td>
<td>0 (0 - 40)</td>
<td>0 (0 - 25)</td>
</tr>
<tr>
<td>EX-SMOKER</td>
<td>0 (0 - 63)</td>
<td>33 (6 - 73)</td>
<td>22 (4 - 56)</td>
</tr>
<tr>
<td>SMOKER</td>
<td>23 (7 - 52)</td>
<td>42 (22 - 66)</td>
<td>34 (18 - 54)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>14 (4 - 34)</td>
<td>32 (16 - 51)</td>
<td>25</td>
</tr>
</tbody>
</table>

**PHLEG =** Answering yes to the question: "Do you usually bring up phlegm from your chest for as much as three months each year?"

**SUMMARY:** Increased prevalence with age only among both smoking categories in North Carolina; ex-smokers and nonsmokers in Texas.

Association with smoking in Montana and North Carolina. Ex-smokers highest and smokers lowest prevalence in Texas.

No statistically significant differences by age or smoking.
TABLE 8

PREVALENCE OF PHLEGM BY EXPOSURE AND REGION

<table>
<thead>
<tr>
<th>YEARS WORKED</th>
<th>MONTANA % (95% C.I.)</th>
<th>TEXAS % (95% C.I.)</th>
<th>NORTH CAROLINA % (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 years</td>
<td>19 (13 - 27)</td>
<td>20 (10 - 34)</td>
<td>20 (7 - 41)</td>
</tr>
<tr>
<td>5-9 years</td>
<td>12 (4 - 26)</td>
<td>9 (6 - 37)</td>
<td>33 (12 - 65)</td>
</tr>
<tr>
<td>10 years</td>
<td>30 (14 - 53)</td>
<td>13 (1 - 50)</td>
<td>25 (10 - 47)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CUMULATIVE EXPOSURE</th>
<th>MONTANA % (95% C.I.)</th>
<th>TEXAS % (95% C.I.)</th>
<th>NORTH CAROLINA % (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW (2)</td>
<td>17 (8 - 30)</td>
<td>7 (1 - 22)</td>
<td>13 (5 - 29)</td>
</tr>
<tr>
<td>MEDIUM (2-6)</td>
<td>18 (9 - 30)</td>
<td>27 (11 - 64)</td>
<td>50 (22 - 78)</td>
</tr>
<tr>
<td>HIGH (6)</td>
<td>17 (8 - 30)</td>
<td>17 (5 - 38)</td>
<td>33 (10 - 71)</td>
</tr>
</tbody>
</table>

PHLEG = Answering yes to the question: "Do you usually bring up phlegm from your chest for as much as three months each year?"

SUMMARY: No consistent tendency to increase with years worked. Higher prevalence in Medium and High Exposure groups in Texas and North Carolina, but not statistically significant.
TABLE 9

PREVALENCE OF DYSPNEA AMONG TALC WORKERS BY AGE, SMOKING, AND REGION

<table>
<thead>
<tr>
<th></th>
<th>40</th>
<th>≥40</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (95% C.I.)</td>
<td>% (95% C.I.)</td>
<td>% (95% C.I.)</td>
</tr>
<tr>
<td>MONTANA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NONSMOKER</td>
<td>2 (1 - 19)</td>
<td>6 (0 - 25)</td>
<td>3 (0 - 11)</td>
</tr>
<tr>
<td>EX-SMOKER</td>
<td>6 (0 - 24)</td>
<td>10 (2 - 28)</td>
<td>8 (2 - 21)</td>
</tr>
<tr>
<td>SMOKER</td>
<td>2 (0 - 9)</td>
<td>5 (0 - 21)</td>
<td>2 (0 - 8)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>2 (0 - 6)</td>
<td>7 (2 - 16)</td>
<td>4</td>
</tr>
<tr>
<td>TEXAS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NONSMOKER</td>
<td>10 (1 - 40)</td>
<td>25 (1 - 75)</td>
<td>14 (3 - 39)</td>
</tr>
<tr>
<td>EX-SMOKER</td>
<td>0 (0 - 29)</td>
<td>20 (4 - 60)</td>
<td>11 (2 - 32)</td>
</tr>
<tr>
<td>SMOKER</td>
<td>0 (0 - 12)</td>
<td>8 (3 - 45)</td>
<td>5 (0 - 16)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>2 (0 - 12)</td>
<td>19 (3 - 37)</td>
<td>9</td>
</tr>
<tr>
<td>NORTH CAROLINA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NONSMOKER</td>
<td>0 (0 - 50)</td>
<td>17 (0 - 40)</td>
<td>9 (0 - 37)</td>
</tr>
<tr>
<td>EX-SMOKER</td>
<td>0 (0 - 63)</td>
<td>33 (6 - 73)</td>
<td>22 (4 - 56)</td>
</tr>
<tr>
<td>SMOKER</td>
<td>0 (0 - 23)</td>
<td>0 (0 - 15)</td>
<td>0 (0 - 10)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>0 (0 - 14)</td>
<td>10 (3 - 24)</td>
<td>6</td>
</tr>
</tbody>
</table>

DYSPNEA = Answering yes to the question: "Do you get short of breath walking with people your own age on level ground?"

SUMMARY: Prevalence increased with age.

No association with smoking.

None of the differences were statistically significant.
TABLE 10
PREVALENCE OF DYSPNEA BY EXPOSURE AND REGION

<table>
<thead>
<tr>
<th></th>
<th>MONTANA</th>
<th>TEXAS</th>
<th>NORTH CAROLINA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (95% C.I.)</td>
<td>% (95% C.I.)</td>
<td>% (95% C.I.)</td>
</tr>
<tr>
<td><strong>YEARS WORKED</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5 (3 - 12)</td>
<td>10 (4 - 22)</td>
<td>5 (0 - 22)</td>
</tr>
<tr>
<td>5-9</td>
<td>0 (0 - 9)</td>
<td>0 (0 - 25)</td>
<td>0 (0 - 24)</td>
</tr>
<tr>
<td>≥10</td>
<td>5 (0 - 22)</td>
<td>13 (0 - 50)</td>
<td>10 (2 - 29)</td>
</tr>
<tr>
<td><strong>CUMULATIVE EXPOSURE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOW (2)</td>
<td>6 (1 - 16)</td>
<td>7 (1 - 22)</td>
<td>3 (0 - 17)</td>
</tr>
<tr>
<td>MEDIUM (2-6)</td>
<td>2 (0 - 7)</td>
<td>8 (1 - 23)</td>
<td>0 (0 - 27)</td>
</tr>
<tr>
<td>HIGH (&gt;6)</td>
<td>3 (0 - 11)</td>
<td>11 (2 - 33)</td>
<td>22 (4 - 56)</td>
</tr>
</tbody>
</table>

DYSPNEA = Answering yes to the question: "Do you get short of breath walking with people your own age on level ground?"

SUMMARY: No consistent increase with increasing years worked.
          No consistent increase with increasing cumulative exposure.
TABLE 11

PREVALENCE OF PLEURAL THICKENING AMONG TALC WORKERS BY AGE, SMOKING AND REGION

<table>
<thead>
<tr>
<th>Region</th>
<th>Non-smoker</th>
<th>Ex-smoker</th>
<th>Smoker</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montana</td>
<td>0 (0 - 11)</td>
<td>14 (1 - 55)</td>
<td>2 (0 - 10)</td>
<td>2 (0 - 7)</td>
</tr>
<tr>
<td>Texas</td>
<td>0 (0 - 27)</td>
<td>13 (1 - 50)</td>
<td>0 (0 - 12)*</td>
<td>2 (0 - 12)*</td>
</tr>
<tr>
<td>North Carolina</td>
<td>0 (0 - 50)</td>
<td>33 (2 - 87)</td>
<td>8 (0 - 33)</td>
<td>10 (2 - 28)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>% (95% C.I.)</th>
<th>% (95% C.I.)</th>
<th>% (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SUMMARY:** Increased prevalence with increased age (significant only in Texas among smokers and combined).

No relationship with smoking (lowest prevalence in non-smokers, highest prevalence in ex-smokers).

*95% confidence intervals do not overlap.
### TABLE 12

**PREVALENCE OF PLEURAL THICKENING AMONG TALC WORKERS BY EXPOSURE AND REGION**

<table>
<thead>
<tr>
<th>YEARS WORKED</th>
<th>MONTANA % (95% C.I.)</th>
<th>TEXAS % (95% C.I.)</th>
<th>NORTH CAROLINA % (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3 (1 - 8)</td>
<td>10 (7 - 33)</td>
<td>15 (4 - 35)</td>
</tr>
<tr>
<td>5-9</td>
<td>5 (0 - 17)</td>
<td>18 (3 - 50)</td>
<td>18 (3 - 50)</td>
</tr>
<tr>
<td>≥10</td>
<td>11 (2 - 33)</td>
<td>29 (5 - 66)</td>
<td>21 (8 - 43)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CUMULATIVE EXPOSURE</th>
<th>MONTANA % (95% C.I.)</th>
<th>TEXAS % (95% C.I.)</th>
<th>NORTH CAROLINA % (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW (2)</td>
<td>2 (0 - 11)</td>
<td>4 (0 - 19)</td>
<td>13 (5 - 29)</td>
</tr>
<tr>
<td>MEDIUM (2-6)</td>
<td>2 (0 - 11)</td>
<td>23 (11 - 42)</td>
<td>10 (0 - 40)</td>
</tr>
<tr>
<td>HIGH (&gt;6)</td>
<td>7 (2 - 16)</td>
<td>6 (0 - 25)</td>
<td>--</td>
</tr>
</tbody>
</table>

**SUMMARY:** Tendency for prevalence to increase with increasing years worked.

No consistent increase with increased cumulative exposure.
TABLE 13
PREVALENCE OF OBSTRUCTION (FEV₁/FVC .70) AMONG TALC WORKERS BY AGE, SMOKING AND REGION

<table>
<thead>
<tr>
<th>AGE</th>
<th>40 % (95% C.I.)</th>
<th>40 % (95% C.I.)</th>
<th>TOTAL % (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MONTANA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NONSMOKER</td>
<td>2 (0 - 11)*</td>
<td>29 (12 - 54)*</td>
<td>10 (4 - 21)</td>
</tr>
<tr>
<td>EX-SMOKER</td>
<td>6 (0 - 25)</td>
<td>33 (14 - 55)</td>
<td>21 (10 - 36)</td>
</tr>
<tr>
<td>SMOKER</td>
<td>14 (6 - 25)*</td>
<td>45 (26 - 67)*</td>
<td>22 (14 - 33)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>9 (4 - 17)*</td>
<td>37 (25 - 51)*</td>
<td>18</td>
</tr>
<tr>
<td>TEXAS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NONSMOKER</td>
<td>10 (0 - 40)</td>
<td>50 (10 - 90)</td>
<td>21 (6 - 50)</td>
</tr>
<tr>
<td>EX-SMOKER</td>
<td>0 (0 - 50)</td>
<td>10 (0 - 40)</td>
<td>5 (0 - 22)</td>
</tr>
<tr>
<td>SMOKER</td>
<td>4 (0 - 19)</td>
<td>33 (12 - 65)</td>
<td>14 (5 - 30)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>5 (1 - 17)</td>
<td>27 (11 - 47)</td>
<td>13</td>
</tr>
<tr>
<td>NORTH CAROLINA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NONSMOKER</td>
<td>0 (0 - 50)</td>
<td>33 (6 - 73)</td>
<td>18 (3 - 50)</td>
</tr>
<tr>
<td>EX-SMOKER</td>
<td>0 (0 - 63)</td>
<td>33 (6 - 73)</td>
<td>22 (4 - 56)</td>
</tr>
<tr>
<td>SMOKER</td>
<td>0 (0 - 29)</td>
<td>39 (16 - 63)</td>
<td>26 (11 - 44)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>0 (0 - 16)*</td>
<td>37 (21 - 56)*</td>
<td>23</td>
</tr>
</tbody>
</table>

SUMMARY: Obstruction increased with age in all smoking categories (significant in Montana nonsmokers, smokers and combined, and combined in North Carolina).

Obstruction had a tendency to be higher in smokers, but only in Montana.

*95% confidence intervals do not overlap.
TABLE 14
PREVALENCE OF OBSTRUCTION (FEV₁/FVC .70) AMONG TALC WORKERS BY EXPOSURE AND REGION

<table>
<thead>
<tr>
<th></th>
<th>MONTANA % (95% C.I.)</th>
<th>TEXAS % (95% C.I.)</th>
<th>NORTH CAROLINA % (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YEARS WORKED</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>15 (9 - 23)</td>
<td>16 (7 - 30)</td>
<td>17 (5 - 38)</td>
</tr>
<tr>
<td>5-9</td>
<td>22 (10 - 38)</td>
<td>9 (0 - 37)</td>
<td>20 (4 - 60)</td>
</tr>
<tr>
<td>&lt;10</td>
<td>30 (14 - 53)</td>
<td>0 (0 - 32)</td>
<td>32 (15 - 57)</td>
</tr>
<tr>
<td>CUMULATIVE EXPOSURE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOW (2)</td>
<td>28 (16 - 43)</td>
<td>11 (3 - 27)</td>
<td>23 (10 - 40)</td>
</tr>
<tr>
<td>MEDIUM (2-6)</td>
<td>8 (3 - 18)</td>
<td>15 (5 - 33)</td>
<td>29 (5 - 66)</td>
</tr>
<tr>
<td>HIGH (_6)</td>
<td>17 (9 - 29)</td>
<td>11 (2 - 33)</td>
<td>22 (4 - 56)</td>
</tr>
</tbody>
</table>

SUMMARY: Tendency to increase with years worked in Montana and North Carolina.

No association with cumulative exposure.
### TABLE 15
CHARACTERISTICS OF COMPARISON POPULATIONS FOR TALC STUDY

<table>
<thead>
<tr>
<th></th>
<th>POTASH</th>
<th>ABOVEGROUND COAL</th>
<th>UNDERGROUND COAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>875</td>
<td>509</td>
<td>5722</td>
</tr>
<tr>
<td>AGE (S.D.)</td>
<td>41 (13)</td>
<td>44 (12)</td>
<td>39 (13)</td>
</tr>
<tr>
<td>HEIGHT (cm)</td>
<td>176 (6)</td>
<td>175 (6)</td>
<td>174 (6)</td>
</tr>
<tr>
<td>YEARS WORKED (S.D.)</td>
<td>16 (13)</td>
<td>18 (13)</td>
<td>15 (13)</td>
</tr>
<tr>
<td>(RANGE)</td>
<td>0-50</td>
<td>0-55</td>
<td>0-56</td>
</tr>
<tr>
<td>NONSMOKERS (%)</td>
<td>20</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>EX-SMOKERS (%)</td>
<td>28</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td>MEAN PACK YEARS</td>
<td>23 (20)</td>
<td>24 (19)</td>
<td>17 (18)</td>
</tr>
<tr>
<td>(S.D.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN CIGARETTES/DAY (S.D.)</td>
<td>25 (14)</td>
<td>23 (12)</td>
<td>19 (12)</td>
</tr>
<tr>
<td>SMOKERS (%)</td>
<td>52</td>
<td>46</td>
<td>56</td>
</tr>
<tr>
<td>MEAN PACK YEARS</td>
<td>28 (23)</td>
<td>27 (18)</td>
<td>17 (14)</td>
</tr>
<tr>
<td>(S.D.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN CIGARETTES/DAY (S.D.)</td>
<td>25 (12)</td>
<td>22 (9)</td>
<td>17 (8)</td>
</tr>
<tr>
<td>MEAN CURRENT NO₂ CONCENTRATION (ppm)</td>
<td>*0.90</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>MEAN CURRENT TOTAL DUST (mg/m³)</td>
<td>*3.45</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>RESPIRABLE DUST</td>
<td>N.A.</td>
<td>1.44 ++</td>
<td>1.36 ++</td>
</tr>
</tbody>
</table>

* Personal samples, from (1,2)

N.A. = Not available.

++ Collected between the first and second rounds of the National Coalworkers' Study. The 25 coal mines were in both the first and second rounds of examinations of the coal study.
TABLE 16

COMPARATIVE RATES (%) OF COUGH AND PHLEGM AMONG TALC WORKERS COMPARED TO POTASH MINERS, ABOVEGROUND AND UNDERGROUND COAL MINERS, STRATIFIED BY AGE AND INDIRECTLY ADJUSTED FOR SMOKING.

| AGE | COUGH | | | PHLEGM | | |
|-----|-------|------------------|------------------|------------------|------------------|
|     |       |       |       |       |       |
|     | <40   | >40   | TOTAL | <40   | >40   | TOTAL |
|     | % (95% C.I.) | % (95% C.I.) | % (95% C.I.) | % (95% C.I.) | % (95% C.I.) | % (95% C.I.) |
| COUGH |       |       |       |       |       |       |
| MONTANA | 16 (10 - 24) | 25 (14 - 37) | 21 (15 - 28) | 21 (14 - 29) | 18 (9 - 30) | 19 (13 - 26) |
| TEXAS | 13 (5 - 27) | 21 (7 - 42) | 17 (9 - 28) | 14 (6 - 28) | 21 (7 - 41) | 16 (8 - 26) |
| NORTH CAROLINA | 28 (11 - 52) | 24 (10 - 43) | 26 (15 - 40) | 13 (2 - 35) | 32 (16 - 51) | 15 (6 - 29) |
| POTASH | 20 (16 - 24) | 30 (26 - 34) | 25 (21 - 29) | 25 (21 - 29) | 30 (21 - 30) | 29 (25 - 33) |
| ABOVEGROUND COAL | 16 (10 - 23) | 35 (30 - 41) | 25 (21 - 29) | 18 (13 - 25) | 41 (35 - 47) | 29 (25 - 33) |
| UNDERGROUND COAL | 18 (16 - 20) | 45 (43 - 46) | 30 (29 - 32) | 32 (31 - 35) | 50 (46 - 53) | 41 (39 - 41) |

SUMMARY

COUGH: All talc populations <underground coal in >40 age group and overall. No difference among talc and other comparison populations.

PHLEGM: <40 - Underground coal had greater prevalence than all populations except North Carolina. No differences among the other populations.

>40 - Talc populations no different from each other. Montana and Texas <underground coal. Montana <aboveground coal.

Total - All populations <underground coal but no different from each other.
### TABLE 17

Comparative rates (%) of dyspnea and pleural thickening among talc workers compared to potash miners, aboveground and underground coal miners, stratified by age and indirectly adjusted for smoking

<table>
<thead>
<tr>
<th></th>
<th>AGE</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;40</td>
<td>&gt;40</td>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% (95% C.I.)</td>
<td>% (95% C.I.)</td>
<td>% (95% C.I.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DYSPNEA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montana</td>
<td>3 (1 - 8)</td>
<td>7 (2 - 16)</td>
<td>5 (2 - 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texas</td>
<td>4 (0 - 15)</td>
<td>15 (4 - 35)</td>
<td>9 (4 - 18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North Carolina</td>
<td>0 (0 - 16)</td>
<td>14 (4 - 31)</td>
<td>6 (1 - 17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potash</td>
<td>5 (3 - 7)</td>
<td>12 (9 - 16)</td>
<td>8 (6 - 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aboveground Coal</td>
<td>2 (1 - 7)</td>
<td>28 (23 - 34)</td>
<td>14 (11 - 17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underground Coal</td>
<td>10 (9 - 11)</td>
<td>41 (40 - 42)</td>
<td>24 (23 - 25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PLEURAL THICKENING</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montana</td>
<td>2 (0 - 6)</td>
<td>11 (4 - 22)</td>
<td>6 (3 - 11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texas</td>
<td>3 (0 - 14)</td>
<td>32 (15 - 53)</td>
<td>17 (9 - 28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North Carolina</td>
<td>12 (2 - 34)</td>
<td>25 (11 - 44)</td>
<td>18 (9 - 31)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potash</td>
<td>0 (0 - 1)</td>
<td>3 (2 - 4)</td>
<td>2 (1 - 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aboveground Coal</td>
<td>0 (0 - 3)</td>
<td>1 (0 - 3)</td>
<td>0.3 (0 - 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underground Coal</td>
<td>0.2 (0.3)</td>
<td>1 (.5 - 1.5)</td>
<td>1 (.5 - 1.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SUMMARY**

**DYSPNEA:**

*<40* - No difference among talc, potash, and aboveground coal populations. Montana less than underground coal.

*≥40* - No difference among talc and potash populations, and all had less dyspnea than underground coal. Montana had less prevalence than aboveground coal.

*Total* - No difference among talc and potash populations. Underground coal had greater prevalence than all populations. Montana had less dyspnea than aboveground coal.

**PLEURAL THICKENING:**

*<40* - No difference among talc populations. North Carolina elevated compared to potash and underground coal.

*≥40* and *Total* - No differences among talc populations. All populations had greater prevalence than nontalc populations.
TABLE 18

COMPARATIVE RATES OF OBSTRUCTION (FEV₁/FVC < .70) AMONG TALC WORKERS COMPARED TO OTHER MINING POPULATIONS. STRATIFIED BY AGE AND INDIRECTLY ADJUSTED FOR SMOKING

<table>
<thead>
<tr>
<th>Age</th>
<th>&lt;40 % (95% C.I.)</th>
<th>&gt;40 % (95% C.I.)</th>
<th>Total % (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MONTANA</td>
<td>8 (4 - 15)</td>
<td>37 (25 - 50)</td>
<td>22 (16 - 29)</td>
</tr>
<tr>
<td>TEXAS</td>
<td>5 (5 - 17)</td>
<td>29 (15 - 51)</td>
<td>16 (9 - 27)</td>
</tr>
<tr>
<td>NORTH CAROLINA</td>
<td>0 (0 - 17)</td>
<td>36 (21 - 56)</td>
<td>17 (8 - 30)</td>
</tr>
<tr>
<td>POTASH MINERS</td>
<td>9 (6 - 12)</td>
<td>33 (29 - 37)</td>
<td>20 (17 - 23)</td>
</tr>
<tr>
<td>UNDERGROUND COAL</td>
<td>11 (10 - 12)</td>
<td>32 (31 - 33)</td>
<td>21 (20 - 22)</td>
</tr>
<tr>
<td>ABOVEGROUND COAL</td>
<td>8 (4 - 14)</td>
<td>31 (26 - 36)</td>
<td>19 (16 - 23)</td>
</tr>
</tbody>
</table>

SUMMARY:  
<40 - No differences  
>40 - No differences  
Total - No differences


### TABLE 19

**SUMMARY OF PULMONARY FUNCTION REGRESSION MODELS AMONG TALC WORKERS (COMBINED AND BY REGION)**

**MODEL:** $PF_f = \alpha + \beta_1(\text{sex}) + \beta_2(\text{mine}) + \beta_3(\text{age}) + \beta_4(\text{height}) + \beta_5(\text{pack years}) + \beta_6(\text{cumulative exposure})$

$$
\begin{array}{ccccccc}
\text{SEX} & \text{MINE} & \text{AGE} & \text{HEIGHT} & \text{PACK YEARS} & \text{CUMULATIVE EXPOSURE (95% CONFIDENCE INTERVAL)} & \underline{r^2} \\
\hline
\text{FEV}_1 (\text{mL}) & -572 & \text{N.S.} & -30 & +46 & -7 & (-3 \text{ to } +3) & .64 \\
\text{Montana} & -608 & \text{N.S.} & -30 & +40 & -8 & (-27 \text{ to } +1) & .71 \\
\text{Texas} & --- & \text{N.S.} & -26 & +33 & (-8) & (-4 \text{ to } +3) & .48 \\
\text{North Carolina} & (-212) & --- & -25 & +77 & (-9) & (-31 \text{ to } +61) & .60 \\
\text{FVC} (\text{mL}) & -865 & \text{N.S.} & -19 & +64 & (-3) & (-5 \text{ to } +5) & .65 \\
\text{Montana} & -1038 & \text{N.S.} & -13 & +53 & (-3) & (-35 \text{ to } -6*) & .74 \\
\text{Texas} & --- & * & -21 & +57 & (-8) & (-5 \text{ to } +3) & .48 \\
\text{North Carolina} & (-341) & --- & (-9) & +108 & (-6) & (-84 \text{ to } +26) & .62 \\
\text{PEAK FLOW} (\text{mL/sec}) & -1592 & \text{N.S.} & -39 & +80 & -15 & (-12 \text{ to } +5) & .47 \\
\text{Montana} & -1674 & \text{N.S.} & -35 & +74 & (-13) & (-57 \text{ to } +56) & .50 \\
\text{Texas} & --- & * & -25 & +63 & (-17) & (-13 \text{ to } +7) & .21 \\
\text{North Carolina} & (-1298) & --- & -54 & +115 & (-25) & (-179 \text{ to } +137) & .54 \\
\text{FEF}_{50} (\text{mL/sec}) & \text{N.S.} & \text{N.S.} & -51 & +27 & -20 & (-4 \text{ to } +11) & .36 \\
\text{Montana} & \text{N.S.} & \text{N.S.} & -57 & +30 & -19 & (-36 \text{ to } +32) & .40 \\
\text{Texas} & --- & * & -33 & (-5) & (-22) & (-6 \text{ to } +12) & .22 \\
\text{North Carolina} & \text{N.S.} & --- & -60 & (+36) & (-17) & (-55 \text{ to } +94) & .44 \\
\text{FEF}_{75} (\text{mL/sec}) & \text{N.S.} & \text{N.S.} & -37 & +12 & -9 & (-4 \text{ to } +3) & .51 \\
\text{Montana} & \text{N.S.} & \text{N.S.} & -42 & (+14) & -10 & (-18 \text{ to } +14) & .55 \\
\text{Texas} & --- & \text{N.S.} & -26 & (-2) & (-8) & (-5 \text{ to } +3) & .40 \\
\text{North Carolina} & \text{N.S.} & --- & -40 & +23 & (-6) & (-7 \text{ to } +52) & .63 \\
\end{array}
$$

N.S. or ( ) = not statistically significant.

If * or no ( ), then $p < .05$. 

TABLE 20
MEAN ADJUSTED VALUES OF PULMONARY FUNCTION OF TALC WORKERS (n = 292)

MODEL: \[ \text{PFT} = \alpha + \beta_1(\text{sex}) + \beta_2(\text{age}) + \beta_3(\text{height}) + \beta_4(\text{smoking status}) + \beta_5(\text{region}) + \beta_6(\text{cumulative exposure group}). \]

<table>
<thead>
<tr>
<th>MEAN (S.E.)</th>
<th>FEV(_1) (mL)</th>
<th>FVC (mL)</th>
<th>PEAK FLOW (mL/sec)</th>
<th>FEF(_{50}) (mL/sec)</th>
<th>FEF(_{75}) (mL/sec)</th>
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</thead>
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<tr>
<td><strong>SMOKING STATUS</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NONSMOKERS</td>
<td>3.72* (.09)</td>
<td>4.59 (.10)</td>
<td>8.32 (.23)</td>
<td>4.63 (.19)</td>
<td>1.66 (.09)</td>
</tr>
<tr>
<td>EX-SMOKERS</td>
<td>3.59 (.09)</td>
<td>4.50 (.09)</td>
<td>8.62* (.25)</td>
<td>4.57 (.21)</td>
<td>1.47 (.10)</td>
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<tr>
<td>SMOKERS</td>
<td>3.50 (.08)</td>
<td>4.48 (.08)</td>
<td>7.94 (.21)</td>
<td>4.20 (.18)</td>
<td>1.40 (.08)</td>
</tr>
<tr>
<td><strong>REGION</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MONTANA</td>
<td>3.58 (.06)</td>
<td>4.65* (.06)</td>
<td>8.51 (.16)</td>
<td>4.07* (.13)</td>
<td>1.41 (.06)</td>
</tr>
<tr>
<td>TEXAS</td>
<td>3.52 (.10)</td>
<td>4.35 (.11)</td>
<td>8.45 (.27)</td>
<td>4.72 (.23)</td>
<td>1.51 (.10)</td>
</tr>
<tr>
<td>NORTH CAROLINA</td>
<td>3.71 (.11)</td>
<td>4.57 (.12)</td>
<td>7.92 (.29)</td>
<td>4.61 (.24)</td>
<td>1.61 (.11)</td>
</tr>
<tr>
<td><strong>CUMULATIVE EXPOSURE GROUP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOW</td>
<td>3.10 (.08)</td>
<td>4.64* (.08)</td>
<td>8.27 (.20)</td>
<td>4.23 (.17)</td>
<td>1.42 (.08)</td>
</tr>
<tr>
<td>MEDIUM</td>
<td>3.68 (.08)</td>
<td>4.59* (.09)</td>
<td>8.28 (.23)</td>
<td>4.57 (.19)</td>
<td>1.59 (.09)</td>
</tr>
<tr>
<td>HIGH</td>
<td>3.53 (.09)</td>
<td>4.35 (.10)</td>
<td>8.33 (.25)</td>
<td>4.59 (.21)</td>
<td>1.53 (.10)</td>
</tr>
</tbody>
</table>

*Significant difference at .05 level.
TABLE 21

MEAN PERCENT PREDICTED PULMONARY FUNCTION OF MONTANA, TEXAS, NORTH CAROLINA TALC WORKERS COMPARED TO COMPARISON GROUPS, ADJUSTED FOR AGE, HEIGHT, AND SMOKING

<table>
<thead>
<tr>
<th>COMPARISON POPULATIONS</th>
<th>% PREDICTED PULMONARY FUNCTION = (observed/predicted) x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEV₁ % (S.E.)</td>
</tr>
<tr>
<td>MALES ONLY (n = 251)</td>
<td></td>
</tr>
<tr>
<td>POTASH</td>
<td>98.85 (1.01)</td>
</tr>
<tr>
<td>UNDERGROUND COAL</td>
<td>97.55 (1.01)*</td>
</tr>
<tr>
<td>ABOVEGROUND COAL</td>
<td>96.60 (1.01)*</td>
</tr>
</tbody>
</table>

* = >2 S.E. less than 100
+ = >2 S.E. greater than 100
Dr. Fraumeni (NCI): As this study progresses, will you be able to shed any light on the relationship of talc exposure and cancer.

Dr. Gamble (NIOSH): The population here is probably not too good for a mortality study at this point. The exposure histories, as we showed, are relatively short, even in North Carolina where it is only ten years. I think this population should be followed-up because of the pleural thickening and the concern for possible mesothelioma. But I do not know that we are going to have any answer for that for awhile.

I think the pleural thickening is of possible concern because of the relationship of pleural thickening in asbestos exposure. It is interesting that in Montana, where no asbestos fibers have been found, there was still an increase in pleural thickening. These populations should be followed.
LUNG CANCER IN
THE NATIONAL COAL WORKERS' AUTOPSY STUDY

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Institute of Occupational Safety and Health, 944
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26505

and

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Virginia University Medical Center, Morgantown,
West Virginia 26506
Summary

The 2410 cases in the National Coal Workers' Autopsy Study were analyzed to determine whether factors in the underground mining environment influenced the incidence or histogenesis of lung cancer. The major factor in the development of lung cancer in coal workers appeared to be cigarette smoking. We could find no effect due to duration of underground exposure. An interesting finding was an apparent increase in the number of cases with adenocarcinoma. This latter finding supports the concept that the histogenesis of bronchial neoplasm is influenced by environmental factors.
Introduction


There are many reasons for suspecting that the environment of the coal mine may influence the incidence of lung cancer. Several studies have been reported in the literature mainly based on death certificate and necropsy data (Kennaway and Kennaway, 1947; 1953; James, 1955; Carroll, 1963; Enterline, 1964; 1972; Liddell, 1973; Costello, et al. 1974; Ortmeyer, et al. 1974; Rooke, et al. 1979; Scarano, et al. 1972; Mooney, 1975; Abraham, 1978; Cochrane, et al. 1979). The majority of these studies have shown a slightly decreased incidence of lung cancer in coal workers. It is difficult to draw firm conclusions from these studies as most of them have suffered from important epidemiological limitations, for example, the majority of studies have not been adequately controlled for smoking.

In the United States squamous cell carcinoma is considered the most common type of lung neoplasm (Clifton and Luomanen, 1968). Also, squamous cell carcinoma is known to be the most prevalent
type of cancer in smokers (Kreyberg, 1962; Doll, et al. 1957; Vincent, et al. 1965; Weiss, et al. 1972). On the other hand, small cell carcinoma has been reported to be the most prevalent type of lung neoplasm in uranium miners (Archer, et al. 1973; Auerbach, et al. 1975) and adenocarcinoma is more prevalent in asbestos workers (Spencer, 1977; Whitwell, et al. 1974; Heuper, 1966; Hourihane and McCaughey, 1966). Thus, knowledge of the frequency distribution by histological type in a given population may provide valuable clues to the etiology of lung cancer.

The purpose of this study was to determine whether there is an association between histological type of coal workers' lung cancer and factors in the underground mining environment. The study was based on the material in the National Coal Workers' Autopsy Study (NCWAS). Comparisons were made between histological type, years of underground exposure, specific occupation within the mine and the type and severity of coal workers' pneumoconiosis (CWP).

**Materials and Methods**

In 1969 the United States Congress passed the Coal Mine Health and Safety Act, which provides free autopsies for all underground coal workers. Since its inception from 1972 through 1977 we have collected 2410 cases from 22 states. Each case submitted to this program included full demographic data, occupational and smoking histories together with a detailed autopsy report and pulmonary tissue. The NCWAS population is similar to the general working miner population with regard to geographic distribution, occupation within the mines and smoking history. However, the mean age, total number of years worked and the smoking history are higher than the
general working miner population (Abraham, 1978).

The pathological material consisted of 3 or more histological sections (1 x 1.5 cm) with 3 corresponding or different blocks of tissue which had been prepared from post-mortem tissues. Histologic evaluation of the type of neoplasm was determined by 4 pathologists according to the WHO classification (Kreyberg, et al. 1967) with minor changes as outlined below.

1. Epidermoid or squamous carcinoma:
   a. Squamous cells with keratin, keratin pearls and intracellular bridges (well differentiated).
   b. Squamous cells with intracellular bridges or pre-keratin (moderately differentiated).
   c. Squamous cells with characteristic growth pattern, sheet-like arrangements and occasional cells with pre-keratin (poorly differentiated).

2. Small cell anaplastic carcinoma.
   a. Small cell lymphocytic (oat cell) type.
   b. Small cell polygonal type.
   c. Small cell fusiform type.

3. Adenocarcinoma
   a. Acinar type with mucin.
   b. Papillary type bronchoalveolar adenocarcinoma with mucin.
   c. Poorly differentiated acinar type with occasional mucin containing cells.

4. Mixed squamous cell and adenocarcinoma with keratin and mucin.

5. a. Large cell carcinoma with mucin.
   b. Large cell carcinoma without mucin.
c. Large cell carcinoma with giant cells.
d. Clear cell carcinoma.

a. Diffuse.
b. Localized.

7. Others (Leiomyosarcoma, fibrosarcoma, etc.)

In 202 cases there was sufficient material available to make a microscopic assessment. Evaluation on a minimum of 3 histological slides were made independently by all the members of the panel; that is, without prior knowledge of autopsy findings or smoking histories or years of mining history; and results recorded. The same group of slides were re-evaluated simultaneously by the panel one week later using a multi-headed microscope and the results of earlier independent interpretations were compared. When disagreements were found among the members of the panel or when mixed tumor patterns and poor differentiation were observed, additional slides stained with a battery of special stains were obtained. The histological sections, stained with H & E, keratin stain, Fontana stain and mucicarmine stains were re-examined by the panel. When these preparations were studied by the panel a unanimous opinion on the type of carcinoma was usually reached. The total number of cases disputed or demanding further characterization by special stains for a unanimous opinion was 66.

Histopathological evaluation of the pneumoconiosis was performed on all the 202 cases by two of the panel members (V.V. and F.H. Y.G.). In this evaluation it was assumed that at post-mortem the lung tissues selected for microscopic examination by the participating NCWAS pathologists were representative of the whole lung.
for the purposes of determining the extent, severity and type of pneumoconiosis. Classification and grading of coal workers pneumoconiosis was made according to standards established by a panel of the College of American Pathologists (Kleinerman, et al. 1979).

The 221 cases identified with lung cancer were matched with cases without lung cancer. The following groupings were chosen:

1. Lung cancer cases matched with all non-lung cancer cases. The matching variables were: race, exact age, mining regions represented by eastern Pennsylvania (anthracite), the rest of Appalachia and the mid west (bituminous) and west, smoking status (non-smoker, cigarette smoker (current or ex), pipe or cigar smoker). A total of 175 matches were found.

2. The same as (1) above with the exception that smoking status was replaced by pack years (5 pack-year intervals). A total of 135 matches were found.

Statistical tests were performed to determine the relationship between occupational exposure, smoking, job differences and the prevalence of lung cancer. The analyses were made using the matched pair t-tests.

**Results**

Table I presents relevant descriptive information for the entire sample. The 2410 miners in the NCWAS had an average age at death of 64.0 ± 11.0 years with 27.0 ± 13.0 years of underground mining. Seventy-two percent of the total sample had a history of cigarette smoking with a mean pack year smoking history of 25.0 ± 19.0. Two hundred and twenty one (221) cases were identified in which carcin-
oma of the lung was mentioned on the autopsy report. This represented 8.8% of the total sample. The mean age at death of miners with lung cancer was 65.0 ± 10.0 years. In the 221 lung cancer cases 200 (90%) smoked cigarettes, 3 were pipe or cigar smokers only and 18 were non-smokers. In 183 cases lung cancer was determined by the pathologist to be the underlying cause of death. Table II shows the distribution of lung cancer cases and cases with cancer at other sites in 22 coal mining states. The proportion of lung cancer cases in Illinois and Ohio appears to be increased.

Analysis of matched group 1 showed a significant difference (P = 0.03) in mean pack years between the smokers in the two groups. The mean pack years for lung cancer cases was 31.0 ± 20.0 compared to 24.0 ± 22.0 for non-lung cancer cases.

Among the 175 matched lung cancer cases 13 were non-smokers with an average underground work history of 26.0 ± 17.0 years. The average underground work history of the matched control population was 35.0 ± 12.0 years. This difference was not statistically significant (P = 0.12).

As there was a significant difference in the amount smoked between the lung cancer and non-lung cancer cases we felt a more precise matching of smoking history was required to determine an independent effect due to occupation. Group 2 illustrates this matching. In both smoking and non-smoking miners no significant differences were detected between years underground, specific occupation and the presence of lung cancer.

Table III shows the relationship between the histological type of lung cancer and age, mining and smoking histories in 202 cases on which histological material was available. The predominant
cell type was adenocarcinoma (30%), followed by small cell (28%) and epidermoid (24%) carcinomas. For each of these three major histological types of lung cancer no significant differences were detected in mean age, pack years smoking history or years in underground mining.

The type of CWP lesion and its frequency of occurrence with the different cellular types of lung cancer are shown in Table IV. There appears to be a lower incidence of macular CWP in cases with small cell carcinoma.

Due to the unexpectedly high incidence of adenocarcinoma in this series an attempt was made to determine the site or origin of these tumors within the lung. These details were extracted from the clinical, surgical and autopsy reports. Out of a total of 60 cases of adenocarcinoma 23% of the tumors originated in the upper lobes, and 16% originated in the lower lobes. The remaining tumors either originated from the trachea, right middle lobe, main stem bronchi or the site of origin could not be determined.

Discussion

Whether exposure to coal mine dust increases the chances for developing carcinoma of the lung is a matter of considerable importance which has not been resolved in spite of several investigations (Green and Laquer, 1980). There are many reasons for suspecting the environment of the coal mine may influence the incidence of lung cancer. Coal mine dust is known to contain polycyclic aromatic hydrocarbons and several metal carcinogens such as beryllium, cadmium, chromium, cobalt, lead, manganese and nickel (Berg and Burbank, 1972). Moreover, coal dust due to its high adsorbing ability may facilitate the transportation of polycyclic hydrocarbons from
cigarette smoke. It has been shown that cigarette smoking miners have an 8 fold increase of lung cancer deaths as compared with non-smoking coal miners (Jacobson, 1976). Several studies suggest that carcinoma of the lung is less frequent in coal miners than in non-miners of a similar age group (James, 1955; Doll, 1959; Goldman, 1965; Costello, et al. 1974; Ortmeyer, et al. 1974; Liddell, 1973; Kennaway, and Kennaway, 1947; Rooke, et al. 1979; Cochrane, et al. 1979). In contrast to these reports other mortality studies conducted in the United States have shown an increased SMR for lung cancer in coal workers (Enterline, 1964; 1972; Mooney, 1975). The cause of this disparity in findings is a reflection on the complexity of the problem. The difficulties in determining the true incidence of lung cancer in coal miners are mainly due to regional variations in coal mines and dust concentrations, difficulties in obtaining control populations from the same areas, and separation of the influence of mining independent of smoking.

In this study we could find no evidence that the development of lung cancer is influenced by duration of underground exposure. However, we have confirmed the well recognized etiological relationship between cigarette smoking and lung cancer.

We found a greater percentage of adenocarcinoma and small cell carcinoma in the NCWAS cases than reported in comparable studies. There is some evidence that lung cancers due to smoking are mainly squamous or small cell in type and adenocarcinomas are endogenous growths (Kreyburg, 1962; 1967; Weiss, et al. 1972). In this study 90% of the NCWAS cases with lung cancer had a history of cigarette smoking. Thus, the discrepancy noted in this study between predominant histological type and smoking history may be related to
occupation. In a study of histological cell types in asbestos workers with lung cancer, adenocarcinoma was also found to be the most common type (Whitwell, et al. 1974). Ionizing radiation, on the other hand, is known to induce predominantly small cell, undifferentiated type of carcinomas and the relative frequency of this type of tumor rises with cumulative radiation exposure (Saccomanno 1971; Archer, et al. 1973; Horacek, et al. 1977; Auerbach, et al. 1975). The relative predominance of adenocarcinoma in the NCWAS cases raises the interesting possibility that factors in the coal mining environment influence the histogenesis of bronchial carcinomas. However, this conclusion cannot be drawn with certainty as a suitable control group of non-miners with lung cancer were not available for us to study. There is also some evidence that the incidence of adenocarcinoma of the lung in the general population is increasing (Vincent, et al. 1977).

In conclusion, we would like to stress that this report is provisional. We are currently updating the study to include cases submitted during 1978 and 1979, and are tracking down additional material on cases lacking histological sections of the lung tumor. We hope to better define the relationship between smoking and lung cancer in coal workers and to compare the severity of CWP in lung cancer cases with matched controls.
TABLE I
NCWAS CASE DESCRIPTION

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<td>MEAN AGE ± S.D.</td>
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<tr>
<td>SMOKERS</td>
<td>1709 (72%)</td>
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<tr>
<td>NON - SMOKERS</td>
<td>619 (26%)</td>
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<tr>
<td>PIPE SMOKERS</td>
<td>47 (2%)</td>
</tr>
<tr>
<td>MEAN PACK - YEARS ± S.D.</td>
<td>25 ± 19</td>
</tr>
<tr>
<td>MEAN MINING YEARS ± S.D.</td>
<td>27 ± 13</td>
</tr>
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## Table II

<table>
<thead>
<tr>
<th>State</th>
<th>Total Deaths</th>
<th>All Cancers Except Lung</th>
<th>%</th>
<th>Lung Cancer</th>
<th>%</th>
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<td>Virginia</td>
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<td>5</td>
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<tr>
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<td>-</td>
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<tr>
<td>West Virginia</td>
<td>666</td>
<td>91</td>
<td>14</td>
<td>56</td>
<td>8</td>
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<tr>
<td>Wyoming</td>
<td>38</td>
<td>9</td>
<td>24</td>
<td>1</td>
<td>3</td>
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</table>

| Totals           | 2336         | 316                     | 14| 213         | 9 |

286
TABLE III

CARCINOMA OF THE LUNG: CELL TYPES BY AGE, SMOKING AND MINING

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>%</th>
<th>Age</th>
<th>Pack Yrs.</th>
<th>Mining Yrs.</th>
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</thead>
<tbody>
<tr>
<td>Adeno-Carcinoma</td>
<td>30</td>
<td>63±</td>
<td>28±</td>
<td>30±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10*</td>
<td>20</td>
<td>14-</td>
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<tr>
<td>Epidermoid</td>
<td>24</td>
<td>68±</td>
<td>33±</td>
<td>31±</td>
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<tr>
<td></td>
<td></td>
<td>10</td>
<td>19</td>
<td>12-</td>
</tr>
<tr>
<td>Small Cell</td>
<td>28</td>
<td>64±</td>
<td>30±</td>
<td>32±</td>
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<td></td>
<td></td>
<td>9</td>
<td>23</td>
<td>11-</td>
</tr>
<tr>
<td>Large Cell</td>
<td>9</td>
<td>67±</td>
<td>35±</td>
<td>33±</td>
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<td></td>
<td></td>
<td>10</td>
<td>16</td>
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<td>Adeno-Carcinoma</td>
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<td>Broncho-Alveolar</td>
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<td>62±</td>
<td>37±</td>
<td>19±</td>
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<td></td>
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<td>10</td>
<td>27</td>
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<td>31±</td>
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<td></td>
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<td>3</td>
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*MEAN ± 2 STANDARD DEVIATION
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<tr>
<th>Cell Type</th>
<th>No-CWP Lesions %</th>
<th>Macule %</th>
<th>Nodule %</th>
<th>PMF %</th>
<th>Silicosis %</th>
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<td>77</td>
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<td>8</td>
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<tr>
<td>Small Cell (56)</td>
<td>34</td>
<td>64</td>
<td>23</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Large Cell (19)</td>
<td>16</td>
<td>74</td>
<td>32</td>
<td>16</td>
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<td>Mixed</td>
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<tr>
<td>Adenocarcinoma (16)</td>
<td>12</td>
<td>88</td>
<td>44</td>
<td>19</td>
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<tr>
<td>Epidermoid</td>
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<tr>
<td>Broncho-Alveolar (3)</td>
<td>33</td>
<td>67</td>
<td>--</td>
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References


Discussion

Dr. Lingeman: I would like to know if there is any correlation between the type of cancer and the amount of silicon present in the coal. For example, do you know how much silicon is present in the coal mined in Pennsylvania, versus that mined in Ohio or in Illinois? Is there a high level of silicon in the coal from these states?

Dr. Vallyathan (NIOSH): Yes, there is a difference in the amount of silicon present in the different types of coal. We have not determined whether there is any correlation between the frequency or type of cancer and the silicon content of the coal mine dust.

Dr. Spirtas (NCI): From the abstract I take it for granted that this is a case control study. Is that correct?

Dr. Vallyathan (NIOSH): No, it is not a case control study. We have not been able to obtain a suitable control autopsy population. Therefore all our comparisons are internal.

Dr. Spirtas (NCI): What is your study design? Is there some way that you will try to determine whether this series of cases are representative cases or is this a report on a series of cases?

Dr. Vallyathan (NIOSH): The National Coal Workers' Autopsy (NCWAS) cases represent only about ten percent of the deaths in the Nation's coal workers. It is a selected population due to its voluntary nature. The NCWAS population is demographically similar in many respects to the living miners in our National coal study.

Dr. Spirtas (NCI): I take it that the voluntary part of the program is on the part of the physician. Is there some reason to suspect that certain types of physicians or certain types of cases are volunteered for autopsy?

Dr. Vallyathan (NIOSH): No, it is not the physician who determines whether to submit a case or not. An autopsy is requested and submitted by the next-of-kin.

Dr. Spirtas (NCI): Is this for a claim for benefits under the Coal Mining Act?

Dr. Vallyathan (NIOSH): Yes.

Dr. Marcy (EPA): I presume these cases represent a stable population in terms of their work history in the industry and residency in a state. Have you verified their residency and other occupational exposures?
Dr. Vallyathan (NIOSH): Yes, these cases do represent a stable population with a coal mining history of 10 or more years in the individual states. We have ascertained the residency of all cases included in this study. However, details of occupational histories other than in the coal mining industry are not available.

Dr. Kraybill (NCI): Did you make any radioactivity measurements? I understand when coal is burned an effluent of radioactive material is released. Is there any radium or uranium type ores connected with coal? Is there a difference in the radioactivity levels of coal mine to coal mine from state to state?

Dr. Vallyathan (NIOSH): No, we did not make any radioactivity measurements in the different mines. There is some information available to us on the radon daughters in different coal mines. This level of radioactivity seems to be insignificant. I am not aware of the presence of any uranium or radium ores in the coal mining areas.

Dr. Blot (NCI): Other than the proportional histology analyses that you have presented today, could you say again how you might be able to use this data to get at the question of whether or not coal miners have an increased lung cancer risk?

Dr. Vallyathan (NIOSH): We hope to answer that question by a case control epidemiological study. However, I have mentioned some of the difficulties that we have encountered. If all the variables are adequately controlled, the question of whether the coal miners have an increased risk of lung cancer can be ascertained. Some of the earlier studies have attempted to differentiate the effect of cigarette smoking and a limited number of cases have shown a low incidence of lung cancer in coal miners. In this respect it is important to note that the latency period for the inorganic type of minerals to induce lung cancer is probably in the range of 20 to 30 years. Induction of cancer by coal dust may not be evident if other causes such as smoking or shortened lifespan from pneumoconiosis occurs before the expression of the cancer.

Dr. Blot (NCI): How are you going to use the data that you have available? I think I am getting at the question that Bob Spirtas was bringing up. How are you going to use that data to answer this question?

Dr. Vallyathan (NIOSH): It can be answered only by a case control study.

Dr. Blot (NCI): A case control study among all coal miners and their autopsies and then looking for differences in lengths of employment?

Dr. Vallyathan (NIOSH): Yes, that is what we plan to do, and we are also in the process of getting a case control series of lung cancer cases from non-mining populations.
Dr. Fraumeni (NCI): I would just like to ask one question for the future. I would be very cautious in saying, as you have a number of times, that you have evaluated the incidence of lung cancer. The data that you have, is some sort of a proportionate frequency series; but does NIOSH have any plans to conduct a cohort analysis of coal miners to determine once and for all whether or not the risk of lung cancer is increased or decreased or the same as the general population? Because I think without a cohort study and lifetable analysis, you will never be able to evaluate the issue. Can anybody answer that?

Dr. Vallyathan (NIOSH): As far as I know, there are no cohort studies which have been initiated. Regarding your first comment, I definitely agree that incidence is the wrong term to be used. I probably should have said "prevalence".

Dr. Bridbord (NIOSH): First of all, there was a recent cohort mortality study sponsored by NIOSH, which did not clarify the lung cancer issue but did suggest an increase in stomach cancer. The second point to note is that the recent Mine Safety and Health Amendments Act does give NIOSH considerable new responsibilities in the area of mining in general, which is not exclusively coal mining but really reaches out to the total spectrum of mining. I think we would have to weigh the answer to that question in terms of the opportunities that would be available to do that additional study and look at our total mining responsibilities. But I do not think that the Institute is completely satisfied that we have resolved the question. So, at some point in the future, I think there might be a chance to embark upon a larger study, but that would be a competing issue in terms of the total mining research needs.

Dr. Fraumeni (NCI): Is there any evidence with regard to the relationship between the cell types and the amount of pulmonary fibrosis? For example, with the cases of adenocarcinoma of the lung, did they have more pulmonary fibrosis or any evidence of what has been called Caplan's syndrome, which is a hypersensitivity reaction with pulmonary fibrosis and rheumatoid arthritis?

Dr. Vallyathan (NIOSH): This was illustrated by the last slide. May I have the last slide again, please? The extent and severity of pneumoconiosis, which has been graded based on the type and presence of the different types of lesions, that is; simple macule, nodule, PMF, which is progressive massive fibrosis and silicosis, are scored for each type of cancer. We have not found any definite association with adenocarcinoma and PMF. However, there is a lower prevalence of pneumoconiosis with small cell carcinoma. In small cell carcinoma only 64% of the cases had pneumoconiosis. Caplan's syndrome was not observed in these coal workers.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Wednesday Morning, May 7

METHODOLOGY/EXPERIMENTAL MODELS SESSION

SESSION CHAIRPERSON

Dr. Carl Morris
Environmental Protection Agency
In the past few years, considerable effort has been directed to defining batteries or combinations of in vitro test methods which could provide rapid, sensitive, and reliable means for assessing the carcinogenic potential of chemical compounds. These various in vitro test systems can be broadly divided into three major categories, namely (1) those which detect mutagenic or chromosomal changes in micro-organisms or mammalian cells; (2) those in which there is induction of morphological transformation in mammalian cells in culture; and (3) those in which interactions between the chemical and target macromolecules such as DNA can be assessed. Experimental evidence has been accumulated which shows that there is a positive correlation between the in vivo carcinogenicity of many chemicals and their capacity to elicit a response in in vitro systems.

Approximately one hundred different model systems have been identified as having been used, in varying degrees, for assessing the carcinogenic and/or mutagenic potential of chemicals. In some instances, limited numbers of chemicals have been tested and in many cases they have been restricted to well characterized direct-acting agents. Although such a review and such compilations of test data are extremely valuable, certain problems are encountered with data derived solely from the literature. For any single test method, there can be small but critical differences in the methods used which can result in apparent non-reproducibility of results. Another problem relates to the fact that the tests, in general, have been performed with the full knowledge of the in vivo carcinogenic activity of the chemicals. Such a situation can introduce a certain amount of bias.
In the utilization of any in vitro method as a valid indicator of the carcinogenic potential of chemicals, there is a basic requirement to know that (1) the methodology is well-defined and the critical elements of the procedure are recognized; (2) reproducible results can be obtained not only within a specific laboratory but can be obtained equally well among different laboratories; (3) there is experience with a broad spectrum of chemicals of diverse structure and biological activity in order to recognize that certain types of chemicals may give unique negative or positive results in some tests; and (4) there are methods and approaches for analysis and interpretation of the experimental results. The existence of well-defined and evaluated assay methods, which can be exploited for the assay of carcinogenic potential, would then provide the means to examine a large number of chemicals and aid in setting priorities for long-term animal carcinogenicity bioassays.

The purpose of this project is to evaluate and determine the usefulness and reliability of an in vitro transformation assay using C3H 10T½ cells as a candidate for one of a battery of short-term assays for the initial determination of the carcinogenic potential of chemicals. In addition the reproducibility of the system will be assessed since the studies are being conducted in two laboratories simultaneously.

The workscopes of the two contracts for this effort were sharply defined so as to initially emphasize the methodological aspect. The specific objectives to be approached in parallel were as follows:

1) Propagate and store a large quantity of mycoplasma-free, low passage 10T½ cells.
2) Characterize the behavior of the cell population with respect to plating efficiency, generation time, saturation density, karyotype stability, cell morphology, cell size distribution, absence of growth in soft agar, and absence of tumor formation in C3H mice.

3) Identify and obtain large lots of fetal bovine serum that yield optimum growth characteristics and transformation response with direct acting carcinogens and polycyclic aromatic hydrocarbons.

4) Establish the 10T ½ cell transformation assay and its concomitant plating efficiency in the absence of an exogenous metabolic activation system, using model chemical compounds.

5) Evaluate the transformation assay using selected known chemical carcinogens and non-carcinogens by scoring for Type III foci and by determining the ability of cells from Type III foci to grow in soft agar and produce tumors when injected into irradiated C3H mice.

6) Develop and characterize a mamalian S-9 activating system for incorporation into the C3H 10T ½ transformation assay.

7) Determine whether the sensitivity and reproducibility of the assay can be further improved by examination of certain baseline factors such as plating density and assay interval, passage number of target cells, and step-down of serum concentration.
8) Test a series of chemicals (supplied under code) consisting of both carcinogens and non-carcinogens for their transforming capacity in 10T ½ cells. The chemicals will be tested with and without an exogenous metabolic activation system.

The progress made in these areas by the two contractors has been satisfactory. Early passage (P-5) cells received from Dr. Heidelberger were subcultured by the prescribed protocol (once every 10 days at $5 \times 10^4$ cells/dish) and large aliquots of cells from P6, 7 and 8 frozen in liquid nitrogen to form a uniform stock for future studies. The cells were determined to have the culture characteristics for C3H 10T ½ cells previously reported in the literature. In addition, lots of fetal bovine serum have been identified that provide the expected saturation density and chemically-induced morphological transformation.

These cells have been characterized and it has been found that they have a plating efficiency (100 cells/60 mm dish) of 23%, a saturation density of $5.9 \times 10^5$ cells/60 mm dish and a generation time of approximately 16 hours. The cells were also examined by transmission electron microscopy and it was observed that there is overlapping and underlapping of cytoplasm between cells. This suggests that cell contact may not be a controlling variable in the density dependent inhibition of cell division.

Other significant findings have been reported by these two contractors.

Transformation assays with eight known chemical compounds showed that benzo(a)pyrene (B(a)P), 3-methylcholanthrene (3-MC), dibenz(a,h)anthracene and 7,12-dimethylbenz(a)anthracene gave a positive response by producing Type III foci in the
absence of an exogenous metabolic activation system. Chemicals not active in the assay were the non-carcinogens, anthracene and phenanthrene, and two known carcinogens, 2-acetylaminofluorene (2-AAF) and N-methyl-N-nitro-N-nitrosoguanidine (MNNG). In the assays positive for transformation, the absolute transformation frequency and the dose at which a positive response was induced varied from experiment to experiment. In general, 3-MC induced a more reproducible transformation response and dose-dependent effect than B(a)P, although variation from experiment to experiment was still evident.

An acceptable lot of rat liver S-9 has been prepared from Aroclor-1254 induced Fischer 344 male rats and is being used to determine the critical parameters for chemically transforming 10T½ cells in the presence of such an exogenously supplied source of mammalian metabolizing activity. Cytotoxicity and transformation assays have been conducted using B(a)P, 2-AAF, diethylnitrosamine (DEN), and 3-MC. When the suspension assay was used, only those cells exposed to B(a)P in the presence of a metabolically active S-9 preparation exhibited morphological transformation. The negative results obtained thus far with the other compounds tested may be attributable to various factors such as (1) the relatively short exposure period (2-4 hours) used; (2) the limitations associated with a suspension assay; (3) failure of the S-9 to activate the chemicals to forms capable of transforming 10T½ cells; (4) metabolic inactivation and detoxification of the chemicals by the S-9 preparation; or (5) the failure of transformed cells or cells in the process of being transformed to exhibit the transformed phenotype.

An amplification assay (Level II transformation assay) is also being assessed to determine whether non-expressed transformed cells are present in the standard
transformation assay. This assay involves replating of the treated cell population when they just reach confluency and scoring the replated cells for Type III foci in 2-4 weeks.

MNNG has been tested using this amplification modification. At a dose of 0.5 ug/MNNG/ml, a single Type II focus was detected in the standard transformation assay. The Level II transformation assay gave rise to numerous Type III foci. The positive control, 3-MC (2.5 ug/ml) induced the formation of 17 Type III foci in the standard transformation assay and no augmentation in transformation was obtained in the Level II assay.

Studies have also been conducted to test various factors which may affect and/or optimize the transformation assay. It was observed with 3-MC treatment that the total number of foci (II and III) increased with increase in exposure time to this chemical, but there was no enhancement of transformation frequency when the treated cultures were maintained in medium containing 2% and 5% serum rather than the standard 10% serum concentration.

The constitutive activity of arylhydrocarbon hydroxylase of the C3H 10T ½ cells was also determined by measuring the conversion of $^3$H-B(a)P and it was compared to the activity in BALB/C-3T3 clone 1-13 cells. The time course of B(a)P metabolism (0.3 ug/ml) with $2 \times 10^5$ cells is linear with incubation time for both 3T3 and 10T ½ cells. However, the 10T ½ cells possess a 15-fold higher activity than the 3T3 cells.

For the next contract period the overall objective will be to further develop and validate a reliable, reproducible assay system for neoplastic transformation using
C3H 10T½ cells, specifically identifying those factors which influence the assay and then determining those procedures which would lead to their standardization. Emphasis will be placed on developing the exogenous enzyme activation system because of absent or insufficient enzyme levels in the target cells.

Simultaneously, because of the progress already made by two laboratories in standardizing, we are moving ahead on phase II, the initiation of assays of coded samples supplied by the NCI. Cytotoxicity effects have already been run on more than 10 of the coded samples and we expect that this effort will move forward rapidly in the final contract year FY 81.
Dr. Page, EPA: On these assays, does there appear to be any good correlation between the cytotoxicity and transformation capability? Is there any positive correlation you could see?

Dr. Cameron, NCI: Transformation and the cytotoxicity?

Dr. Page, EPA: Cytotoxicity for the cell cultures?

Dr. Cameron, NCI: I don't have an answer for that. I would mention that the samples came out of the bioassay program.

Dr. Kelsey, NCI: Was I correct in hearing that they did not pick up AAF and MNNG in the standard assay?

Dr. Cameron, NCI: Right.

Dr. Kelsey, NCI: Would that not bother you in terms of giving them coded samples?

Dr. Cameron, NCI: It bothers the contractors.

Dr. Kelsey, NCI: I mean until they can get some knowns to work, or at least some basic knowns, wouldn't it be advisable?

Dr. Cameron, NCI: I think it would bother anybody using the system.

Dr. Kelsey, NCI: Are there plans to use maybe hepatocytes or something like that as an activating system?

Dr. Cameron, NCI: Not in the protocol.

Dr. Hegyeli, NCI: Dr. Alcian made a study to compare in vitro and in vivo toxicity, and he inferred that the distribution of a certain substance between optimal and the aqueous phase has a very important role in determining the ratio that was detected from in vitro to in vivo. My question is whether there was any study done as far as the solubility and what kind of solvent was used for the different kinds of chemicals?

Dr. Cameron, NCI: The solubility factors were determined by a different laboratory, a chemical analysis laboratory, and they were supplied by that laboratory. So that is worked out for the contractor. He receives the chemical and the solubility data simultaneously. I hope that answered the question.
I. TITLE

Human Epithelial Cell Metabolic Activation Systems for Use with Human Cell Mutagenesis (R80556310-02)*

J. Justin McCormick and Veronica M. Maher
Carcinogenesis Laboratory
Michigan State University

II. ABSTRACT

The need to develop ever more adequate test systems which can reliably determine the possible toxic, mutagenic, teratogenic and/or carcinogenic effects of exposure of the human population to chemicals in the environment is becoming more and more evident. It is, of course, not possible to carry out such testing in humans, and therefore, information on risk estimates has to be extrapolated to man from results obtained in test systems which utilize microorganisms, mammalian cells in culture, or animals. Mammalian cells in culture have increased in importance in biological testing because they retain many of the characteristics of the target cells at risk and, yet, can be grown into very large populations and analyzed and characterized by a wide range of biochemical and genetic techniques that are not possible with whole animals. We have developed a quantitative system for measuring the cytotoxic and mutagenic effect of radiation and direct-acting chemicals in diploid human skin fibroblasts in culture and have shown that these effects are directly related to the capacity of human cells to repair damage to DNA. Since the human population is far more likely to be exposed to parent compounds than to direct-acting model compounds, we are extending the capability of the diploid human fibroblast cell mutagenesis system to include parent compounds, or mixtures of chemicals, which require metabolic activation by coupling it with cell-mediated activation. Cell lines derived from human tumors are being utilized as feeder layers to provide metabolic activation of carcinogenic agents. To find cells capable of metabolizing the various test chemicals, we prescreen our series of tumor cell lines for evidence that the chemical causes interference with DNA synthesis. (The compound under investigation is administered to the candidate metabolizing cells over a wide range of concentrations. After 48 hrs. of incubation, the amount of semiconservative DNA synthesis is measured by incorporation of radioactively-labeled thymidine and compared with that of untreated control cells.) When a cell line appears to activate a particular chemical to a form which interferes with DNA synthesis, it is then examined for ability to act as a metabolizing layer for target cells. To enhance the sensitivity, the target cells in this co-cultivation system are usually excision repair defective XP cells which are examined for evidence of cell killing (loss of colony formation). We have found that normal fibroblasts may be used as target cells once conditions are optimized. Using this prescreen, we have identified cells capable of activating a wide range of agents, including polycyclic aromatic hydrocarbons, aflatoxins, aromatic amides and amines, nitrosamines, nitrogen-containing polycyclics, etc. We are now investigating the usefulness of these cells lines: (a) to quantitate the cytotoxic effect of environmental agents by measuring cells' loss of ability to clone, (b) to quantitate the induction of mutations using several markers, and (c) using radioactive parent compounds to determine the number and kinds of adducts formed on DNA by covalent attachment of chemical residues. The activation systems developed are not limited in application to the human fibroblast mutation assay and can be expected to have more general applicability.

*Project officer, Dr. Michael D. Waters, Environmental Protection Agency.
III. INTRODUCTION

It is now realized that most chemical carcinogens require metabolic activation before they can exert their carcinogenic effect. James and Elizabeth Miller were the first workers who clearly realized the importance of metabolic activation and its near universal application to chemical carcinogens in their studies of the 1960's (1). This led to a realization that assays of the mutagenic or carcinogenic potential of compounds using bacteria, fungi, or mammalian or human cells in culture required a source of metabolic activation of the carcinogens if the cells being used were not able to metabolize the compounds themselves as was most frequently the case. This led Malling to develop the use of liver homogenates to provide the metabolic activation for such in vitro systems (2). These liver homogenates have been widely used and adapted as in the Ames test with Salmonella and may consist of microsomal preparations or of S-9 preparations of liver homogenates (3). Once the need for metabolic activation was realized, a parallel development also took place in mammalian cell culture where researchers made use of feeder layers of metabolizing cells such as primary liver hepatocytes which could metabolize many of the carcinogens that one wished to examine (4-6). In practice, the target cells which one wishes to mutagenize or transform into cancer cells are placed in contact with the feeder layer cells and carcinogen is added for 24 or 48 hours. The cells are then trypsinized and replated for determination of the mutagenic or transformation response. The studies with feeder layers have generally been qualitative. That is, the authors have shown a plus or minus response but have not usually shown a dose response or other more elaborate kinetic analysis of the interaction of the carcinogen with the cells.

The value of using activation systems derived from liver or other cell homogenates has been questioned since high concentrations of benzo(a)pyrene (BP), e.g., 1,320 nmol per mg of microsomal protein, produced extensive DNA adducts, but only a small percentage of these represented the principal cellular DNA adduct formed from BP, viz., the N₂-guanine adduct formed by the anti 7,8-diol 9,10-epoxide of BP (7). In contrast, low concentrations of BP, e.g., 15 nmol per mg of protein, produced the diol epoxide DNA adduct of BP exclusively (8). A recent report by Santella, et. al. (9) indicates that with appropriate induction of microsomal enzymes, an S-9 fraction can also be shown to catalyze the binding of BP to DNA exclusively through the diol epoxide pathway when a low substrate concentration (12.5 nmol per mg of protein) is used. The maximum extent of DNA modification in these latter studies was 8.1 per 10⁷ nucleosides. Using diploid human fibroblasts which are totally lacking in excision repair of BP adducts from DNA we have determined that 250-fold greater levels of DNA binding are necessary to see significant induction of mutations to thioguanine resistance. Even higher initial levels of bound adducts are required to observe mutations in normally-excising human fibroblasts. The only practical way to obtain greater binding with S-9 fractions is to use higher concentrations of BP since the S-9 fraction itself is toxic. Unfortunately, as noted above, this higher concentration can be expected to generate the wrong adducts. Similar problems have been noted with dimethylbenz(a)anthracene (DMBA) metabolism by Bigger, et. al. (10) when the rat liver microsomes were used for carcinogen metabolism. At a high concentration of DMBA, K region DNA adducts were formed while at low concentrations, bay region adducts were formed. Most significantly, they found that when intact mouse cells were used for metabolism, no such qualitative differences were found in the DNA adducts formed over a 40 fold dose range. Since the K region as well as the bay region adducts of BP and DMBA are mutagenic (11), it seems likely that
many of the reports of mutagenicity of these compounds in short term tests which make use of S-9 fractions or microsomal protein for metabolic activation are the result of formation of K region adducts. Thus, it appears that the assays used have given the "correct" answers but for the wrong reasons. It should, therefore, be clear that at least for metabolism of polycyclic aromatic hydrocarbons, intact cells are to be preferred to microsomal or S-9 preparations. Detailed comparative studies of metabolism of other compounds have not yet been carried out, so the problem may well extend to other classes of compounds.

We have extensively studied the effect of active derivatives of carcinogenic agents on human fibroblasts derived from normal individuals as well as repair deficient fibroblasts derived from xeroderma pigmentosum patients (II-15). Because such fibroblasts have either no or extremely low levels of metabolic activating activity for carcinogenic agents, it is necessary to utilize direct acting carcinogens with such cells to see their effect. However, to make broader use of this assay, as well as to explore the effect of agents that require metabolic activation or of unknown chemical agents (such as in complex mixtures), we have been studying the ability of feeder layers derived from various cell types to provide metabolic activation for this cell system. Because we were working with human fibroblasts as target cells, it seemed ideal to select human cells for metabolic activation. Primary epithelial cells derived from various tissue of man would seem likely to provide the ideal system for metabolic activation. However, there are a number of problems connected with obtaining and standardizing any such epithelial cell system. First, it is difficult to obtain pure populations of epithelial cells in culture since fibroblasts tend to overgrow the epithelial cell cultures. Second, it is extremely likely that epithelial cells derived from various human donors (and even from the same donor at different times) will show different levels of metabolizing ability for a particular carcinogen. This will make it extremely difficult to standardize any assay based on the use of primary epithelial cells. Third, epithelial cells from organs such as liver which might be extremely useful for metabolizing carcinogens are not readily available for use, show extreme variability between various donors, and are not easily adapted to cell culture. In addition, working with human liver material poses the health threat of hepatitis infection. To get around all the limitations of metabolic activation systems for cells in culture discussed above, we have chosen to use tumor cell lines derived from various human tissues. These human cell lines maintain the ability to activate various carcinogens and, have an infinite lifespan in culture. These properties suggested they would be extremely useful as feeder layers.

IV. OVERALL OBJECTIVE

The objectives of the research are to develop procedures that will allow for more adequate in vitro testing of environmental chemicals and to shed light on the mechanisms involved in mutagenic processes and related events. The use of diploid human cells in culture for environmental research is very useful first of all, because of the relevance to man of results obtained with such cells and secondly, because the existence of DNA repair deficient human cells derived from xeroderma pigmentosum (XP) patients which have been characterized and shown to be unable to remove many different types of carcinogen residues covalently bound to DNA (II, 12) allows one to determine the potency of various chemicals without the interference of excision processes operating during the period when the target cells are being incubated with the chemical. Furthermore, comparing the effects of various agents in excision repair-proficient and deficient human target cells allows one to study the biological effects of DNA excision repair on the mutagenic process.
A. Development of a Short Term Assay for Identifying Human Epithelial Cell Lines Capable of Metabolizing Parent Carcinogens into Reactive Forms:

In our initial studies of human cell-mediated metabolism, we made use of benzo(a)pyrene BP, as our model compound. One of the reasons for choosing BP is that there is a simple rapid technique for measuring its metabolism, viz., conversion of tritiated BP into the water soluble product. We used this assay to screen 17 cell lines and found several which could metabolize BP. When these were identified we assayed them for ability to serve as a metabolizing layer capable of activating not only BP but a whole series of polycyclic hydrocarbons into forms which are cytotoxic to repair deficient diploid human skin fibroblasts.

However, not all carcinogens which require metabolic activation for activity can easily be obtained in tritiated form nor is metabolism of a compound to a water-soluble form necessarily an indication of activation to a form which can bind to DNA. Therefore, we modified a DNA synthesis inhibition assay developed by Painter (17) which uses the inhibition of incorporation of $^3$H-thymidine into DNA as an indication of metabolic activation of carcinogen and resulting DNA damage.

In this method, $5 \times 10^6$ actively growing cells to be assayed for ability to metabolize a carcinogen are plated per 16mm well (Costar 3524 Tissue Culture Cluster) in 1 ml medium. Approximately 24 hr later, these cells are treated with carcinogen by adding 1 ml of carcinogen solution to the 1 ml of medium already present in the well. After 48 hr incubation, the treatment is removed and replaced with 1 ml of medium containing $^3$H-Tdr (5uCi/ml). After a two hour labeling period, the medium is removed and the cells are rinsed with PBS. The cells are removed in 1 ml of trypsin-EDTA, diluted to 4 ml with phosphate buffered saline (PBS), rinsed with PBS two times on a glass membrane filter, and then rinsed two times with 10% chilled TCA. The filters are then removed, treated in 1 ml 0.5N HCl for 60 minutes at 90° and counted in a liquid scintillation counter. The data are calculated and presented as percentage of the amount incorporated by the untreated control cells.

B. Metabolizing Strains Identified:

1. Interference with DNA Synthesis:

Using the DNA synthesis inhibition assay described above, many human carcinoma cell lines were examined for their ability to metabolically activate several classes of carcinogens to forms which would interact with cellular DNA and thus inhibit DNA synthesis. Two cell lines which we had already found to be capable of metabolizing benzo(a)pyrene were examined in this assay as positive controls. Figure 1 shows the results of this experiment. Both cell lines show inhibition of DNA synthesis, 703 to a greater extent than 835. This inhibition data agrees with the level of metabolism of BP to water-soluble compounds found in these cells, i.e. 703 is greater than 835. Figure 2 shows the results of exposing PC-3, 703, XP and normal human fibroblasts (NF) to dimethylnitrosamine (DMN) and diethylnitrosamine (DEN) and measuring the inhibition of thymidine incorporation.
Figure 1. Inhibition of tritiated thymidine incorporation in tumor lines 835 and 703 after a 48 hour pre-treatment with BP at various concentrations.
Figure 2. Inhibition of tritiated thymidine incorporation in tumor lines □ 562, ▲ PC-3, and ○ 703, and in ○· XP and ★ normal human fibroblasts after a 48 hour treatment with various doses of DEN or DMN.
2. Direct Cell Killing:

To further examine the biological consequences of the metabolic activation of the carcinogens discussed above, we examined the cytotoxicity of the compounds directly on the metabolizing cells themselves by treating the cells with carcinogens at the indicated concentrations for 48 hr at 37° in Eagles Minimum Essential Medium containing 10% fetal bovine serum and antibiotics. At the end of the exposure period, the cells were trypsinized, replated at cloning density, and cultured for approximately two weeks until macroscopic clones developed. The results of exposing 703, 835 and 549 cells to BP are shown in Figure 3. It is obvious that 703 cells shows the greatest toxicity per dose. These results were the results obtained above with 703 and 835 cells. The results of exposing 703 cells (polycyclic hydrocarbon metabolizing cells) to aflatoxin B\textsubscript{1} are shown in Figure 4. The aflatoxin B\textsubscript{1} appears to be very cytotoxic to these cells.

3. Cell-mediated Cytotoxicity in Xeroderma Pigmentosum Cells:

Since it is always possible that direct cytotoxicity assays are not as sensitive as they could be due to DNA repair in the carcinoma cell lines, we used the cell-mediated assay previously developed in this laboratory in which lethally irradiated metabolizing cells are co-cultivated with target xeroderma pigmentosum cells (XP) in the presence of the carcinogen requiring activation for a period of 48 hr at 37°. At the end of the exposure period the cells are trypsinized, replated, and the XP cells allowed to clone. The percent survival of the cloning ability of XP cells was determined by dividing the cloning efficiency of co-cultivated XP cells in the presence of carcinogen by the cloning efficiency of co-cultivated XP cells in the absence of carcinogen, multiplied by 100.

Optimal conditions for co-cultivation were previously established using BP as the model carcinogen. The effect of increasing the number of metabolizing cells and changing the concentration of BP was investigated. The cytotoxicity of BP in the XP target cells was found to increase with increasing number of metabolizing cells and BP concentration. In addition, the number of induced mutations to thioguanine resistance in the XP target cells also increased with increasing BP concentration in the presence of metabolizing cells. Using the cell-mediated cytotoxicity assay system developed for BP other carcinogens and metabolizing cells were examined. (Figure 5-8)
Figure 3. Direct cytotoxic action of BP on 549, 835 and 703 tumor cells. The cells were incubated for 48 hours with BP at various concentrations and then assayed for cloning ability.

Figure 4. Direct cytotoxic action of AFB₁ on 703 tumor cells. The cells were incubated for 48 hours with AFB₁ at various concentrations and then assayed for cloning ability.
Figure 5. Cell-mediated cytotoxicity of XP cells coincubated for 48 hours with lethally-irradiated 835 tumor cells in the presence of various concentrations of polycyclic aromatic hydrocarbons or their derivatives. After the 48 hour coincubation, the cells were trypsinized and the XP cells assayed for cloning ability.
Figure 6. Cell-mediated cytotoxicity of XP cells coincubated for 48 hours with lethally-irradiated 835 or 703 tumor cells in the presence of various doses of aflatoxin B₁. After the 48 hour coincubation, the cells were trypsinized and XP cells assayed for cloning ability.
Figure 7. Cell-mediated cytotoxicity of XP cells coincubated for 48 hours with lethally-irradiated PC-3 or 700 tumor cells in the presence of various concentrations of DEN or 4,4' methylene bis (2-chloroaniline). After 48 hours of coinoculation, the cells were trypsinized and the XP cells assayed for cloning ability.
Figure 8. Cell-mediated cytotoxicity of XP cells coincubated for 48 hours with lethally-irradiated 549 or 703 tumor cells in the presence of various concentrations of polycyclic aromatic hydrocarbons or their derivatives. After 48 hours coincubation, the cells were trypsinized and the XP cells assayed for survival.
4. Cell-mediated Mutagenicity in Xeroderma Pigmentosum Target Cells:

We have tested the system for use as source of metabolic activation of two parent polycyclic aromatic hydrocarbons viz., benzo(a)pyrene and dimethylbenz(a)anthracene. To assay the co-cultivated target cells for the frequency of BP-induced mutations to 6-thioguanine resistance, cells were trypsinized and counted as above and plated into 250 ml flasks to allow for expression of mutations at cell densities which allowed surviving target cells to replicate without reaching confluence. (Sufficient numbers of flasks were employed for each determination to insure a minimum of $10^5$ surviving target cells at the beginning of the expression period.) The cells were trypsinized, pooled, and replated into flasks one or more times to insure logarithmic growth. The total length of the expression period was adjusted for each experimental determination to allow the cells to undergo at least 4.5 population doublings before selection was begun, i.e., 7 to 10 days (15).

At the end of the expression period, the cells were trypsinized, pooled, and 1-2 x $10^6$ cells plated into selective medium at a density of 500 cells/sq cm (180 x 60mm dishes per point). To determine their cloning efficiency at the time of selection, these cells were further diluted and plated at cloning densities into medium identical to selective medium, but lacking 6-thioguanine. A reconstruction experiment with HPRT$^-$ Lesch Nyhan cells (18) accompanying each determination indicated that the efficiency of recovery of 6-thioguanine resistant colonies in the experiments reported here was 85%. Selection was continued for 14 to 18 day one refeeding. The frequency of mutations to 6-thioguanine resistance was calculated from the probability of a mutant per dish using the $P(0)$ method (13). The results are shown in Figure 9.

It will be seen that DMBA is more mutagenic than BP when these are compared as a function of the concentration administered. This is also true as a function of the cytotoxic effect of the hydrocarbons. (Compare these data with those of Fig. 8 for the percent survival of cells exposed to DMBA and in Fig. 11 for that of cells exposed to BP for 48 hrs.)
Figure 9. Induced mutation frequency of XP cells per 10^6 cells. XP cells were coincubated for 48 hours with lethally-irradiated 703 tumor cells in the presence of various doses of BP or DMBA and then assayed for 6-thioguanine resistance as described in the text.
High Pressure Liquid Chromatography Analysis of B(a)P-DNA Adducts Produced in Co-cultivated Human Cells

Since previous data had shown that BP induces an increase in the frequency of mutations to thioguanine resistance in target human diploid fibroblasts co-cultivated with human tumor cells capable of metabolizing BP, this suggested an interaction of BP metabolites with cellular DNA. To investigate the extent and nature of this interaction, we determined the number of BP-DNA adducts formed during a 48 hr exposure of co-cultivated target cells and metabolizing cells to tritiated BP per 10^7 moles of DNA nucleotides were found to be, respectively, 1.0, 4.1, and 12. These results confirm that metabolites of BP were binding to the cellular DNA and showed a direct relationship between the concentration of BP in the medium and the number of BP-DNA adducts formed.

To identify these BP-DNA adducts, we analyzed those formed at the highest concentration using high pressure liquid chromatography (HPLC). Figure 10 shows the HPLC elution profile obtained. The major peak, 82% of the radioactivity associated with the adducts, co-chromatographed with the optical standard-adduct formed between deoxyguanosine (dG) and anti BPDE. This cell-mediated BP-DNA adduct also co-chromatographed with the adduct formed in normal diploid human skin fibroblasts exposed for 2 hr to anti BPDE (12). The minor radioactive peak in Figure 12 co-chromatographed with the optical standard formed between dG and syn BPDE. These results are consistent with reports on BP-DNA adducts formed in human explant tissue from lung (19), colon (20), and bronchus (21) as well as cells derived from human lung carcinoma, A549, in which the N2-dG-anti BPDE adduct was the major adduct observed.

Figure 10. HPLC profile of the DNA nucleoside adducts formed in 835 cells coincubated with XP cells. The major peak is the N2-dG-anti BPDE adduct and the minor peak is the N2-dG-syn BPDE adduct.
C. Time-Dependent Dose Response of XP Cells to BP Using Cell-Mediated Metabolic Activation:

To further characterize our assay system to changing doses of BP for varying times of exposure, logarithmically growing XP cells were plated at 200,000 per 30mm diameter culture dish. After 24 hours, X-irradiated transformed epithelial cells were plated at a final attached cell density equal to that of the XP cells. The cultures were exposed to increasing concentration of BP for 24 or 48 hours and then replicate sets of treated cells were trypsinized and replated at cloning densities. In order to determine the number of XP cells in co-cultivated treatment dishes accurately, a dish containing XP cells alone without epithelial cells was counted. As shown in Figure II, the percent survival of XP cells decreased with increasing concentration of BP and in addition, the 48 hr incubation resulted in greater cytotoxicity than the 24 hr.

![Figure II](image)

Figure II. Cell-mediated cytotoxicity of XP cells incubated for 24 or 48 hours with lethally-irradiated 835 tumor cells in the presence of various concentrations of BP. After the coincubation, the cells were trypsinized and the XP cells assayed for cloning ability.
VII. SIGNIFICANCE

In vitro assays of mutagenicity and transformation are used as short term tests to determine the potential danger of unknown agents as well as to carry on mechanistic studies regarding the mechanisms of mutagenesis and transformation. In both types of studies, one is frequently faced with the need to utilize agents which require metabolic activation. It is clear from published studies that the incubation of target cells with preparations from liver homogenates such as S-9 fractions or microsomal preparations, results in the production of potentially mutagenic and transforming adducts in the DNA of the cells, but that these may be different from those actually produced in the DNA of the cells used to make the homogenate. Thus, the conclusion that a particular compound is potentially mutagenic may be the correct one, but for the wrong reasons, i.e., the wrong DNA adduct was produced. It is obvious that any reliance on short term assays which makes use of the metabolizing ability of cell homogenates may be held up to question precisely because of this problem. This poses potential problems for regulating agencies as well as for those interested in mechanistic studies. The use of cells able to metabolize carcinogens as feeder layers allows one to produce the expected DNA adducts in target cells which are the same as those produced in vivo and will therefore be useful for many types of studies.
REFERENCES


Discussion

Dr. Morris, EPA: Certainly within the regulatory sphere we are interested in batteries of tests and so on for predictors and do you envision then that as we proceed, certainly in the rule-making exercise for Section 4 of TSCA we might be involved in not only selecting the cell type for the mutation or transformation, whatever assay but we are, also, maybe wanting to hone in on the specificity of the cell line for metabolizing? Is that the direction we are going in?

Dr. Waters, EPA: Yes, I think that is definitely true, especially as we progress to higher levels of testing. I think what I have said in terms of the specificity of metabolism should not be taken as an indictment of an Ames test and its S-9 preparation because I still believe that this is a very good detection system. However, as we attempt to extend results from detection systems to confirmatory in vitro bioassays, and to the level of the in vivo bioassay we must be much more concerned about the specificity of the metabolism. We must be concerned about it if we are to have really relevant confirmatory bioassays. So, I would see the kind of work that is being performed under this grant at Michigan State University as being indicative of the type of testing that ought to be carried out in the confirmatory phase of assessment of compounds. Does that answer your question?

Dr. Morris, EPA: Yes.

Dr. Kraybill, NCI: I am Dr. Kraybill, NCI. I am still not quite clear then because I feel that using the S-9 fraction here, you are getting the result, but the relevancy of that result to an in vivo system; after all, that is what we are interested in, what happens in man, how do you look then upon the Ames system with the S-9 fraction here, as an indicator or--

Dr. Waters, EPA: Yes, as an indicator system.

Dr. Kraybill, NCI: Just simply that?

Dr. Waters, EPA: Right, and in point of fact, it is the correlation that has been developed between Ames test results for many compounds and results of whole animal carcinogenesis bioassays that is the real strength of that assay. I think that these correlations argue very strongly for the use of this kind of test as a detection system. However, when the question of specificity of metabolism arises, as we progress from the detection level to the confirmatory level of testing we should be concerned about the formation of specific metabolic products in the intact animal and possibly in man. I think that is the reason that we need to be doing this kind of research--to solidify our confidence in the kind of metabolism carried out in confirmatory bioassays.
Dr. Chandler, NIOSH: Jerry Chandler, NIOSH. Mike, there are two facts that we have to keep in mind, I suppose. One is that many of the reactive intermediates that are generated by microsomal oxidase systems are extremely short-lived and may not be as long as the BAP half life or reactive intermediate. Secondly, when you use two cell systems, one is an activation system. You are requiring that the compound must diffuse out of the one cell across the other membrane into another cell. Do you think this is going to be generally applicable?

Dr. Waters, EPA: The data that has been obtained up to now by a number of investigators, Huberman and Sacks in Israel, Weibel in Germany, indicate that despite our concerns, the ones that you mentioned, that a compound has to be metabolized in one cell, get out of that cell and into another to show its effect, despite those concerns, in many cases, with a number of compounds that require metabolic activation, the systems are working. I believe it must not be terribly difficult for this cell-cell interaction to occur. The key element may be the proximity of the two cell types. In fact, it appears that they must be in close proximity to one another. I don't know how far one can separate the activation cell on the one hand and the indicator cell on the other. We have performed some experiments in our laboratory where we have interposed dialysis membranes, and it is still possible under those circumstances, in the case of compounds, for the metabolites to get out and to enter the indicator cells. So I don't yet know what the limits are, but I do think, that based on the results obtained thus far, that it does seem feasible to use these kinds of systems. Also, it is indicative, I think, of an important concern that we probably should have, and that is that metabolism that occurs in one cell type in an intact organism may be highly influential on another cell type. That is something we need to keep in mind.

Dr. Hegyeli, NCI: Hegyeli, NCI. Mike, I see from your description of the procedure that these cell lines, the feeder cell lines are derived from human tumors. Did you ever try to use primary cells?

Dr. Waters, EPA: This is, of course, not our own work but that of Drs. McCormick and Maher. We have used primary cells in our laboratory, yes, and they have as well but not as a part of this particular program to date. We have used primary hepatocytes, and we are using primary bladder cells and primary lung cells. As a specific example, Dr. Robert Langenbach in our laboratory has worked with a series of nitrosamine analogs. He finds that using whole cell hepatocyte activation, the correlation that is obtained for mutagenic activity in V-79 cells and carcinogenic activity in whole animal systems is much better than that obtained if mutagenic activity in an Ames test using S-9 activation is compared with carcinogenic activity in the intact animal. Please, let me say again, I am not indicting the Ames test. I am simply indicating that for certain kinds of chemicals whole cell metabolism may be more relevant to the intact animal, and I agree with you that primary cell
metabolism may even be better than that observed in many cell lines. However, primary cells in culture do have some significant disadvantages. They are much harder to reproducibly prepare and control. If we can select cell lines that can carry out a broad range of metabolic activities, then I think they should be, in theory, preferable to the primary cells because of the difficulty of having to recover cells from the intact animal for each experiment.

Dr. Herberman, NCI: Herberman, NCI. Is there some concern that different cell lines might metabolize the same agent somewhat differently and that if you use as the primary screen the direct cytotoxicity that that might not reflect the metabolites that you would be interested in for mutagenic or carcinogenic effects?

Dr. Waters, EPA: Yes, the Painter screen was used primarily because of its rapidity, and they were able to examine a series of 19 different cell lines very quickly. However, before they would recommend the use of these cell lines for screening purposes it would be essential to confirm that the metabolic activation capability possessed by these lines is indicative of the type of metabolism that we are looking for in vivo. So, this is, in fact, what they have done in one part of the study. Did I answer the first part of your question? Would you repeat that? Maybe I did not quite catch all of it.

Dr. Herberman, NCI: The question was whether the primary screen that was being used was the best one or whether it would be better to go directly to a mutagenic or carcinogenic one?

Dr. Waters, EPA: I think it would, but of course, it takes a lot longer to do that, and they are doing it secondarily, and as long as it is done before you propose using those cells, I think it is probably okay.

Dr. Morris, EPA: Thank you, Mike.
STUDIES OF ORGAN-ASSOCIATED ANTIGENS AND OTHER MARKERS IN HUMAN TUMORS WHICH MAY BE USEFUL FOR THE DIAGNOSIS OF MALIGNANT DISEASES

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The main rationale for these projects is to help identify test procedures that could pick out individuals in a population who might have been exposed to carcinogenic agents and would be the ones who would actually be coming down with malignant disease. This is a generally important problem, to be able to screen general populations or high-risk populations and to identify the relatively small numbers of individuals who harbor occult malignancies.

The particular objectives in this area have been to focus on immunologic markers that might be useful to diagnose certain types of human malignancies, particularly in this type of screening approach, and the focus for the contracts that are being supported under this program has been primarily leukemia and breast cancer, but these projects also have possibilities for other types of cancer.

There are some particular goals to keep in mind that are needed to be successful with tests of this type. I might note at the outset that accurate detection of occult malignancies is a very difficult task to accomplish, although it is obviously quite important.

One of the first criteria that one would be concerned with would be to have a test with a high degree of specificity. Particularly, it would be desirable to have a test that would be able to not only detect the presence of cancer but would be able to get some indications for the type of cancer or where it would be arising. This is one of the reasons to put some emphasis on markers that would have some tissue or organ association.

The other side of the coin is to have tests or markers which would have a high level of sensitivity. There are many tests in which the markers are positive with cancers when they are present at a fairly advanced stage, when there is a lot of tumor present. It is considerably more difficult for a test to be positive in patients with small amounts of tumor, when it is early, localized, and at a point that is treatable. That is clearly the stage that one would like to have sensitive tests, in order to be able to begin or alter therapy and thereby affect the course of disease.

Finally, if one is going to develop assays that might be valuable for screening of populations, even quite high-risk populations, this would involve testing of quite large numbers of individuals. Therefore, it would be necessary to have an assay procedure which would be practical for large numbers of specimens and would, also, be quite reproducible.

There has been quite a large effort in this direction. The National Cancer Institute specifically has many grants and contracts that have been designed to develop immunodiagnostic or other diagnostic markers for cancer. The two projects that are being supported under this program essentially are just a small subsegment of this type of approach, but they are ones that were thought at the time of initiation of the program to be ones that were promising for these goals.
The first particular project that we are concerned with is at the University of Minnesota and has focused on leukemias and lymphomas. The investigators involved with this are Drs. Kersey and Tucker Lebien.

The particular approach that they started out with was to prepare heterologous antisera, primarily in rabbits, that would react against antigens that would be associated with various types of human leukemias, either in a quite specific way for the leukemias, or that would see differentiation antigens, that would not be entirely leukemia specific. The latter type might still be quite useful as a detection system since in the normal development of the hematopoietic cells these markers might only be present in a very small proportion of cells or in early stage of development. Thus, in this situation, one would be focusing more on quantitative differences rather than on real qualitative differences.

The approach which has been adopted during the past year or so has been to shift away from the more classical approach of just injecting cellular or subcellular materials into rabbits or other species, and to go to an approach which in general seems to be much more promising for specificity and, also, for developing large amounts of the same reagents. This involves the production of monoclonal antibodies in mice that might be able to see the same or perhaps an additional series of antigens.

It may be worthwhile to first briefly describe the methodology of monoclonal antibodies. Cells or subcellular materials are injected into mice and, at a time thought to be optimal for getting an immune response, spleen cells are taken from the mice. These lymphoid cells are co-cultured with an established myeloma cell line, preferably a myeloma cell line which itself does not make antibodies or immunoglobulins. The transformed cells are fused with the B cells that are in the spleens of the immunized animals; this fusion occurs quite readily in the presence of polyethylene glycol.

After fusion, the cells are cultured for a period of time in so-called "HAT" medium which is a combination of cytotoxic agents to which the parental myeloma line is quite sensitive. Thus, the unfused cells would be eliminated and only the fused products that have resistance to these materials would be able to survive. Supernatants from surviving cells in various wells are screened for the reactivity that one is trying to measure. This is the most critical part of this methodology and one has to be clever enough to have a good screen. The type of screens that the group in Minnesota has used has involved the use of paired cell lines; on the one hand, the leukemia line to look for positive antibody reactivity against leukemia associated antigens and on the other hand, an autologous B cell line transformed with Epstein-Barr virus as the negative part of the screen. Thus, they are looking for antibodies that would react with the leukemic line and not with the B cell line from the same individual. They have been able to derive several clones from these fusions that have looked quite promising for antibodies that could be useful to detect leukemia-associated or differentiation antigens. One of these monoclonal antibodies was raised against a T cell acute lymphocytic leukemia line, HSB. This particular monoclonal antibody has not been nearly as selective as one would like, at least in the peripheral blood since it reacted with a large majority of T cells among peripheral blood mononuclear cells. However, it has reacted with a series of acute leukemias and leukemic cell lines but not with other types of acute leukemias.
There have also been some monoclonal antibodies that have been raised to another acute lymphocytic leukemia cell line, NALM-6. One monoclonal antibody to NALM-6 reacted to both the ALL line and the autologous B cell line, and it appears to be detecting some B cell associated antigens. In the peripheral blood it seems to be reacting with more or all normal B cells, but in the bone marrow it seems to be much more selective, reacting with very few of the cells. For the screen against leukemias it has been quite effective in picking up all of the B type chronic lymphocytic leukemias and a series of acute lymphocytic leukemias.

The third monoclonal antibody that they have raised seems to be the most interesting one. This reacts against an ALL-associated antigen which seems to be very similar, if not identical to one which they had previously identified by heterologous antiserum. The specificity for this antigen seems to be a differentiation antigen associated with pre-B cells. Very small numbers of normal B cells in the bone marrow react with this, but almost no cells in the normal peripheral blood react. Yet, the antiserum reacts with a considerable portion of acute lymphocytic leukemias, mainly those which are classified as the most common type of ALL, the so-called "non-T, non-B" ALL and also reacts with so-called "pre-B ALL" and also with the cells from chronic myelogenous leukemias in blast crisis. This last group is particularly of interest because those types of leukemias have been associated with some of the carcinogenic effects seen with cytotoxic therapies in cancer patients.

Although the first two antibodies that I described have not been nearly as selective as would have been hoped, one of the promising aspects to the overall study is that among 40 different individual leukemias that have been screened, there was essentially no overlapping between leukemias positive with one of these antibodies compared to the others. Thus, by using a panel of these three reagents it was possible to pick up almost all of the leukemias whereas each antibody detected only a subset. This may be the type of approach which will be taken in this area, to combine several markers since one particular reagent may not be sufficiently sensitive or specific.

The other project which is being supported in this area is at Emory University with Dr. R. Chawla as the principal investigator. The focus has been on a marker called EDC1.

EDC1 is a protein which has been found in the urine of cancer patients. It is a relatively low molecular weight glycoprotein. It has been possible to identify EDC1 in the urine of the majority of patients with metastatic breast cancer, and a variety of other types of solid malignancies.

This is not a tumor-specific protein but is actually a degradation product of a normal serum protein, inter-alpha-trypsin inhibitor, that is present in everyone's serum that has a much higher molecular weight (170,000). Under normal circumstances the serum protein is not degraded to produce detectable levels of EDC1. Therefore, the focus in most of the studies of Dr. Chawla has been to screen the urine for the lower molecular weight compound. The problem with this approach has been that the antibodies that he has available have cross-reacted immunologically between the serum protein and EDC1.

Therefore, he was faced with the task of finding a method to more specifically detect EDC1. To a certain extent, screening in the urine provided a biologic distinction because the large molecular weight protein under normal circumstances would not be filtered in the glomeruli, whereas the lower molecular weight one would. The problem with this approach is that with any impairment of glomerular function, there could be some leakage into the urine of the higher molecular weight material.
Dr. Chawla has corrected for that to a large extent by making a ratio of the amount of immunologically active material detected to the creatinine that was excreted into the urine. However, this was not entirely selective. He has been able to develop a radioimmunoassay which is much more sensitive than the early ones. However, it detects both proteins, and there have been some false positives in tests on urine which are probably related to the subtle impairments in renal function which can occur in some tumor-bearing individuals.

Dr. Chawla has been trying other ways to discriminate between the two proteins. One possibility was two-dimensional crossed immunoelectrophoresis, since the charge, as well as the size of these molecules, was different. However, although the initial studies looked very promising, with clear separation of the two proteins, there have been some normal individuals who have had an unexpected degree of heterogeneity, with a peak of an intermediate molecule between the one and the other. This has caused some confusion in the assay.

The more recent thing that Dr. Chawla has come up with has been to precipitate the parent molecule with sulfa-salicylic acid. This seems to be quite effective in precipitating the normal serum protein without affecting the lower molecular weight compound. This seems to be a very good basis for an assay for distinction.

The objective of Dr. Chawla's project, once he works out this technical problem, is to screen a large number of sera and urine which he is currently collecting from patients who are being evaluated for the initial diagnosis for breast cancer. There are specimens from patients with breast cancer and also sera from women who turn out to have only benign breast disease.

At this time, with metastatic breast cancer patients, the large majority have levels of EDC1 that are above the range that is seen in either normal individuals or in patients with a variety of benign diseases, including benign breast lesions. Elevations also have been seen in a variety of other types of malignancies, including acute myelogenous leukemia. The hope is that with the radioimmunoassay, which is much more sensitive than the assay used to generate the current data, and some kind of selection procedure, there may be a specific and sensitive procedure suitable for the ultimate objective of this project.
Dr. Kraybill, NCI: We received a call the other day from an authority in the South Dakota State Department of Health. He has come across some records of 1400 tests that were run with a high indication of association of another biochemical marker, hyaluronidase, that might indicate tumorigenicity in people. Have you heard anything about this particular marker?

Dr. Herberman, NCI: I am not aware of detailed documentation that hyaluronidase is useful. There have been a series of enzymes that have been put forward as possible discriminants. In fact, my experience has been that investigators do a study by comparing advanced cancer with normals and see some very interesting differences.

Unfortunately, you can get the same kind of differences very frequently with the erythrocyte sedimentation rate. The bigger difficulty is to be able to get adequate discrimination between cancer, particularly at a localized stage, and benign diseases, especially of the same organ system as the cancer.

As part of the NCI's program we have a serum bank in which specimens are sent to investigators that have assays that they think are promising for making these kind of discriminations. Over about the five-year period that this has been available, there have been about 200 or so assays that have been screened, including a number of enzymatic assays of the type you have mentioned. Unfortunately, only a handful of them has had significant levels of discrimination when tested with the various groups of patients and controls that I mentioned.

Dr. O'Connor, NCI: I would like to raise a general and somewhat provocative question. I wonder whether you would comment on the potential of the three-dimensional system that Norman Anderson has at Argonne. I think you are familiar with it. Would you comment about where that might fit into this whole problem?

Dr. Herberman, NCI: I think this is a very impressive type of methodology. It has the ability, as I understand it, to look at a very wide cross section of metabolic products and not only immunologic but, also, biochemical types of markers. It might well pull out something which would be quite interesting.

With the serum bank that has been available there have been some things in the sera that have looked to be quite discriminating. For example, Dr. Phyllis Brown in Rhode Island has been looking at liquid chromatographic separation of nucleosides that are in sera. There are several nucleosides that she has been able to identify that seem to have considerably different patterns in cancer patients, including some early stage cancer patients, compared to some benign diseases and normals. Certainly the Anderson type of approach should be able to pick up this as well as a variety of other things. I guess one of the concerns that I have with that approach is that you may be really flooded with information. There will be so much information gathered that it may be difficult to actually sort out the relatively small number of interesting or important differences from the background of so many other nondiscriminating materials. The other thing that I would have to add is that, although this technology is quite intriguing as an approach, I am not aware that it has been validated for this kind of application. We will really just have to wait and see how well it will do. Unfortunately, among the many potential tests screened thus far, there has been a low yield of tests that have held up under scrutiny. A very small proportion of tests can discriminate between known early cancers and known benign diseases and even fewer have the levels of sensitivity and specificity to screen even a high-risk population. For screening you need extraordinarily high levels of
sensitivity and specificity and I am not convinced that there are any tests that are adequate for screening for the types of cancers that we are concerned with in the United States.

Dr. Morris, EPA: As I understand, in the past with breast cancer we had HCG and CEA and one other test which I cannot remember now that was used in a battery approach. Do you feel that we are far enough along to consider some type of battery even at this point or do you feel that is premature?

Dr. Herberman, NCI: I think the battery approach probably will be the way to go. The problem is to decide what to put into the battery. My view is that each of the tests in the battery has to, on its own right, be reasonably powerful.

Some of the evaluations that have been done have included some things in the batteries that are marginal, and if you put together a few marginal tests, you seem to end up with marginal data in the aggregate with their problems adding up as well as their strengths.

In fact, the combination of tests for breast cancer that you are mentioning is an example of the problems in this area. HCG is a marginal marker for breast cancer.

Dr. Morris, EPA: You are saying that for high-risk groups it is far enough along, determining, you know, malignant disease versus benign disease. Do we have enough information or should we start considering this. I was thinking about my ACB population for example that are potentially at risk. Do we have enough information even in the present battery, admitting we may wind up with some benign in the process? How significant would it be, even at this point to approach that with some of these selected populations, not broadly but in select cases?

Dr. Herberman, NCI: I think an example that one can think about specifically in this regard is CEA (carcinoembryonic antigen). If one looks at cancers, carcinomas for example, versus a normal healthy population, it actually performs rather well, in that with colon cancer, breast cancer, lung cancer, at least 90 percent or more of patients have elevated levels of CEA as compared to less than 5 percent of the normal population. That sounds pretty good, but there is, also, a range of benign diseases with elevated levels. The experience has been that when CEA is applied for screening, most of the elevations are due to benign diseases since they are much more prevalent in the population than the cancers. For every cancer that may be picked up, there may be 50 or 100 benign diseases. In addition, the problem with markers of this type is that they tend to increase in their levels or even in their detection rate with more advanced disease. Thus, most of the cancers that were picked up were advanced and untreatable.

The one test which has really shown promise and has been applied at a large-scale level for screening has been alpha fetoprotein. The Chinese have utilized this test for screening in a high-risk area and have shown reasonably well that they could use this in field conditions. They could identify a significant number, at least, of people with liver cancer, including some surgically resectable liver cancers. However, the yield of really treatable cases was very low for the number of people that they had to screen, which has been in the neighborhood of one-half million. At most 10 or 20 treatable liver cancers were picked up.
A research program using aquatic systems and organisms to study the fate and effects of carcinogens in the aquatic environment has been underway during the FY's 78 and 79 at the Gulf Breeze EPA Laboratory. The two major investigative, disciplinary areas have been in pathobiology and biochemistry. A fish-carcinogen assay system has been developed that involves laboratory controlled long-term exposure of fish to suspect carcinogens followed by histopathology and physiology of exposed fish, induced growths, and related disorders. This system appears adequate for carcinogen tests for fish. A two year field study is underway of tumor, disease prevalence, and carcinogen residue or metabolites in fish and shellfish in variously polluted estuaries along the northern Gulf of Mexico. It is too early to predict the significance of the findings in this study, but several new tumor types have been discovered in fish.Biochemical studies have revealed that fish may respond in enzymatic reactions in ways similar to mammals exposed to the same carcinogens, and that fish may prove to be adequate supplemental, biological monitors of carcinogens in the environment. It was shown that aside from induction of oxygenase activity, transferases responsible for detoxification reactions are also induced, the latter by metabolites rather than parent compound. The significance of this is that while the oxygenases are responsible for producing ultimate carcinogens from procarcinogens, the transferases are involved in the excretion and hence detoxification of the oxidized metabolites. An extra-mural (grant) program consisting of eight grants to Academic investigators has supplemented specific areas of investigation in the overall project. Findings from individual grants and cooperative agreements are reported for work completed to date.

Acknowledgement:

This research is supported by an Interagency Agreement between the National Cancer Institute and the Environmental Protection Agency.
III Introduction

A major problem faced by the National Cancer Institute and the Environmental Protection Agency is that of aiding in determining the fate and effects of carcinogenic pollutants in the larger environment. It is one thing to track the routes, fates, and effects of a pollutant in an industrial system or even in a metropolitan area, but quite another to understand the behavior of single pollutants in the total environment. Exposure of human populations to most carcinogens occurs insidiously in the general environments of air, land, and water. This does not minimize, in any way, the necessity to study the behavior of carcinogens in special, limited environments, such as industrial, urban, or suburban complexes, but we know relatively little about the risks of human exposure to pollutants, such as mutagens and carcinogens, in the general environment.

One way to approach the problem of understanding risks of general exposure is to study wildlife populations that are widespread, but which live in environments where exposure to ambient pollutants is certain. The use of wildlife populations as surrogates for human populations may be considered to be a novel expansion or logical extension of the use of laboratory animals and animal models of human diseases as alternatives to use of human subjects. The sharing of biologic characteristics by phylogenetically diverse species makes certain comparative approaches possible.

Problems arise, however, when such factors as selection of sensitive, indicative species, geographically adequate populations, and indicative segments of the air, land, water biosphere are considered. In this regard, Office of Research and Development laboratories, such as the Gulf Breeze Environmental Research Laboratory, have exemplary, pilot research programs that are investigating the use of aquatic animal species as indicators of the presence and potential effects of toxics, particularly of certain car-
cinogens, in the larger environment. The Gulf Breeze pilot research program has been under way since August, 1978 and is supported jointly by the Office of Research and Development and the National Cancer Institute through an interagency agreement. The Gulf Breeze studies are based on the premise that the aquatic portion of the biosphere (water, biota, and sediment) is the ultimate "sink" for the runoff, fallout, and discharge of most toxic pollutants. In addition, animals living in the relatively efficient solvent water are more intimately exposed (total exposure through body surfaces, gills, alimentary tracts) than probably are most species living in terrestrial or air environments and are less likely to easily escape a dissolved or carried pollutant.

At Gulf Breeze, researchers are studying species of fish and shellfish along the Northern Gulf of Mexico (Florida, Alabama, Mississippi) in order to determine which are good indicators of the role of carcinogenic agents in the environment. Fish, oyster, and clam populations are sampled monthly for determination of prevalence of tumors, cellular diseases indicative of pollution, and chemical analyses of residues of potential carcinogenic chemicals. Sampling stations are located in both polluted and clean estuaries, as well as offshore in relatively pristine waters. Select species of fish are exposed in long-term assays in the laboratory to determine their specific tissue, cellular, and biochemical responses to known chemical carcinogens that may occur in the environment.

IV Objectives

Objective 1:
Carcinogenic or suspect carcinogenic substances often enter the aquatic environment as pollutants and pose a multi-threat to aquatic ecosystems. Because most carcinogens are mutagens, the major risks to aquatic ecosystems
and component species are long-term mutagenic and teratogenic effects expressed both at the organismic and population levels. Commercially and ecologically valuable species such as fishes, oysters, and shrimps may be adversely affected by carcinogens in the form of cellular proliferative diseases and mutagenic effects. Populations of valuable aquatic species impacted by mutagenic substances will be studied in order to predict effects on population stability and survival.

Objective 2:
The intake of carcinogens, mutagens, and teratogens by man comes in part through his food. Our chief concern in this area is the need to determine if aquatic species accumulate and convert procarcinogens to proximal carcinogens and thus pose a direct carcinogenic threat to man in his consumption of seafood. It is therefore essential to know the routes, rates, and reservoirs and metabolism involved in the accumulation of these compounds in aquatic food webs.

Objective 3:
Aquatic organisms may be exposed to carcinogens from run-off, fallout and discharge of pollutants into the aquatic portion of the biosphere which behaves as the ultimate pollutant "sink". Select species in the aquatic environment have potential to be used as sentinel or indicator systems to reflect the presence, behavior and effects of carcinogens, mutagens, and teratogens. Comparative laboratory and field studies of select species for uptake, accumulation, and effects of known carcinogens will reveal the best modes for utilization of aquatic species as indicators. Results of studies with sentinel species will be used to determine when further testing is required and may offer pertinent information on mechanisms of effects.
V Methodological Approaches

To contribute to the realization of the preceding goals, we have underway both in-house and extra-mural complementary projects. The overall project is divided into two major disciplinary approaches: 1) Pathobiology and 2) Biochemistry. Therefore, methods outlined below and results in the next section will be reported under these two complementary disciplinary headings. Results of grants in progress during FY 79 will be included at the end of this report. The disciplinary area of each grant will be identified by project office (Couch – pathobiology; Schoor – biochemistry).

1. Pathobiological Methods

a) Fish carcinogen assays, toxicity, and histopathology of induced lesions:
Aquatic species such as oysters and fishes that are exposed to carcinogens in special flow-through laboratory systems will be studied to determine relatively toxicity (lethality and dysfunction as criteria) and structural effects (histopathological and morphological changes as criteria).
Long-term exposures to low concentrations of select carcinogens will be carried out to determine if tumors or cellular proliferative disorders similar to those reported in feral specimens of oysters and fishes can be induced. Exposure of oysters and fish for periods of one year or more are underway. Information from these tests should aid in determining if tumors found in feral invertebrates and fishes in field monitoring studies are indicative of chemical carcinogenesis related to specific pollutants as determined experimentally. Studies similar to this for other systems are being supported through cooperative agreements (Couch, Martin, Hendricks, Sinnhueber – see grant progress reports at end of annual report.)

b) Field epizootiologic survey of cellular diseases and carcinogen residues in feral fish and shellfish: A survey of tumors and cellular
diseases in oysters, clams, and fish concomitant with a point source pollutant survey is underway for a period of at least two years along the Northeastern Gulf Coast between Pensacola, Florida and Pascagoula, Mississippi. Public requests for tumor bearing specimens have also been made. The purpose shall be to see if there is a correlation between tumor prevalence in aquatic species and pollutant prevalence in specific coastal areas. Four stations are sampled monthly for oysters and fish which will be examined grossly and histologically for neoplasms or related disorders. A concomitant survey of industrial, agricultural and domestic pollution at or near the sample stations has been made. Attempts to isolate specific carcinogenic pollutants or complexes will be made by collecting tissue, and sediment samples for chemical analyses as dictated by knowledge of pollutant sources. A 1979-1980 cooperative agreement between Dr. John Laseter and Gulf Breeze will permit the detailed qualitative and quantitative analyses of tissue and sediment samples for the presence of suspect or overt carcinogens. A similar study along the Oregon Coast of shellfish tumors and carcinogens has been supported through a grant from the project to Dr. Michael Mix, (Couch, Mix).

2. Biochemical Methods

a) **Induction Studies**: Aquatic species such as mullet, killifish, flounder, sea catfish and others have been and will continue to be exposed to inducers of microsomal mixed-function oxygenase (MFO) activity either by intraperitoneal injection or direct exposure in seawater. Since times of possibly one year might be necessary to induce MFO activity by water exposure, the direct injection of inducer is used at the start of the investigations in order to optimize other parameters such as metabolite and conjugation reactions. The long-term, low-
exposure route in seawater will follow. (Schoor, Melius, Strength).

b) **Metabolite Identification:** Metabolites from the MFO reactions will be identified using high pressure liquid chromatography coupled with fluorescence detection and confirmed by stopped-flow fluorescence scanning. Metabolite standards for benzo(a)pyrene (BaP) have been obtained from the Illinois Institute of Technology through the courtesy of NCI. All the phenolic compounds have been chromatographed and their fluorescence and emission spectra have been obtained in appropriate solvents. All spectra will be stored on discs for later data manipulation. (Melius, Schoor).

c) **Conjunction and Excretion Studies**

Rats are being used to make a series of conjugation products in vivo by injection of $^{14}$C-labelled BaP. They will include glucuronides, glutathiones, and sulfates. Their occurrence in fish will then be ascertained by comparison to the standards produced in the rat. This will be helpful in determining the final disposition of a carcinogen like BaP within the animal and in what forms the parent compound is finally passed back into the seawater. (Strength, Schoor).

VI **Major Findings and Progress**

As noted earlier under Methodological Approaches results and findings of studies to date are reported under the two disciplinary areas of Pathobiology and Biochemistry with grant cooperative agreement complementary studies reported at the end of this paper.

1. Pathobiology

   a) **Fish carcinogen assay:** Major advances have been made during FY 79 in design, utilization and evaluation of a laboratory, flowing-water, carcinogen assay system. The system is designed to control water
temperature, photoperiod, flow rates, and nutritional status of fish subjects for long periods and is being evaluated in a long-term (11 months to date) exposure of sheepshead minnows to the suspect carcinogenic herbicide, Trifluralin. To date, 70 to 90% of fish exposed continuously from fertilized eggs to 1-3 µg/l Trifluralin have developed abnormal vertebral growths that superficially resemble osteomas, or benign boney tumors during their early juvenile, and young adult periods. Methods for study of these lesions have been developed and include histological, histochemical and radiographic approaches. The vertebral growths can be located and diagnosed in samples of experimental fish taken directly from the assay system, anesthetised and then radiographed in a laboratory x-ray unit. Confirmation of any specific vertebral lesions is then made by histological examination of individual specimens prepared in special ways. Large numbers of control fish and feral fish (sheepshead minnows) have been examined similarly with no findings of growths. We conclude, therefore, that the vertebral growths are experimentally induced in our assay system. This study continues in FY 80.

b) **Field epizootiological survey:** This study has been underway since August, 1978. To date, samples of fish, oysters, and clams have been collected and examined monthly for the prevalence of tumors, or cellular diseases. Thousands of (>40,000) fish have been examined grossly and internally for lesions; and over 7,000 oysters and several hundred clams have been examined for lesions histologically. Fish and oyster tissues, and sediment samples have been collected for chemical analyses, underway at present. Another approach has also yielded good results - that of circulating requests for tumor bearing specimens to the public (fishermen - sport and commercial, clubs
and packing houses). So far several specimens have been brought in with cancer-like or truly neoplastic lesions.

Summarized results are as follow: Fish - numerous tumor-like lesions with several neoplasms diagnosed from field collected specimens; oysters - two cases of leukemic-like (blood cell proliferative) disease - one case from a polluted harbor, another from clean waters; clams - several cases of external growths (polyps) to be diagnosed.

Perhaps the most exciting and interesting finds were the specimens brought to us by an aquaculture group in response to our public circulars requesting specimens. Mr. Bill Tremble of the Mariculture Center at Gulf Shores, Alabama presented us with three specimens of *Fundulus grandis*, the Gulf Killifish, that had large white to yellow growths on their heads and bodies. Histologic examination of these cultured fish revealed that the growths were probably very invasive pigment cell tumors that grew rapidly. These large, older fish had been reared in ponds treated with certain chemicals for control of parasites and receiving ambient water from the intercoastal waterway. The availability of cultured *Fundulus* with a history of invasive neoplasia may provide us with an animal model with which to study certain forms of neoplasia. To date, the tumor has been identified as an erythrophoroma, the tumor cells identified and biochemical characterization of pigments has been completed.

After only one and one-half years of study, from which only a portion of the specimens have been analyzed, we cannot draw conclusions or final correlations concerning disease prevalence and pollutant occurrence.
2. Biochemistry

a) A Schoeffel RRS-1000 Spectrofluorometer was installed in the spring of 1979 and has been used in obtaining the excitation and emission spectra of metabolites of BaP. Twelve different phenols for BaP and all spectra were obtained. The speculation that there would be enough characterizable differences in each compound's spectra was found sound for the phenols. So far, the quinones have been shown to be extremely unstable in light. It was shown that in the case of 3-OH and 9-OH BaP the stopped-flow HPLC scan clearly identified the two isomers. In a mixture of the twelve phenols, ten can now be separated and quantitated.

b) Enzymes studies to date are (1) UDP-glucuronosyl tranferase (E.C.2.4.1.17), (2) 3-phospoadenosine-5-phosphosulfate sulfotransferase (E.C.2.8.2.1), (3) UDP-glucose dehydrogenase (E.C.1.1.1.22), (4) glucose-1-phosphate uridyltransferase (E.C.2.7.7.9), (5) b-glucuronidase (E.C.3.2.1.31), and (6) aryl sulfatase (E.C.3.1.6.1). We have observed the induction of the above transferases in rat tissue, but no induction of the dehydrogenase activity was found. For UDP-glucuronosyl transferase induction by phenobarbital was 3-5 times, by 3-methylcholanthrene 2-3 times, by phenanthrene 1.5-1.8 times, and by BaP 1.5-2 times the value of the control. For the 3-phosphoadenosine-5-phosphosulfate sulfotransferase induction by PB was 2 times, 3-MC 2 times, and weak for both Ph and BaP. All inducers were fed in the diet at 0.1%. It was observed that in the case of 3-MC there was a large increase in liver size, which was not observed in the other cases. It is presently suspected that the transferases are induced by the metabolites of the inducers. (Strength).
c) Our studies have shown that the mullet (*Mugil cephalus*), the sea catfish (*Arius felis*) and the gulf killifish (*Fundulus grandis*) possess MFO systems which are inducible by *Aroclor* 1254 (mullet) and by 3-MC (sea catfish and gulf killifish). We have employed cytochrome b<sub>5</sub> and cytochrome P<sub>450</sub> reductase assays, carbon monoxide difference spectroscopy, high-pressure liquid chromatography, and the *Salmonella* microsome mutagenicity assay to monitor induction and evaluate PAH metabolism. Our studies indicate that these organisms possess MFO systems inducible by *Aroclor* 1254 and 3-methylcholanthrene and capable of benzo(a)pyrene metabolism. B(a)P treated gulf killifish did not appear to metabolize B(a)P as efficiently and seemed to produce lower levels of B(a)P metabolites than did 3-MC treated gulf killifish. This may have resulted because (1) B(a)P is a less effective inducer than 3-MC or (2) in vivo B(a)P metabolite may have induced the conjugation systems which would result in ethyl acetate insoluble and nonmutagenic in vitro B(a)P metabolites. These studies show that certain similarities exist in the mechanics of fish and mammalian MFO systems even though differences exist in the activities of these systems. (Melius).

d) It has been demonstrated that the mullet is able to hydroxylate B(a)P, that this activity is inducible by 3-MC, and that the metabolite profile and enzymatic activity are similar to those found in rats. Proliferation and enlargement of ER strongly suggests that the mullet hepatocytes behave similarly to other vertebrate hepatocytes when exposed to enzyme-inducing chemicals. Nuclear changes in the hepatocytes of the injected fish reflect cellular necrosis due to cellular intoxication. In spite of feeding, early loss of liver glycogen may be indicative of nutritional as well as other stress such as confinement. (Schoor, Couch).
VII Significance to Biomedical Research and Program Needs of NCI and EPA

Progress achieved in FY 79 in this project indicate the following contributions to biomedical interests and needs of NCI and EPA:

1. The fish carcinogen assay system functions well for long-term testing of chemicals against the sheepshead minnow, a common Gulf and Atlantic coastal species. This system can be considered now a complementary test system to those used routinely for rodent assays at NCI and elsewhere. New chemicals and some new species will be tested in the future. The system has been used in FY 79 to demonstrate the induction of a specific tumor-like lesion in a fish with a suspect carcinogen (Trifluralin).

2. To date, the field study has shown that fish and shellfish from the Northern Gulf of Mexico suffer from a variety of lesions similar to lesions found in higher animals such as mammals (i.e. pigment cell tumor in Fundulus). This study is too incomplete, at present, to draw conclusions concerning early warning or sentinel capabilities of the aquatic populations under investigation.

3. Biochemical and correlated structural responses of fish liver systems seem to be relatively similar to responses of mammals to certain carcinogens. This permits future comparative studies to determine if biochemical methods may be incorporated in early warning or sentinel monitoring project with fish. The biochemical studies suggest that fish may serve as animal models in carcinogen (preneoplasia) studies to complement mammalian studies.
We plan to pursue the following efforts in FY 80-81 in the project:

1. Continue and expand our use of the fish carcinogen assay system by testing new compounds and, perhaps, by using Fundulus as a test organism. Carcinogens found in the field monitoring study may also be tested against select fish species. Improve diagnostic techniques for preneoplastic and neoplastic lesions in fish and shellfish.

2. Continue in the second year of the field (epizootiology) study of tumors in feral fish and shellfish populations. Beginning in FY 80 will be the chemical analytical portion of the study.

3. Continue and expand the biochemistry effort in the study of metabolism, conjugation, and excretion of carcinogens in fish and invertebrates. Examine the ways in which man may be exposed to carcinogens or their metabolites via the aquatic environment.

4. Several new cooperative agreements for extramural studies will begin in FY 80.
Date Contract Initiated and Period of Contract Planned

Initiated: October 1978
Expiration Date: September 30, 1984

Contractors Project Director
Dr. Herman Kraybill/NCI

Project Officers for NCI or EPA

NCI: Dr. Herman Kraybill
EPA/ORD: Dr. Wayne Galbraith
EPA/Gulf Breeze: Dr. John A. Couch, Dr. Hank Enos
1) Long-term experiments in progress:

a. *Ictalurus* injected i.p. with 50 µl 10% DENA (diethylnitrosamine) in distilled water.

b. *Cyprinodon* injected i.p. with 50 µl 5% DENA

c. *Cyprinodon* injected i.p. with 50 µl 1% DENA

d. *Ictalurus* and *Cyprinodon* injected i.p. with 125 µl BEN (benzidine).

Saturated solution in distilled water.

e. *Cyprinodon* exposed to weekly contaminations of DENA at 10 ppm.

f. *Cyprinodon* exposed to weekly contaminations of BEN at 1 ppm and at 10 ppm.

g. *Ictalurus* and *Cyprinodon* fed 1 ml DENA/100 gm dry food.

h. *Ictalurus* and *Cyprinodon* fed 0.5 gm BEN/100 gm dry food.

2) Bioaccumulation studies have been conducted in which 50 µl of $^{3}$H-BaP (benzo(a)pyrene) was adsorbed to food pellets fed to *Ictalurus*. The results indicated the highest level of label in the liver at 6 hours after ingestion.

These results, when compared to previously accomplished bioaccumulation studies, suggest that fish are more likely to accumulate polycyclic aromatic hydrocarbons as a result of their being adsorbed to ingested materials than through direct uptake from the water column.

3) A dechlorination technique has been developed that allows increased resolution of cellular morphology of the early embryonic development of *Cyprinodon* (Moreno, M.S. Thesis, Univ. Southern Mississippi, 1979). Experiments are being conducted utilizing this technique to determine the effects of BEN and DENA on early embryonic development.
4) Studies with a sheepshead (*Archosargus probatocephalus*) cell line have established the following levels of acute toxicity: BaP 2.0 µgm per ml media, BEN 0.2 mgm per ml media, and DENA 2.0 mgm per ml media. Subacute toxicity is evidenced by altered cell morphology and reduced growth rate. Vacuolization is a characteristic stress response for SHF-1 and BaP elicits this response at lowest concentrations. After a number of subcultivations, SHF-1 cells exposed BaP in the range of 100 ngm to 20 ngm per ml media developed multilayered foci. These foci of cells that apparently lack contact inhibition are about 1 mm in diameter. Multilayered foci have also been observed in one instance of exposure to BEN. The foci do not appear in controls.

5) Classic acute toxicity studies have established that the LC-50 of BEN for *Ictalurus* is 50 ppm. Studies are underway to make this same determination for BEN with respect to *Cyprinodon*.

6) A thorough histologic study of the G.I. tract of *Cyprinodon* has been completed and is being prepared for publication. A similar study of the blood cell morphology of *Cyprinodon* is nearing completion.

7) Preliminary studies indicate a striking elevation in leukocyte (mostly eosinophil-like cells) counts in *Cyprinodon* exposed to DENA. When *Cyprinodon* are injected with foreign RBC's and later their splenic blood is exposed to the antigenic RBC's, immune rosettes can be observed. Efforts are now underway to use this technique to evaluate the immunocompetence of exposed and unexposed *Cyprinodon*. 

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1. Methodology

We have nearly completed development of a method that has a high degree of precision for measuring polynuclear aromatic hydrocarbons (PNAH) in environmental samples. This method is considered by analytical chemists to constitute the state-of-the-art for analyzing environmental levels of PNAH in shellfish tissues and sediments. We intend to publish this method in Analytical Chemistry.

Eighteen PNAH, including 12 EPA priority pollutants (*) can be quantified using reverse phase high pressure liquid chromatography (HPLC) with UV and fluorescent detectors; the PNAH include fluorene*, phenanthrene*, fluoranthene*, pyrene*, benzo(c)phenanthrene, triphenylene, benzo(a)anthracene, chrysene*, (benzo(j)fluoranthene-benzo(e)pyrene), benzo(b)fluoranthene*, benzo(k)fluoranthene* dibenz(a,c)anthracene, benzo(a)pyrene*, dibenz(a,h)anthracene*, benzo(g,h,i)-perylene*, ideno(1,2,3-c,d)pyrene* (Figure 1).

2. Sampling and Determination of PNAH Body Burdens

Beginning in October, 1978, bivalve mollusks have been sampled bimonthly from three Oregon bays. These include Coos Bay (Mya arenaria and Tresus capax), Yaquina Bay (Mytilus edulis, M. arenaria and Crassostrea gigas) and Tillamook Bay (M. edulis, M. arenaria and C. gigas). Table 1 contains data on PNAH concentrations in samples that have been analyzed to date. All samples will be analyzed by September, 1980.

Tissues from clams (Coos Bay) and mussels (Yaquina Bay) have also
been prepared for histological examination to detect the presence of cellular proliferative disorders. Those slides will be examined during the summer (1980).

3. Trace Metal Analysis

During 1979, trace metals were measured in mussels using atomic absorption spectrophotometry (AA) and neutron activation analysis (NAA). The purposes of the study were to determine: if there were correlations between quantities of trace metals and concentrations of PNAH; and if NAA and AA could be used to supplement HPLC data for identifying or "fingerprinting" point sources of PNAH. The data obtained was not sufficient to identify any correlations. However, it was determined that there were correlations between metal and PNAH body burdens. Recently, these studies have been extended to measure levels of trace metals as a function of season and degree of metabolic activity (Table 2). The purpose of this study is to identify those factors that may influence uptake and retention of various contaminants. Finally, we became interested in measuring two inorganic carcinogens, nickel and arsenic and determined that AA is suitable for Ni while modified NAA methods can be used for As. We will conduct preliminary studies on As levels in shellfish this summer.

4. Other studies

The following studies are in progress or will be initiated this spring: determining the existence and prevalence of cellular proliferative disorders in bivalve mollusks; Pollicipes polymerus preliminary bioassay studies; and measurement of PNAH levels in other marine organisms.
Figure 1

METHOC 5

INJECT 15 41:23 1

Fluorine
Phenanthrene

Fluoranthene
Pyrene

Benzoc[6] Phenanthrene

Tr[phenylene]

Benzoc[7] Anthracene

Benzo[6] Pyrene

Benzoc[6] Anthracene

Dibenzo[6:7] Anthracene

Benzoc[9h] Heptalene

Indeno[1,2,3:cd] Pyrene

Coronene
ns of PNAH in Mussels (*Mytilus edulis*) and Oysters (*Crassostrea gigas*) from Yaquina Bay, Oregon.

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Table 2. Concentrations of trace metals in soft tissues of *M. edulis* in Yaquina Bay at Station Y-1. Values are in µg/g.

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In compliance with the special conditions required in the Cooperative Agreement Act of 1977, the following is a quarterly progress report for the above project, CR-807016-01-1.

Methods and Materials

Three compounds (aoclor 1260, toxaphene, benzo(a)pyrene), each at 2 dose levels, were fed to groups of 80 rainbow trout for 7-9 weeks to determine maximum tolerated doses and effects on selected liver mixed function oxidase (MFO) parameters. Protein levels, cytochrome P-450 content, benzo(a)pyrene monooxygenase activity, ethoxyresorufin-O-deethylase (EROD) and ethoxycoumarin-O-deethylase (ECOD) activities were determined at 1, 3, 5, 6, 7 and 9 week intervals and compared to levels in control fish fed our standard basal diet. The groups of fish were started on the following staggered schedule to facilitate sampling: benzo(a)pyrene 2-13-80; aoclor and toxaphene 2-20-80. Dietary levels of 500 and 1,000 ppm were used for each compound. However, partway through the short-term feeding trials both dose levels of toxaphene were found to be toxic for rainbow trout. Affected fish became anorexic and hyperactive with a few mortalities occurring. On 3-7-80 two new groups of fish were started on diets containing toxaphene at lower levels of 250 and 125 ppm, respectively.

Maximum tolerated doses of each compound determined from the short-term feeding trials were used for initiating a long-term diet study. On 4-7-80, 6 duplicate lots (12 total) of 100 rainbow trout fingerlings were placed in perforated buckets (2 per tank) and started on separate diets each containing one of the three compounds at the following dose levels: benzo(a)pyrene -
1,000 ppm; aroclor 1260 - 500 ppm; toxaphene - 100 ppm. Control fish received our standard basal diet. These diets will be fed for 18 months to determine if there is a carcinogenic response to any of the three compounds. Samples of 20 fish will be removed from each tank at 6 (10-6-80) and 12 (4-6-81) months for histopathology and bioaccumulation assays. The remaining 60 fish will be killed at 18 months (10-5-81). Multiple tissue samples will be taken from all fish. Other data will include diet consumption, fish weight gain and liver weight to body weight ratios.

Two additional routes of exposure to benzo(a)pyrene will be used to determine its carcinogenicity in rainbow trout. Fifty gram rainbow trout will be injected subcutaneously with benzo(a)pyrene, dissolved in propylene glycol and observed for 12 months; fertile rainbow trout embryos will be exposed to a solution of benzo(a)pyrene and held for 12-18 months.

Results

Rainbow trout tolerated the higher dose levels of aroclor and benzo(a)pyrene during 7 weeks of feeding. However, the lower dose level of aroclor 1260 was used for the long-term feeding trial due to the following criteria:

1) Experiments in rats have shown that lower doses of 200-300 ppm are sufficient carcinogenic levels

2) Slower weight gain in our fish on the higher dose suggested that a lower dose may be more tolerable during an 18 month feeding trial

3) Other research at our laboratory will involve the use of additional aroclor compounds in diets at levels of 500 ppm. The use of a 500 ppm level of aroclor 1260 would facilitate comparison with these other results.

Both the adjusted lower dose levels of toxaphene were tolerated for at least 31 days although fish did not feed as vigorously as control fish and
body weights remained stationary. Therefore, a still lower dose level of 100 ppm was chosen for the long-term feeding trial of toxaphene.

Results of the liver MFO determinations from trout on the short term feeding trials are presented in Table 1. Due to the breakdown of a fluorimeter used in both deethylase assays, no 3 week samples were taken of trout fed the benzo(a)pyrene and control diets. Early in the experiment a basic modification in the preparation of liver microsomes used in the 4 assays invalidated comparison of the 1st week samples of benzo(a)pyrene and control fish with subsequent samples. Despite these difficulties sufficient data was obtained for a valid analysis. Data in Table 1 indicate the following:

1) All 4 liver MFO parameters were higher in fish fed diets containing the 3 compounds than in fish on the control diet
2) The levels of all MFO parameters appeared to be dose responsive except in fish fed the toxaphene diet where levels remained similar regardless of dose.

Conclusions

All three compounds appeared to be inducers of the 4 liver MFO parameters that were examined. Increased induction appeared early (1-3 weeks) in the experiment with benzo(a)pyrene being more potent as an inducer than aroclor 1260. Because toxaphene dose levels were considerably lower, its induction potential could not be compared to the other two compounds. However, at the dose levels used, MFO induction in fish fed toxaphene was considerably lower than in fish receiving aroclor 1260 or benzo(a)pyrene. Induction of drug metabolizing enzymes by some compounds has been recognized as an early step towards carcinogenesis. The outcome of our long-term feeding trials will determine if such a correlation can be made with aroclor 1260, benzo(a)pyrene or toxaphene.
Table 1. Mean mixed function oxidase values in livers of rainbow trout fed diets containing benzo[a]pyrene, arochlor 1260 and toxaphene.*

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<th>DATE</th>
<th>WEEK</th>
<th>EROD nM/mg/min**</th>
<th>ECOD nM/mg/min</th>
<th>AHH nM/mg/min</th>
<th>P-450 nM/mg</th>
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<tr>
<td>2-20-80</td>
<td>1</td>
<td>2.007 ±0.361</td>
<td>0.2575±0.0219</td>
<td>0.3744±0.0110</td>
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<tr>
<td>3-05-80</td>
<td>3</td>
<td>1.5310±0.5766</td>
<td>0.2431±0.0744</td>
<td>0.2902±0.1496</td>
<td>0.5595±0.0632</td>
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<tr>
<td>3-19-80</td>
<td>5</td>
<td>0.7010±0.293</td>
<td>0.0767±0.0104</td>
<td>0.2872±0.0034</td>
<td>0.4037±0.0349</td>
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<tr>
<td>4-02-80</td>
<td>7</td>
<td>1.5310±0.5766</td>
<td>0.2431±0.0744</td>
<td>0.2902±0.1496</td>
<td>0.5595±0.0632</td>
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<tr>
<td>4-09-80</td>
<td>9</td>
<td>0.7010±0.293</td>
<td>0.0767±0.0104</td>
<td>0.2872±0.0034</td>
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<td>1000 ppm BENZ[A]PYRENE</td>
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<td>7.752 ±5.179</td>
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<td>0.0412±0.008</td>
<td>0.0106±0.0149</td>
<td>0.4367±0.015</td>
</tr>
<tr>
<td>4-02-80</td>
<td>7</td>
<td>0.0199±0.0028</td>
<td>0.0249±0.003</td>
<td>0.0096±0.0029</td>
<td>0.3640±0.0286</td>
</tr>
<tr>
<td>4-16-80</td>
<td>9</td>
<td>0.0456±0.0031</td>
<td>0.0384±0.0145</td>
<td>0.0141±0.0057</td>
<td>0.4693±0.0082</td>
</tr>
</tbody>
</table>

* ± = standard deviation
** nano moles per mg of protein per minute
The oxidative metabolism of polycyclic aromatic hydrocarbons (PAH) results in products of high cellular toxicity or mutagenicity. The types of metabolites identified in several other laboratories include phenols, dihydrodiols and quinones. In addition, several secondary metabolites of PAH compounds have been identified; these include glucuronide, sulfate sulfhydryl and DNA conjugates. The primary purpose of this project is to identify the types of oxidation products produced from selected PAH compounds in certain fish and shellfish. The extent and types of conjugation products are under study and the enzyme systems involved in the conjugation process are under study to ascertain the effects of exposure of the organisms to PAH upon their metabolism. The results of this investigation indicate that the glucuronosyltransferase and sulfotransferase enzymes are induced by exposure of rats to PAH compounds by feeding or injecting phenobarbital, 3-methylcholanthrene, phenanthrene and benzo(a)pyrene. Exposure time required for induction was between 8 and 12 days; requirements for the induction of the enzymes involved in the conjugation process suggested that metabolites (possibly oxidation products of the PAH compounds) may be the actual inducers of the enzymes catalyzing the conjugation reactions. Phenobarbital, 3-methylcholanthrene, phenanthrene and benzo(a)pyrene were inducers of the transferases and are listed in the order of most effective to least effective as inducer.

The enzyme systems, UDP-glucuronosyl transferase, sulfate transferase, UDPG-dehydrogenase, β-glucuronidase and arylsulfatase were each studied.
in tissues of rat, mullet, oyster, clam and mussel. Attempts to demonstrate induction of the transferases in the marine animals involved mandatory limitations to exposure time and dosage. Generally UDPG-dehydrogenase, arylsulfatase and 3-glucuronidase were not observed to be induced by PAH compounds. In marine animals, unequivocal induction of the transferases has not been demonstrated. Modifications in exposure time and dosage in channel catfish and Fundulus are currently in progress to enhance the conditions favorable to induction. Additional studies are in progress to develop conditions for the maintenance of cells from several species in order to expose the cells directly to the PAH compounds and their metabolites. Liver cells from rat livers and channel catfish livers have been successfully maintained for periods sufficient to effect exposure and observe effects.

3-Methylcholanthrene administered to mullet as a single dose or fed to rats has a profound effect upon the mass and gross appearance of the liver. The mass expressed as g of liver/100g of body of animal, both rat and mullet is significantly greater in treated animals than in their respective controls.

Oxidation products of phenanthrene and benzo(a)pyrene produced by incubation of the compounds with microsomes of rat liver and microsomes of channel catfish liver, respectively form conjugates with $^{35}$SO$_4$ that can be demonstrated by separation on thin layer chromatograms. Arylsulfatase liberates the radioactive sulfate from the bound, mobile radioactive fraction recovered from the thin layer chromatograms. Indications are that hydroxyaryl metabolites of phenanthrene and benzo(a)pyrene are conjugated with sulfate by the enzyme of the cytosol or rat liver.

Work planned for the future includes the identification of the compounds that conjugate with sulfate and glucuronic acid. Improvements in regulation of treatment time and dose is essential to future studies with marine animals.
Feeding of the compounds or repeated injections will be required to simulate the conditions found to be the most effective for induction of the transferase enzymes in rats.
In the first year of this project we investigated the induction of the mixed function oxidase enzyme system in gulf mullet (Mugil cephalus) and the Killifish (Fundulus grandis). Aroclor 1254 and 3-methylcholanthrene were used as the inducing agents and NADPH-cytochrome P450 reductase and cytochrome P450 were measured in the fish livers. No significant changes were observed. In in vitro studies, using microsome preparations from the mullet and killifish it was found that benzo(a)pyrene (B(a)P) was oxidized to a variety of metabolites comparable to those found when rat microsomes are used. The microsomes from the livers of induced fish gave positive results in the Ames Salmonella test for mutagenicity. This work was reported at the "4th International Symposium on Polynuclear Aromatic Hydrocarbons" held at Ohio State University in 1979.

We reported on the metabolite patterns of B(a)P in mullet liver microsome preparations compared to the rat at the ASTM 4th Symposium on Aquatic Toxicology held in Chicago, October 16, 17, 1979. The total metabolites of B(a)P in the mullet were formed at about one fifth the amount as the rat under the in vitro conditions we used. We were able to detect increases in the 9,10-diol B(a)P, 4,5-diol-B(a)P, 7,8-diol-B(a)P, quinones, 9-OH-B(a)P and 3-OH-B(a)P.

More recently we have initiated studies with the Tilapia aurea. The inducing agents were trans-stilbene oxide (TSO), Aroclor 1254, β-napthoflavone (BNF) and 3-methylcholanthrene (3-MC). Cytochrome P450 and b5, NADPH-cytochrome P450 and NADH cytochrome b5 reductases, glutamate-pyruvate
and glutamate-oxaloacetate transaminases, N-demethylase and cholinesterase were assayed in control and induced fish. Enzyme induction decreases in the following order: Aroclor 1254 (200 mg/kg) > TSO (200 mg/kg), BNF (40 mg/kg) > 3 MC (20 mg/kg). Male fish had greater induction capacity than the female fish. The results of these experiments have been submitted to Biochemical Pharmacology and Toxicology.

We have developed a new analytical procedure to assay for epoxide hydrase by determining 1,2-diols using lead periodate and atomic absorption spectrophotometry. Epoxide hydrase is one of the enzymes of the mixed function oxidase system. This work has been reported in Analytical Chemistry 52, 602, 1980.

An extensive study of the kinetic product patterns and mechanistic pathways of B(a)P metabolism in Aroclor-treated mullet has been made and a manuscript has been submitted to Biochemical Journal. The aryl hydrocarbon hydroxylase activity has been found to be constant for all metabolites studied except 3-OH-B(a)P and B(a)P-9, 10-diol which increased non-linearly to 22-fold and 17 fold at higher concentration, respectively. Incubation temperature greatly altered metabolite patterns; phenols and diols required longer reaction times at lower temperature to obtain optimum activity, quinones changed from a steady state at 25°C to optimised kinetics at 37°C, triols and tetrols were relatively unchanged with temperature. A steady state intermediate radical, 6-oxobenzo(a)pyrene was postulated for mediating the enzymatic formation of the quinones as has been previously suggested by Lesko et al (1975).
This analytical study was undertaken as part of the Pathobiology Section of the EPA/NCI Collaborative Carcinogens in the Aquatic Environment Program. The primary objective of the study is to obtain quantitative measurements of specific organic pollutants with carcinogenic potential in sediment and biota samples collected in three Gulf Coast bays and in an offshore comparison site. As originally conceived, the program consisted of two phases. In Phase I, replicate analyses of eight specific organic carcinogens would be carried out in sediment, oyster tissue, and fish tissue. These data would then be used to estimate the number of samples required to achieve the desired analytical precision in Phase II of the program. A qualitative screening of the three matrices collected at each bay and the offshore site for 112 of the EPA organic unambiguous priority pollutants, and any other potentially carcinogenic organic pollutant detected, would also be conducted in Phase I. Phase II of the program would be devoted to monitoring the carcinogenic compounds detected during Phase I in samples collected periodically throughout a one year period.

The results of our initial analyses indicated that the samples were too complex to analyze for the eight specific carcinogens by gas chromatography without the use of specific detectors. This restriction dictated that the emphasis of Phase I be shifted away from replicate analyses of the eight selected carcinogens, towards the screening of the samples for potential organic carcinogens. The screening, which was to be done by combined gas chromatography
and mass spectrometry (GC-MS), was upgraded from a qualitative analysis to a qualitative/semi-quantitative analysis. The screening exercise was constructed around but not limited to analysis of the organic priority pollutants. That is, volatile (purgable), base-neutral extractable, acid extractable, and pesticide fractions were collected from representative samples of each of the three matrices from the three bays and the offshore comparison site. Quantitative analytical procedures were available for pesticides in sediments and in tissues, but not for the other (semi-volatile) fractions. This necessitated a period of development prior to the beginning of analyses of the volatile base-neutral extractable, and acid extractable organics.

The pesticide analyses, which consisted of qualitative and quantitative GC analyses, are in their final confirmation stage. Thirty-three pesticide fractions from eleven representative samples have been analyzed by GC-EC (electron capture detection). Polychlorinated biphenyls (PCB) were not detected in any of the samples analyzed. Of the 28 priority pollutant pesticides and PCB's searched for, B-BHC, heptachlor, DDE, and trifluralin (not a priority pollutant) were identified by GC-EC in the samples treated. Several unidentified halogenated compounds were also detected and will be analyzed by GC-MS during the confirmation phase of the pesticide analyses.

Two computerized mass spectral search procedures were sequenced on the GC-MS data system in such a manner that specific priority pollutant organics were identified and quantitated and then all peaks above threshold were searched against the NIH-EPA mass spectral library and quantitated, if successfully identified. These two search routines were used in the analysis of the volatile, the base-neutral extractable, and the acid extractable organic fractions. The total processing time for a complete detailed mass spectral search using both search procedures ranged from 6 to 12 hours and yielded data on from 50 to 200
or more peaks per run.

Because of the problems with emulsions, it was necessary to use two separate aliquots of each sample for extraction of the base-neutral and acid organics. To improve recovery, acid-neutral rather than just acid extractable organics were extracted from the sediment samples. Neutral compounds of biogenic origin such as fatty acid methyl esters, normal alkanes, and fatty acid ethyl esters dominated the analyses of the base-neutral and the acid-neutral extracts. The acid extractable fractions contained free fatty acids as well as fatty acid methyl esters (probably due to overloading the system) of biogenic origin. The sediment extracts contained low molecular weight hydrocarbons, probably of petroleum origin, as well as several polynuclear aromatic hydrocarbons apparently derived from pyrolytic sources. Without counting extraction, fractionation, or actual instrument analytical time, approximately 150 hours of data treatment time have been consumed on these analyses.

Volatile organics from sediments, oyster tissue, and fish tissue were analyzed using a dynamic headspace technique which involved a total purging, trapping and drying time of six hours per sample. These data will be processed using the sequenced mass spectral search procedures described above.

Upon completion of the screening portion of this program, specific organic carcinogenic pollutants will be selected for analysis during Phase II. The carcinogens selected for quantification will be analyzed in replicate samples collected quarterly from the four bays and the offshore comparison site.
XII. PUBLICATIONS RESULTING FROM PROGRAM

Bunting, D.B. 1979. An Evaluation of Benzo(a)pyrene Metabolism in an Oyster (Ostrea edulis)-Bacteria System. M.S.


Hemingway, S.J. 1979. (1) Storage Sites of Benzo(a)pyrene in contaminated Bay Mussels (Mytilus edulis) inhabiting different sites in Yaquina Bay, Oregon. (2) Uptake and depuration of Benzo(a)pyrene in contaminated Bay Mussels (Mytilus edulis) inhabiting different sites on Yaquina Bay. M.S.


Hendricks, J.D., R.O. Sinnhuber, M. Henderson, and D.R. Buhler. 1980. Liver and kidney pathology in rainbow trout (Salmo gairdneri) exposed to dietary pyrrolizidine (Senecio) alkaloids. (to be submitted to Am. J. Pathol.)


Mix, M.C. and W.P. Breese. A cellular proliferative disorder in *Ostrea chilensis* from Chiloe, Chile, South America. Submitted to Journal of Invertebrate Pathology, In Press.

Mix, M.C. Cellular proliferative disorders in bivalve mollusks from contaminated marine environments. To be submitted to Journal of Environmental Pathology and Toxicology.


Riley, R.T. and M.C. Mix. An ion-exchange technique for the determination of ammonia in small volumes of seawater. Submitted to Marine Chemistry.


Riley, R.T. and M.C. Mix. The pathways of glucose metabolism in the oyster, Ostrea edulis. To be submitted to Comparative Biochemistry and Physiology.


Schaffer, R.L. and M.C. Mix. A method for determining environmental levels of polycyclic aromatic hydrocarbons in bivalve mollusks by reverse phase liquid chromatography. To be submitted to Analytical Chemistry.


Tan, B. Responses of hepatic enzymes of tilapia to biphenyls and polyaromatic hydrocarbons. Submitted to Biochem. Pharmacology.


Tan, B. Comparative metabolism of polyaromatic hydrocarbons in marine organisms. Invited review article being prepared for Journal of Comparative Biochemistry and Physiology.


Tan, B., M.V. Kilgore, D. Elam and W.P. Schoor. Metabolites of benzo(a)pyrene in Aroclor 1254 treated mullet. 4th Symp. on Aquatic Toxicology, ASTM (In press).


Tan, B. 1980. Benzo(a)pyrene Metabolism in PAH and Biphenyl treated Tilapia. To be presented Oct. 28-30, 5th Intl. Symp on PAHs, Columbus, Ohio.


Discussion

Dr. Cooper, NCI: In one of your early slides you showed us a very nice schematic of the equipment you use to expose marine organisms to environmental hazards, and at the bottom of that slide was a little arrow that said, "Effluent line." What is on the other end?

Dr. Couch, EPA: That question invariably comes up. Anyway, we have an effluent pond next to the laboratory. It is an evaporation pond. It receives the effluent from our test system. So, effluent does not get out into the natural system bay, we monitor this periodically, and we have wells around the pond to test for seepage. We use low concentrations (micrograms/liter) and in our test system that I showed you, there is a very low flow rate. The fish we selected are good because they can live in a very low flow-through of water; the turnover of the water and the concentration is very small in that regard. If you used larger fish, you would have to use a greater flow rate.

Dr. Morris, EPA: Yes.

Dr. Baumel, NIOSH: I am not that familiar with the dynamics and all the pharmacokinetics of the metabolism of aquatic organisms. It seems to me you have an interesting problem of re-uptake of excreted metabolites. You have somewhat a potential for some steady state or diffusion dynamics that go on that don't normally happen, say, in a normal environmental system with airborne concentrations.

Have you looked at any of this? Have you looked at the formation of metabolites or re-uptake possibilities as factors affecting the outcome of your experiment?

Dr. Couch, EPA: In a flow-through system, when we expose the animals, much of the excreted metabolites would be removed by the flowing water turning in the tanks. There may be some re-uptake of excreted metabolites by the test animals. We have done no work on this specifically yet.

We have looked at the disappearance and persistence of the parent compound in the water column as I showed you. Some of the disappearance is due to adsorption to the glass of the aquarium or the system itself. For example, if we put oysters in the system and expose them to benzopyrene, a certain amount of the finite concentration that we put in will be taken up by the oyster, and an equal amount or an even larger amount may be taken up, adsorbed by the glass of the system. So, we do have adsorption and desorption as a problem. Dr. Schoor has done some solubility studies and fate studies in these systems to try to keep track of the compound, but we have not addressed what you mentioned specifically in terms of metabolites being taken up again. Dr. Schoor can talk with you about that in more detail and tell you what his opinion is on that.

Dr. Hegyeli, NCI: Bivalves are excellent bioconcentrators, but at the same time they flush out the toxic materials or corpuscular elements very easily. So, my question is whether this was considered when you were collecting the samples and keeping them in flowing fresh water, and the other idea is about the resistance of bivalves to carcinogenic substances.
Dr. Couch, EPA: That is a very complex set of questions. We do know that oysters, for example, which we are very familiar with in our work, will accumulate two different toxicants or carcinogens at different rates, depending on the kind of chemical to which you are exposing them. For example, with some of the pesticides such as DDT and some of the PCBs in earlier work at our laboratory, we found that oysters would concentrate up to several hundred thousands times ambient, but when we exposed oysters to benzopyrene at environmentally realistic levels of four parts per billion (benzopyrene in a flow-through system), we found that oysters only accumulated 200 to 240 times ambient. So, it depends on the kind of chemical to which you are exposing them. The natural cellular and functional excretory mechanisms, secretory mechanisms of the animals involved, in bivalves, oysters for example, involve a phenomenon that vertebrates do not have. If they take up macromolecules or molecular substances or even larger than macromolecular substances into their system, their leukocytes may pinocytose or phagocytize these substances and actually crawl across the epithelial layers, throw themselves out into the mantle cavity, and rid themselves of large quantities of these substances. Therefore, uptake and retention of different substances by bivalves depends entirely upon the substance and the bivalve involved.

In answer to the second part of your question, the bivalves have not proven to be as good an assay organism for carcinogenesis in the laboratory as have fish. So, we are concentrating more on fish for many different reasons. There are several interesting neoplastic diseases of bivalve mollusks that occur in nature, including both carcinoma-like disorders and sarcoma-like disorders. We have read a report from Russia recently that Kudolay has been able to induce a leukemia condition in freshwater mussels with chemical carcinogens. So far this has not been substantiated in this country; in our work with benzopyrene, we were able to induce various cellular inflammatory reactions in oysters, but we have not successfully induced a tumor in oysters, but we have only really started in that area.

Dr. Morris, EPA: I was intrigued by your model earlier where you showed the relationships of man and the rat and fish and showing these overlaps. This is an interesting concept because I think as we collect, and certainly in our agency, human health effects data, ecotox data, at some point in time we may want to put this together and ask questions about these inter-relationships. I think this kind of approach is very useful to that way of thinking.

Dr. Couch, EPA: One word of caution. I think that with any phylogenetic or comparative approach you have to be familiar enough with the two organisms that you are comparing to know where the differences begin and the similarities end.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCUPATIONAL CANCER STUDIES

Wednesday Morning, May 7

METHODOLOGY/EXPERIMENTAL MODELS SESSION (CONTINUED)

SESSION CHAIRPERSON

Dr. John Cooper*
National Cancer Institute

* Dr. Cooper substituted for Dr. Umberto Saffiotti, NCI
METABOLISM OF AZO DYES TO CARCINOGENIC AMINES

Larry K. Lowry, Ph.D.
William P. Tolos, B.S.

Division of Biomedical and Behavioral Science
National Institute for Occupational Safety and Health

Mark Boeniger, M.S.
Division of Surveillance,
Hazard Evaluations, and Field Studies
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and

Charles Nony, B.S.
Malcolm Bowman, B.S.

National Center for Toxicological Research
Jefferson, Arkansas  72079

FDA 224-78-0004
The metabolism of the purified benzidine-based azo dye Direct Black 38 (DB-38) and the 3-3'dichlorobenzidine-based Pigment Yellow 12 (PY-12) was studied in the hamster. A single oral dose of DB-38 containing 3.0 ppm benzidine (Bzd), 6.0 ppm 4-aminobiphenyl (4-ABP) and 670 ppm of 2,4-diaminoazobenzene (DiAmAzBz) was administered at 100 mg/kg to 18 male Syrian golden hamsters. Urine specimens collected over a period of 8 days and analyzed by electron capture-gas chromatography and high pressure liquid chromatography showed significant total amounts of Bzd (10 µg), monoacetylbenzidine (MoAcBzd, 535 µg), diacetylbenzidine (DiAcBzd, 28 µg) and 4-ABP (11 µg). Levels of metabolites peaked at 8-16 hours with MoAcBzd, the major metabolite, still quantitated after 7 days. In addition, alkaline hydrolyzable conjugates of Bzd (328 µg) and 4-ABP (613 µg) were found. The level of excreted metabolites far exceeded the levels of Bzd and 4-ABP present as impurities in the dye and represent metabolic breakdown of the dye. Mutagenic potential of DB-38 and the major metabolites evaluated with the Ames Salmonella test indicated: MoAcBzd and DiAcBzd - strong (TA 1538); 4-ABP - moderate (TA 98 and 100); DB-38, Bzd, DiAmAzBz - weak (TA 100); hamster urine, 8-16 hours containing 26 µg MoAcBzd - moderate (TA 1538). No compounds were mutagenic in the absence of S-9 fraction.

In contrast to DB-38, studies with PY-12 (100 mg/kg) did not produce any detectable levels of the hypothetical metabolites indicating either no metabolism or little absorption of the pigment.
ABBREVIATIONS

DB-38, Direct Black 38; PY-12, Pigment Yellow 12; Bzd, benzidine; 4-ABP, 4-aminobiphenyl; DiAmAzBz, 2,4-diaminoazobenzene; MoAcBzd, monoacetylbenzidine; DiAcBzd, diacetylbenzidine; EC-GC, electron-capture gas chromatography; HFB, heptafluorobutyrl; HPLC, high pressure liquid chromatography.

ACKNOWLEDGMENT:

This effort was supported by an interagency agreement with the National Cancer Institute.
INTRODUCTION

Epidemiological investigations of workers in the dye industry have shown an increase in bladder tumors over that expected in age-adjusted cohort populations (1,2). The benzidine-based dyes represent one class of dyes and include many of those most commonly used.

Benzidine (Bzd), a structural component of these dyes, is also an impurity and is a known human bladder carcinogen (3). The hypothesis that the dyes may be metabolized back to Bzd prompted studies on the carcinogenicity and metabolism of these dyes.

Studies sponsored by the National Cancer Institute have shown that Direct Black 38, Direct Brown 95, and Direct Blue 6 produced liver tumors in rats as early as five weeks after continuous dosing (4). Urine specimens contained Bzd in the parts per billion (ppb) range. Okajima, et al., found that Direct Black 38 (DB-38) produced bladder, liver, and colon tumors in 46% of male rats given 500 parts per million (ppm) of the dye in drinking water for 60 weeks (5). Bzd was not detected in urine specimens.

The metabolism of DB-38, Direct Brown 95, Direct Blue 6, and Direct Red 28 was studied by Rinde and Troll (6). When single oral doses of the dyes were given to Rhesus monkeys, Bzd and monoacetylbenzidine (MoAcBzd) were found in the urine. It is known that these commercial dyes contain many impurities, including Bzd.
The discovery that these dyes were carcinogenic in rats, together with the knowledge that they may be metabolized to Bzd in animals and humans, has lead to the recommendation by the National Institute for Occupational Safety and Health that workers no longer be subject to the adverse health effects of these dyes and that these dyes no longer be used (7,8).

Other concerns have been raised on the potential metabolic fate of the dichlorobenzidine-based pigment, Pigment Yellow 12 (PY-12) (9). Previous research on the metabolism and carcinogenesis of benzidine-based azo dyes has been done with commercial dyes containing unspecified impurities. The question of the origin of Bzd in the urine of animals dosed with these dyes has not been answered. This report describes a definitive metabolism study in the hamster of DB-38 containing defined levels of amine impurities. Specific methodologies were developed and used to identify and quantitate dye metabolites in urine. Selected metabolites and the dye were also evaluated for mutagenic potential. Preliminary metabolism studies were also done with PY-12.
METHODS AND MATERIALS

DB-38 was purchased from GAF Corporation, New York, N.Y. PY-12 was purchased from the Dry Color Manufacturers' Association, Nutley, N.J. Other chemicals were obtained from commercial sources or synthesized (9). Purification of the dye and pigment was conducted manually by exhaustive liquid-liquid extraction. Amine impurities were converted to their heptafluorobutytyl (HFB) derivatives and analyzed by electron-capture gas chromatography (EC-GC). Stability and recovery studies of the dye and potential metabolites in urine were conducted prior to the dosing of animals. Details of these procedures are reported by Nony and Bowman (10).

Potential metabolites of the dye, Bzd, and the pigment were synthesized and HFB derivatives prepared. Structures were confirmed by gas chromatography-mass spectrometry. Urine metabolites were extracted, derivatized and analyzed by EC-GC using a 5% Dexil 300 on Anakrom Q glass column and a Ni$^{63}$ detector. High pressure liquid chromatography (HPLC) was also used to analyze underivatized urine extracts using a reverse phase column ($\mu$ Bondapak C$_{18}$, Waters Associates, Milford, MA.) and an ultraviolet detector at 295 nm. Conjugated metabolites were first hydrolyzed with sodium hydroxide, then extracted and analyzed by EC-GC and HPLC. Details of the metabolite analysis are as reported by Nony and Bowman (11).

Male Syrian golden hamsters obtained from ARS Sprague-Dawley, Madison, WI., weighing between 104-128 g were housed three to a cage with a total of 18...
animals (6 cages) used with the dye. Control urines were collected for 24 hours prior to dosing. Urine samples were collected at intervals of 0-8, 8-16, 16-24, 24-32, 32-48, 48-72, and 144-168 hours after dosing. All collections were done in the presence of dry ice. Hamsters received a single oral dose of DB-38 in water at 100 mg/kg body weight.

In a limited experiment, three hamsters were dosed orally with 100 mg/kg of PY-12 in trioctanoin. Urines were collected as above. Details of the experimental protocol are reported by Nony, et al. (12).

Mutagenicity of urinary metabolites was evaluated using the Ames Salmonella test with and without mouse liver microsomal metabolic activation (9).

RESULTS

The Dyes

Figure 1 shows the structures of DB-38 and related substances with their abbreviations. DB-38 was analyzed upon receipt and was found to contain traces of Bzd and significant amounts of 4-aminobiphenyl (4-ABP) and diaminoazobenzene (DiAmAzBz), both known carcinogens. The latter two compounds have not been previously reported as contaminants of azo dyes. Table 1 shows the levels of impurities before purification and immediately before use. Note that the levels of non-benzidine impurities were significantly reduced while the level of Bzd actually increased.
Figure 2 shows the structures of PY-12 and related substances along with their abbreviations. The pigment was analyzed and contained 89 ppm of 3,3'-dichlorobenzidine. After purification, the level of dichlorobenzidine was 0.3 ppm. No other amine impurities were detected.

Table I

<table>
<thead>
<tr>
<th>Impurity, ppm</th>
<th>Benzidine</th>
<th>4-Aminobiphenyl</th>
<th>Diaminoazobenzene</th>
</tr>
</thead>
<tbody>
<tr>
<td>As Received</td>
<td>&lt;0.1</td>
<td>150</td>
<td>9,200</td>
</tr>
<tr>
<td>After Purification</td>
<td>3.0</td>
<td>6.0</td>
<td>670</td>
</tr>
</tbody>
</table>

Urinary Metabolites - Direct Black 38

Bzd (see Figure 1), monoaocetylbenzidine (MoAcBzd), diacetylbenzidine (DiAcBzd), and 4-ABP were positively identified in the urine of hamsters fed DB-38. These compounds were analyzed by both HPLC and EC-GC of HFB derivatives. Table II shows the results of HPLC analysis of urine from hamsters given DB-38. Bzd excretion peaked at 0-8 hours but fell to control levels by 48 hours. MoAcBzd also peaked at 0-8 hours, but was 100 times more concentrated than Bzd. Its excretion returned to control levels after 168 hours. DiAcBzd and 4-ABP peaked at 16 and 8 hours, respectively, before returning to control levels by 24 hours. No DiAmAzBz was detected.
Table II

HPLC Analysis of Major Metabolites in Urine of Hamsters Fed One Dose (100 mg/kg) of Direct Black 38

<table>
<thead>
<tr>
<th>Sampling Interval (hr)</th>
<th>Volume (ml) of Urine (x ± SD)b/</th>
<th>Total Amount Excreted (µg) of Indicated Metabolites (x ± SD)a/</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benzidine</td>
<td>Monoacetyl-Benzidine</td>
</tr>
<tr>
<td>Pretreatment (24 hr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-8</td>
<td>9.1 ± 5.4</td>
<td>0.093 ± 0.014</td>
</tr>
<tr>
<td>8-16</td>
<td>2.3 ± 0.1</td>
<td>2.33 ± 2.16</td>
</tr>
<tr>
<td>16-24</td>
<td>3.7 ± 3.2</td>
<td>1.89 ± 2.04</td>
</tr>
<tr>
<td>24-32</td>
<td>2.7 ± 0.7</td>
<td>1.89 ± 2.19</td>
</tr>
<tr>
<td>32-48</td>
<td>1.9 ± 0.6</td>
<td>0.258 ± 0.000</td>
</tr>
<tr>
<td>48-72</td>
<td>6.2 ± 2.6</td>
<td>0.524 ± 0.000</td>
</tr>
<tr>
<td>144-168</td>
<td>12. ± 7.8</td>
<td>ND</td>
</tr>
<tr>
<td>144-168</td>
<td>8.5 ± 1.3</td>
<td>ND</td>
</tr>
</tbody>
</table>

a/ Mean and standard deviation from five cages of three hamsters each. Results were corrected for pretreatment sample background and recovery.

b/ Mean and standard deviation from five cages of three hamsters each.

ND None detected above background.
**Figure 3** shows an EC-GC chromatogram from urine collected 8-16 hours after dosing with DB-38. The chromatogram clearly shows 4-ABP (1.4 ppm), Bzd (1.7 ppm), and MoAcBzd (78.7 ppm). DiAcBzd and the alkaline hydrolyzable conjugates of Bzd and 4-ABP were analyzed following hydrolysis to free amine metabolites as previously described (9).

**Table III** shows the composite results of an EC-GC analysis of hamster urine from dosed animals. The results are similar to those from HPLC analysis but, because of higher sensitivities, levels of metabolites have been detected at longer intervals after dosing. **Table IV** shows the results for alkaline hydrolyzable conjugates analyzed by EC-GC after pretreatment. It can be seen that Bzd conjugates are present at about 30 times the level of free Bzd but that 4-ABP conjugates are present at about the same levels as the free metabolites. No DiAmAzBz was found in hamster urine by either HPLC or EC-GC.

**Urinary Metabolites - Pigment Yellow 12**

The expected metabolites of PY-12 shown in **Figure 2** were not detected in the urine of hamsters dosed with PY-12. The expected metabolites were chemically synthesized, spiked into control urines and analyzed at the ppb level by EC-GC. However, no traces of these expected metabolites were found in dosed hamster urine.
### Table III

**EC-GC Analysis of Major Metabolites in Urine of Hamsters Fed Direct Black 38**

<table>
<thead>
<tr>
<th>Sampling Interval (hr)</th>
<th>Volume (ml) of Urine ($\bar{x} \pm SD$)$^b/$</th>
<th>Benzidine</th>
<th>Monoacetyl-Benzidine</th>
<th>Diacetyl-Benzidine</th>
<th>4-Aminobiphenyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment (24 hr)</td>
<td>9.1 ± 5.4</td>
<td>0.151 ± 0.217</td>
<td>0.361 ± 0.103</td>
<td>0.087 ± 0.061</td>
<td>0.152 ± 0.155</td>
</tr>
<tr>
<td>0-8</td>
<td>2.3 ± 0.1</td>
<td>4.33 ± 2.39</td>
<td>216. ± 133.</td>
<td>10.7 ± 7.1</td>
<td>6.62 ± 1.72</td>
</tr>
<tr>
<td>8-16</td>
<td>3.7 ± 3.2</td>
<td>3.08 ± 2.44</td>
<td>208. ± 177.</td>
<td>10.3 ± 2.5</td>
<td>3.26 ± 3.02</td>
</tr>
<tr>
<td>16-24</td>
<td>2.7 ± 0.7</td>
<td>2.16 ± 2.33</td>
<td>95.8 ± 133.</td>
<td>5.69 ± 5.11</td>
<td>1.17 ± 1.52</td>
</tr>
<tr>
<td>24-32</td>
<td>1.9 ± 0.6</td>
<td>0.259 ± 0.205</td>
<td>5.59 ± 4.88</td>
<td>0.064 ± 0.063</td>
<td>0.162 ± 0.084</td>
</tr>
<tr>
<td>32-48</td>
<td>6.2 ± 2.6</td>
<td>0.470 ± 0.506</td>
<td>7.39 ± 7.82</td>
<td>0.223 ± 0.212</td>
<td>0.236 ± 0.078</td>
</tr>
<tr>
<td>48-72</td>
<td>12. ± 7.8</td>
<td>0.226 ± 0.184</td>
<td>1.56 ± 0.994</td>
<td>0.626 ± 0.000</td>
<td>ND</td>
</tr>
<tr>
<td>144-168</td>
<td>8.5 ± 1.3</td>
<td>ND</td>
<td>0.474 ± 0.393</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

$^a/$ Mean and standard deviation from five cages of three hamsters each. Results were corrected for pretreatment sample background and recovery.

$^b/$ Mean and standard deviation from five cages of three hamsters each.

ND None detected above background.
Table IV

EC-GC Analysis of Alkaline Hydrolyzable Conjugates in the Urine of Hamsters Fed Direct Black 38

<table>
<thead>
<tr>
<th>Sampling Interval (hr.)</th>
<th>Benzidine (µg)</th>
<th>4-Aminobiphenyl (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment (24 hrs.)</td>
<td>0.034</td>
<td>1.16</td>
</tr>
<tr>
<td>0-8</td>
<td>103.0</td>
<td>2.57</td>
</tr>
<tr>
<td>8-16</td>
<td>154.0</td>
<td>2.56</td>
</tr>
<tr>
<td>16-24</td>
<td>45.5</td>
<td>ND</td>
</tr>
<tr>
<td>24-32</td>
<td>5.59</td>
<td>ND</td>
</tr>
<tr>
<td>32-48</td>
<td>13.9</td>
<td>ND</td>
</tr>
<tr>
<td>48-72</td>
<td>6.41</td>
<td>ND</td>
</tr>
<tr>
<td>144-168</td>
<td>0.019</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND – None detected above background.

Mutagenicity Testing

The mutagenic potential of the identified metabolites of DB-38 is shown in Table V. The purified synthetic metabolites were assayed with and without S-9 fraction. None of the compounds tested were mutagenic without activation. All compounds showed some degree of mutagenic activity in the presence of S-9 fraction with at least one of the tester strains. The degree of mutagenicity based on the number of revertants per plate were: MoAcBzd and DiAcBzd - strong; 4-ABP - moderate; Bzd and DiAmAzBz - weak. Mutagenic evaluation of DB-38 hamster
urine collected at pretreatment, 0-8 and 8-16 hours after dosing, showed no mutagenic activity without activation but mutagenic activity up to 10 times background in the 0-8 hour urine with activation. These results are consistent with the level of MoAcBzd in the urine, the major metabolite.

Table V

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mutagenic Potential of Direct Black 38 and Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compound</td>
</tr>
<tr>
<td>Direct Black 38</td>
<td>TA 98</td>
</tr>
<tr>
<td></td>
<td>TA 100</td>
</tr>
<tr>
<td>Benzidine</td>
<td>TA 98</td>
</tr>
<tr>
<td></td>
<td>TA 100</td>
</tr>
<tr>
<td>Monoacetylbenzidine</td>
<td>TA 1538</td>
</tr>
<tr>
<td>Diacetylbenzidine</td>
<td>TA 1538</td>
</tr>
<tr>
<td>4-Aminobiphenyl</td>
<td>TA 98</td>
</tr>
<tr>
<td></td>
<td>TA 100</td>
</tr>
<tr>
<td>Diaminoazobenzene</td>
<td>TA 98</td>
</tr>
<tr>
<td></td>
<td>TA 100</td>
</tr>
</tbody>
</table>

- Negative
+ Weak (2 x background)
++ Moderate (5 - 10 x background)
+++ Strong (40 x background)

CONCLUSIONS

Purified DB-38, containing low defined levels of free Bzd as an impurity, was extensively metabolized to Bzd, the N-acetylbenzidine metabolites and to
unspecific Bzd conjugates in hamsters. About 8-10% of the Bzd contained in
the dye in azo linkage could be accounted for in the urine as Bzd or its
metabolites. In addition, 4-ABP was found in amounts much greater than the
level of impurity, along with its conjugates. These findings indicate that
considerable risk may exist for humans exposed to DB-38 since both Bzd and
4-ABP are well established bladder carcinogens. In addition, MoAcBzd, the
major metabolite, was found to be mutagenic with activation using the Ames
Salmonella test.

In contrast, PY-12 did not produce detectable levels of expected metabolites,
indicating either no metabolism or little absorption of the pigment.
REFERENCES


FIGURE LEGENDS

Figure 1 - Direct Black 38 and Related Compounds.

Figure 2 - Pigment Yellow 12 and Related Compounds.

Figure 3 - Electron-Capture Gas Chromatograms of Derivatized Extracts of Urine Collected From Hamsters 8-16 Hours After Treatment With Direct Black 38.
Diaminoazobenzene (DiAmAzBzl)  
Benzidine (Bzd)  
Diacetylbenzidine (DiAcBzd)  
Monoacetylbenzidine (MoAcBzd)  
4-Aminobiphenyl (4-ABP)  

Figure 1
C.I. Pigment Yellow 12

3,3'-Dichlorobenzidine

Monoacetyldichlorobenzidine

Diacetyldichlorobenzidine

Figure 2
URINE COLLECTED FROM HAMSTERS 8-16 HRS AFTER TREATMENT WITH DIRECT BLACK 38

220°C
5.0 µg-equiv/inj.

260°C
6.25 µg-equiv/inj.

280°C
2.5 µg-equiv/inj.

4-ABP (1.4 ppm)

Bzd (1.7 ppm)

Bzd (1.7 ppm)

MoAcBzd (78.7 ppm)

MoAcBzd (78.7 ppm)

RESPONSE (2x10^-9 AMP FULL SCALE)

MINUTES
Discussion

Dr. Jenkins, EPA: I would like to ask one question about the problems you would have in the HPLC analysis of benzidine and I believe you said that you converted that to the appropriate derivative. The reason for my asking would be what other types of compounds would interfere, come out at about the same peak, and what you are converting that to? I am interested in the congeners.

Dr. Lowry, NIOSH: The derivative you mentioned was a heptafluorobutyryl anhydride reaction with an aromatic amine to produce a fluoro-electron capture sensitive group that would be picked up with the electron capture detector of the gas chromatograph.

The HPLC analysis of benzidine was done without derivatization using a reverse phase C18 column. The congeners of benzidine are separated from benzidine under these conditions. More details of the methodology can be found in the paper written by C. R. Nony and M. C. Bowman published in the February issue of the Journal of Chromatographic Science (Vol. 18, pages 64-75, 1980).

Dr. Cooper, NCI: It was curious that on the benzidine results in the minus 24 to zero time period you showed excretion of benzidine and yet it fell later to undetectable levels. Where was that benzidine coming from?

Dr. Lowry, NIOSH: It is possible you could call that noise.

Dr. Hegyeli, NCI: Was inhalation considered in this case as most of the workers working with these types of chemicals were inhaling instead of ingesting them.

Dr. Lowry, NIOSH: Inhalation was not considered primarily for reasons of getting the work done with the amount of money that was available to support the work. Further work is being done on two congeners of the benzidine-based dyes, one toluidine-based dye, Direct Red 2 and one dianicidine-based dye, Direct Blue 15. NCTR is about to start some work using some radio labeled material to look more thoroughly at metabolism, absorption of the material, and tissue distribution.
THE EFFECT OF DIETARY DISULFIRAM UPON THE TISSUE DISTRIBUTION
AND EXCRETION OF $^{14}C$-1,2-DIBROMOETHANE IN THE RAT

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DONALD E. RICHARDS, AND KENNETH L. CHEEVER

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
U.S. Public Health Service, Center for Disease Control
National Institute for Occupational Safety and Health
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ABSTRACT

Dietary disulfiram enhances the toxicity of inhaled 1,2-dibromoethane in rats. This study was undertaken to determine whether the differential toxicity noted was associated with alterations in the levels of the compound and/or its metabolites in the target organs. A comparison of the levels of $^{14}C$ in selected tissues of male rats, with and without dietary disulfiram, following the oral administration of $^{14}C$-1,2-dibromoethane was made. The results indicated that levels of radioactivity in the target organs of animals in the disulfiram group were significantly elevated both at 24 and 48 hours following compound administration. The data indicate a direct correlation between tissue levels and the enhancement of toxicity noted in the disulfiram-treated rats in the inhalation study. A significant elevation in the levels of radioactivity in washed liver nuclei obtained from animals receiving dietary disulfiram was also noted, suggesting a relationship between nuclear uptake and the increased incidence of liver tumors appearing in the disulfiram group in the inhalation study.

INTRODUCTION

A study of the toxicity of 1,2-dibromoethane in rats, exposed at the current U.S. occupational standard of 20 ppm, with and without disulfiram in the diet, has recently been completed by the Midwest Research
Institute, Kansas City, Missouri (NIOSH, 1979). Preliminary results noted at the end of the eleventh month of the 18-month study were reported earlier (Plotnick, 1978). The final results of this study indicate that 1,2-dibromoethane, at an exposure level of 20 ppm under simulated occupational exposure conditions (7 hours/day, 5 days/week), is a carcinogen in both male and female Sprague-Dawley rats. A most interesting finding in this study was an enhancement of carcinogenic and other toxic effects in the disulfiram-treated rats. The combined exposure resulted in significant increases in tumors of the liver, spleen, kidney, and thyroid as well as atrophy of the testes when compared with those animals exposed to 1,2-dibromoethane alone. The most profound disulfiram-related effect was an increase in the incidence of hepatocellular carcinoma. The present study was undertaken to determine whether dietary disulfiram modifies the tissue distribution and excretion of orally-administered 1,2-dibromoethane, utilizing $^{14}$C-labeled material, in an attempt to explain the interaction noted. Special attention was given to the organs affected in the chronic inhalation study. In addition, liver nuclei were isolated for quantitation of radioactivity to determine whether the disulfiram diet was associated with a preferential distribution to this organelle.

MATERIALS AND METHODS

Animals. Male rats of the Sprague-Dawley strain, weighing 100-125 g, were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. For a one-week acclimation period, the animals were housed two per cage, under controlled environmental conditions, with a 12-hr light-dark schedule with the light cycle beginning at 7:00 a.m. Pelletized Rodent
Laboratory Chow (Ralston Purina Co., St. Louis, MO) and tap water were available ad libitum during this acclimation period.

**Chemicals.** Tetraethylthiuram disulfide (disulfiram) was obtained from the Sigma Chemical Co., St. Louis, MO. $[^{14}C]_{\text{U}}$1,2-dibromoethane was purchased from New England Nuclear, Boston, MA. Analysis of the radioisotope by the supplier prior to shipment showed this compound to have a radiochemical purity of 98% and a specific activity of 9.35 mCi/m mole. 1,2-Dibromoethane (98%) was obtained from MC/B Manufacturing Chemists, Norwood, OH.

**Treatment Solution.** The treatment solution was prepared by mixing the $[^{14}C]_{\text{U}}$1,2-dibromoethane with sufficient "unlabeled" 1,2-dibromoethane in corn oil (MC/B Manufacturing Chemists, Norwood, OH) to yield a concentration of 3.75 mg 1,2-dibromoethane/g of treatment solution with a specific activity of 6800 dpm/µg of 1,2-dibromoethane.

**Animal Treatment.** Upon initiation of the experiment, 24 rats were weighed, randomly assigned to two experimental groups of 12 animals each, and placed in individual stainless-steel metabolism cages designed for the separate collection of urine and feces. The rats in one group received ground Rodent Laboratory Chow containing 0.05% disulfiram, while those in the second group received plain ground Rodent Laboratory Chow, as a control diet. All animals had unlimited access to tap water and their respective diets. After 12 days on these diets, the rats were fasted overnight and subsequently given single 4-g/kg (15 mg 1,2-dibromoethane/kg) doses of the treatment solution by oral intubation. The rats were returned to their metabolism cages, and urine and feces were collected from each rat at 24-hour intervals from
the time of treatment with 1,2-dibromoethane until the animal was killed. Six rats from each experimental group were killed at 24 and 48 hours after administration of the treatment solution by exsanguination by cardiac puncture with a heparinized syringe following anesthetization by 100-mg/kg intraperitoneal injections of sodium pentobarbital (Nembutal Sodium, Abbott Laboratories, North Chicago, IL). In addition to blood, the liver, kidneys, spleen, testes, brain and suprarenal fat were removed at autopsy for analysis of $^{14}$C activity. Plasma was obtained by centrifugation of an aliquot of the heparinized blood.

Isolation of Nuclei from Rat Liver. Livers taken at autopsy were immediately placed in ice-cold 0.25M sucrose in TKM buffer solution (0.05M tris-(hydroxymethyl)-aminomethane, 0.025M KCl, and 0.005M MgCl$_2$, adjusted to pH 7.5 at 20°C). The livers were individually homogenized in two volumes of 0.25M sucrose in TKM and the nuclei from 9.0 ml of the homogenate (equivalent to 3 g of whole liver) were isolated according to the method of Blobel and Potter (1966). The nuclear pellets were resuspended in 36 ml of TKM buffer and subsequently resedimented by centrifugation at 5000 x g for 10 minutes. These washed nuclear pellets were then analyzed quantitatively for $^{14}$C. A 2-ml aliquot of each liver homogenate was taken as the liver sample to be analyzed for $^{14}$C as part of the tissue distribution studies.

Radioactivity Assays. All biological samples, including urine and feces, were prepared and analyzed for $^{14}$C activity by the method of Weigel, Plotnick, and Conner (1978) using a Beckman LS 8100 Liquid Scintillation System.

Statistical Analysis of Data. The significance of differences between
groups was determined by Student's t-test at a probability level of 0.05.

RESULTS
At both time intervals studied, tissue concentrations of $^{14}$C in the liver, kidneys, spleen, testes, and brain were significantly higher in the rats receiving disulfiram in the diet. Urinary excretion of radioactivity during the first 24 hours after compound administration was significantly depressed in the disulfiram diet group. A comparison of tissue levels at 24 and 48 hours indicates that the rate of clearance of $^{14}$C from liver, kidneys, spleen, testes, and brain was appreciably lower in the disulfiram group. This was particularly true for clearance from the testes. While the levels (µg/g) in the testes of the animals in the control group at 24 and 48 hours were significantly different ($p < 0.001$), a comparison of the corresponding levels in the testes of the disulfiram group at the two time intervals indicated that they did not differ significantly ($p > 0.2$). There were no significant differences between the groups with respect to levels of $^{14}$C in the fat and whole blood at either time interval studied. The tissue distribution data, expressed both as tissue concentrations and as a percentage of the administered dose, appear in Tables 1 and 2. The levels of radioactivity in the washed liver nuclei obtained from the disulfiram-treated animals were significantly higher than those of the controls at both 24 and 48 hours. These data appear in Table 3.
Table 1 - Effect of Dietary Disulfiram Upon the Distribution of $^{14}$C in Selected Tissues and Body Fluids of Male Rats 24 Hours After a Single Oral Dose of 15 mg/kg [U-$^{14}$C]1,2-Dibromoethane

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>CONTROL DIET</th>
<th>DISULFIRAM DIET</th>
<th>PERCENT OF DOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIVER</td>
<td>4.78 ± 0.24</td>
<td>6.52 ± 0.39$^6$</td>
<td>1.79 ± 0.07</td>
</tr>
<tr>
<td>KIDNEYS</td>
<td>3.32 ± 0.42</td>
<td>6.82 ± 1.37$^6$</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>SPLEEN</td>
<td>1.00 ± 0.03</td>
<td>1.56 ± 0.14$^6$</td>
<td>0.02 ± &lt;0.01</td>
</tr>
<tr>
<td>TESTES</td>
<td>0.49 ± 0.05</td>
<td>0.88 ± 0.10$^6$</td>
<td>0.04 ± &lt;0.01</td>
</tr>
<tr>
<td>BRAIN</td>
<td>0.41 ± 0.04</td>
<td>0.64 ± 0.08$^6$</td>
<td>0.02 ± &lt;0.01</td>
</tr>
<tr>
<td>FAT$^3$</td>
<td>0.35 ± 0.04</td>
<td>0.48 ± 0.07</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>BLOOD$^4$</td>
<td>0.90 ± 0.05</td>
<td>0.95 ± 0.09</td>
<td>0.59 ± 0.03</td>
</tr>
<tr>
<td>PLASMA</td>
<td>0.46 ± 0.04</td>
<td>0.56 ± 0.05</td>
<td>--</td>
</tr>
<tr>
<td>URINE$^5$</td>
<td>--</td>
<td>--</td>
<td>72.38 ± 0.98</td>
</tr>
<tr>
<td>FECES$^5$</td>
<td>--</td>
<td>--</td>
<td>1.65 ± 0.28</td>
</tr>
</tbody>
</table>

1. Values represent mean concentrations in µg/g or µg/ml (expressed as parent compound) ± S.E.M. of duplicate determinations on six animals.
2. Values represent the mean percentage of the administered radioactive dose ± S.E.M. of duplicate determinations on six animals.
3. 6% of body weight (Donaldson, 1924).
4. 9% of body weight (Donaldson, 1924).
5. n = 12 (includes 24 hour samples obtained from rats killed 48 hours after compound administration).
6. Significantly different from control values (p < 0.05).
Table 2 - Effect of Dietary Disulfiram Upon the Distribution of $^{14}$C in Selected Tissues and Body Fluids of Male Rats 48 Hours After a Single Oral Dose of 15 mg/kg [U-$^{14}$C]1,2-Dibromoethane

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>CONTROL DIET</th>
<th>DISULFIRAM DIET</th>
<th>PERCENT OF DOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIVER</td>
<td>2.87 ± 0.33</td>
<td>5.23 ± 0.38$^6$</td>
<td>1.10 ± 0.12</td>
</tr>
<tr>
<td>KIDNEYS</td>
<td>1.06 ± 0.16</td>
<td>4.31 ± 0.40$^6$</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>SPLEEN</td>
<td>0.66 ± 0.03</td>
<td>1.29 ± 0.12$^5$</td>
<td>0.01 &lt; 0.01</td>
</tr>
<tr>
<td>TESTES</td>
<td>0.19 ± 0.02</td>
<td>0.72 ± 0.08$^6$</td>
<td>0.01 &lt; 0.01</td>
</tr>
<tr>
<td>BRAIN</td>
<td>0.17 ± 0.02</td>
<td>0.50 ± 0.03$^6$</td>
<td>0.01 &lt; 0.01</td>
</tr>
<tr>
<td>FAT$^3$</td>
<td>0.44 ± 0.06</td>
<td>0.53 ± 0.05</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>BLOOD$^4$</td>
<td>0.64 ± 0.07</td>
<td>0.81 ± 0.05</td>
<td>0.43 ± 0.04</td>
</tr>
<tr>
<td>PLASMA</td>
<td>0.22 ± 0.02</td>
<td>0.39 ± 0.03$^6$</td>
<td></td>
</tr>
<tr>
<td>URINE$^5$</td>
<td></td>
<td></td>
<td>73.54 ± 2.80</td>
</tr>
<tr>
<td>FECES$^5$</td>
<td></td>
<td></td>
<td>2.42 ± 0.54</td>
</tr>
</tbody>
</table>

1. Values represent mean concentrations in µg/g or µg/ml (expressed as parent compound) ± S.E.M. of duplicate determinations on five animals.
2. Values represent the mean percentage of the administered radioactive dose ± S.E.M. of duplicate determinations on five animals.
3. 6% of body weight (Donaldson, 1924).
4. 9% of body weight (Donaldson, 1924).
5. Cumulative 48 hour excretion.
6. Significantly different from control values (p < 0.05).
Table 3 - Effect of Dietary Disulfiram Upon the $^{14}$C Content of Liver Nuclei Isolated 24 or 48 Hours After Administration of a Single Oral Dose of 15 mg/kg [U-$^{14}$C]1,2-Dibromoethane

<table>
<thead>
<tr>
<th>TIME INTERVAL</th>
<th>CONTROL</th>
<th>DISULFIRAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 Hours</td>
<td>687 ± 82$^1$</td>
<td>1773 ± 314$^2$</td>
</tr>
<tr>
<td>48 Hours</td>
<td>460 ± 42</td>
<td>1534 ± 197$^3$</td>
</tr>
</tbody>
</table>

1. Results are expressed as disintegrations per minute per pellet (Mean ± S.E.M.) of duplicate determinations on six animals per group at 24 hours and five animals per group at 48 hours.
2. Significantly different from control values (p < 0.01).
3. Significantly different from control values (p < 0.001).

**DISCUSSION**

The study performed by Midwest Research Institute established that the addition of disulfiram to the diet of rats enhanced the toxicity of inhaled 1,2-dibromoethane. The study reported here demonstrates that addition of disulfiram to the diet of male rats results in significant increases in the tissue levels of subsequently administered $^{14}$C-1,2-dibromoethane in the organs studied. This increase in tissue levels was evident at both 24 and 48 hours following administration of the halogenated hydrocarbon and probably accounts for the interaction noted. Particularly noteworthy is the increase in the amount of radioactivity in washed nuclei isolated from the livers of rats in the disulfiram group. At 24 and 48 hours there are 1.4-fold and 1.8-fold increases, respectively, in the levels of radioactivity in the livers of animals in the disulfiram group as compared with the control animals. The corresponding ratios obtained for the nuclear pellets are 2.6 and 3.3, respectively, indicating a non-uniform, preferential distribution to this organelle. This increased nuclear uptake of $^{14}$C in the liver may
account for the high percentage of hepatocellular carcinomas found in the inhalation study in the group exposed to 1,2-dibromoethane while receiving disulfiram in the diet when compared to those exposed to 1,2-dibromoethane and receiving a control diet (70% vs. 5%). Slower clearance of $^{14}C$ from the testes in the disulfiram group may explain the significantly greater incidence of testicular atrophy observed in the rats receiving the combined exposure in the inhalation study when compared with those exposed to the halogenated hydrocarbon alone (90% vs. 4%). The mechanism of the enhancement of carcinogenicity by disulfiram is presently unclear. Researchers at the Southern Research Institute (Hill et al., 1978) have identified bromoacetaldehyde as an intermediate of 1,2-dibromoethane metabolism in the rat. Based upon this observation, one could speculate that disulfiram, a known inhibitor of aldehyde dehydrogenase, blocks the further oxidation of the bromoacetaldehyde formed, resulting in increased tissue levels of this intermediate. While little is apparently known about the toxicity and biological reactivity of bromoacetaldehyde, such information is available on another $\alpha$-haloaldehyde, chloroacetaldehyde. In the Ames mutagen assay, employing tester strain TA100 without activation, chloroacetaldehyde was found to be 746 times more active as a mutagen, per µmole, than vinyl chloride (McCann et al., 1975). In addition, it is known that chloroacetaldehyde reacts non-enzymatically with the nucleic acid bases adenine and cytosine to form so-called "etheno" derivatives (Secrist et al., 1972). Such a reaction, if it occurs in vivo, could produce significant alterations in nucleic acids. It is also possible that disulfiram merely inhibits the excretion of 1,2-dibromoethane.
metabolites and that the increased tissue levels reflect this interference with excretion. This alternate hypothesis is supported by a recent study which suggests that disulfiram interferes with the excretion of barbital, a hypnotic agent which does not undergo any significant biotransformation, resulting in an increased barbital sleeping time in rats (Sharkawi and Cianflone, 1978). Studies of the biochemical mechanism of this interaction are currently in progress in our laboratory.

REFERENCES


Mention of company or product names does not constitute endorsement by the National Institute for Occupational Safety and Health.

From the Mechanism of Industrial Disease Section, Experimental Toxicology Branch, Division of Biomedical and Behavioral Science

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Discussion

Unidentified Speaker: I was interested in the finding with dibromoethane and I am wondering whether or not the finding might be extrapolated to bromomethanes or bromopropanes. Do you think that that might be the case?

Dr. Plotnick, NIOSH: You don't get the same type of metabolites. So, I would doubt it. With bromomethane, is this CH3BR you are talking about?

Unidentified Speaker: For example, some the compounds that Dr. Bull was talking about - dibromochloromethane is one of them.

Dr. Plotnick, NIOSH: They don't undergo the same type of biotransformation - through that alcohol aldehyde type of thing. They are two carbon compounds rather than one carbon. Propanes, yes, possibly because I really think that things like DBCP act after loss of one of their carbons.

Dr. Bull, EPA: I thought those were very interesting results you had. Trichloroethylene, as you noted, does go through a similar kind of metabolic path, and do you have an epidemiologic cohort, or whatever, identifying disulfiram?

Dr. Plotnick, NIOSH: I am not sure whether we are going to do anything with it at this point. It is very intermittent exposure in very few people.

Dr. Bull, EPA: I mean there is evidence of interaction I think in epidemiologic populations between disulfiram.

Secondly, would there be a possibility of interaction with ethanol, because ethanol obviously goes through the same sort of --- the same enzymes are involved and at least you could see a competitive kind of interaction. Has anyone done any interaction studies between ethanol and ---

Dr. Plotnick, NIOSH: I did precisely the same study with 5% ethanol in drinking water. The only thing I can tell you is that there may have been one defect in experimental design. We stopped the ethanol the night before, and then treated with the ethylene dibromide orally on the following morning. Because of the rapid detoxification of ethanol there is a possibility there was not sufficient ethanol there to result in this modification. Therefore, it really should be done and they should be drinking the ethanol up to the time of treatment.

The other thing is that because of the fact that they are nocturnal maybe what we ought to do, really with that particular study, is treat them after midnight when they would be actively eating and drinking.

There is a study in the literature by Raddicke and Stemmer. It was an EPA-funded study at Cincinnati. In this study six hundred ppm vinyl chloride with 5% ethanol in the drinking water resulted in a shortened latent period and an increased number of liver hemangiosarcomas. We used their method except that, again, you have to have a point at which you can cut off the ethanol and that probably is a problem, but there was no difference and we did the same thing, 24 animals two time intervals with no differences in tissue levels.
Dr. Bull, EPA: How does a regulatory agency deal with an effect of a compound that is produced only in conjunction with a second compound which is rarely used.

Dr. Plotnick, NIOSH: With respect to the ethylene dibromide itself, since our study as well as an NCI gavage study reported in 1975, and a more recent inhalation study at two other levels by NCI, have established that EDB is a carcinogen in the rat, for regulatory purposes there is no problem with that one - at least from OSHA carcinogen policy guidelines. There also is a definition there that anything that enhances carcinogenicity or acts in a promoter type of a way is, also, to be considered a carcinogen. However, while disulfiram is used industrially in rubber manufacturing as an accelerator, a related compound is used as an accelerator or as an antioxidant and also is used in spraying fruit trees, primarily in California. There is alcohol intolerance in workers who spray their fruit trees in that area. You have across-the-board regulatory problems there because some of it is EPA, some of it is OSHA, and a good deal of it because the major use of the tetraethyl is antabuse or related products, is FDA.
IN DEPTH BIOCHEMICAL, PHARMACOLOGICAL AND METABOLIC STUDIES ON TRIHALOMETHANES IN WATER

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INTRODUCTION

The occurrence of trihalomethanes in drinking water represents one of the most fundamental issues in environmental health. These chemicals are produced through reaction of chlorine used in disinfection with organic material present in the source water (Bellar et al. 1974; Rook 1974). This discovery is currently forcing careful consideration of trade-offs between 3 fundamentally different types of risk:

1. Carcinogenic risk associated with disinfection byproducts.

2. Risks associated with microbiologically-induced disease.

3. Non-cancer risks that may be associated with alternate disinfectants to chlorine.

It is clear that a logical framework for making such trade-offs does not currently exist. This is partially due to inadequate information upon which to base estimates of risk. More fundamental is the lack of a means of assigning value to acute vs. chronic effects of disease causing agents. In the final analysis, however, it is the quality of relevant data that describes the dose response relationship involved
with the alternative risks which must be relied upon for decision making. The present project represents one attempt to provide an adequate data base upon which carcinogenic risks associated with disinfection byproducts may be estimated.

The major evidence of carcinogenic risk associated with chlorination reaction products are several reports of chloroform-induced tumors in experimental animals (Eschenbrenner and Miller, 1945; NCI, 1976; Roe et al. 1979). These data do fairly clearly establish that chloroform is carcinogenic in the mouse and the rat. However, the studies provide very little insight into the question of how these data might be relevant to man. It is obvious from the work of Roe et al. (1979) that there are substantial strain as well as species differences in response to chloroform. Additionally, the dose-response information which resulted from these studies has been extremely limited.

The use of bioassay data in which maximally tolerated doses have been utilized to make risk estimates at low doses has been criticized on several grounds. Perhaps the oldest argument is that tumors might arise from epigenetic mechanisms triggered by necrotic tissue damage. Such a relationship has been observed specifically with chloroform, although with limited numbers of animals/group (Eschenbrenner and Miller 1945). A second issue has been the appropriateness of extrapolating data from the rat and mouse to the human when the extent of chloroform metabolism in the three species differs considerably (Reitz
et al. 1978). Third is the possibility that the metabolism of chemical carcinogens may be dose-dependent; raising a second question about whether results obtained at maximally tolerated doses can be extrapolated with confidence to environmental levels.

The above problems are not trivial. At the same time they are not completely answerable because of the ethical limitations rightfully placed on human experimentation. Within that limitation the present project addresses these issues. The results presented here include metabolic work being performed at the Health Effects Research Laboratory of the U.S. EPA in Cincinnati and subchronic and chronic studies being conducted under contract with Stanford Research Institute in Menlo Park under the direction of D.C.L. Jones and T. Jorgensen. These efforts are expected to contribute to better estimates of the risks posed by chloroform and the other trihalomethanes and to form the foundation of better regulatory decisions.

METHODS

Animal Groups and Dose Levels

Three hundred female B6C3Fl mice were obtained from Charles River Breeding Laboratory and 250 male Osborne-Mendel rats from Camm Research Institute. Following two weeks of quarantine the animals were placed on test at 6 weeks of age. The animals were allocated to experimental
groups by using a table of random numbers. Cages were rotated on the racks and racks were rotated within the room once each week throughout the study.

The test groups, levels of chloroform, and number of animals assigned to each group were as follows:

<table>
<thead>
<tr>
<th>Male Osborne-Mendel Rats</th>
<th>Female B6C3F1 Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose Level (ppm)</td>
<td>No. of</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
</tr>
<tr>
<td>0</td>
<td>40*</td>
</tr>
<tr>
<td>0 (match cont.) +30</td>
<td>30</td>
</tr>
<tr>
<td>200</td>
<td>30</td>
</tr>
<tr>
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<tr>
<td>1800</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Includes 10 for day 0 sacrifice.
+ The amount of water consumed by the high dose groups was determined daily and an equivalent amount given to the matched control group the ensuing day.

Clinical Chemistry

Ten animals from each group were sacrificed at 30, 60 and 90 days of the study. Serum glutamate oxalacetate transaminase (SGOT) and lactic dehydrogenase (LDH) activity were measured in the mice using the methods of Karmen (1955) and Wacker, et al. (1956). The clinical chemistry measurements in the rats were measured according to Technicon Manual Technical Publication # UA-3-0306B3, March 1976 and included blood urea nitrogen, lactic dehydrogenase, serum glutamate
oxalacetate transaminase, serum glutamate pyruvate transaminase, and serum triglycerides.

At the time of sacrifice complete necropsies were performed according to the Guidelines for Carcinogen Bioassay in Small Rodents (NCI-CG-TR-1, Technical Report Series #1, February 1976). Major organs and all suspected tumors and gross lesions were examined microscopically.

**Chloroform analysis**

**Drinking water.** Pesticide-quality chloroform from Matheson Coleman Bell all of the same lot number was used throughout this study. Chemical analysis indicated the presence of 30 ppb diethyl carbonate (DEC) in the commerically obtained chloroform. Therefore, the chloroform was distilled just prior to the twice-weekly preparation of the dosing solutions. Fresh solutions of chloroform were prepared on Mondays and Thursdays and the old solutions were discarded. When the solutions were thoroughly mixed, they were transferred to animal water bottles via a syringe-activated teflon siphon. Two samples for each chloroform level were removed for analysis by the method of Bellar and Lichtenberg (1974). Sampling was done both on freshly prepared and discarded water at the twice-weekly change in the animals' drinking water. Second Analysis of solutions being discarded revealed an average of 92.0 ± 1.4% of the initial chloroform concentrations.
Serum. Chloroform levels in serum were assayed using the purge trap method of Bellar and Lichtenberg (1974) as modified by Peoples, et al. (1979).

Organ Fat Analysis

The procedure used to perform the assay of lipids in the rat kidney and mouse liver in this study was as follows. The organ tissue was tamped dry on the exterior and weighed on an analytical balance. The tissue then was homogenized with 4 ml of high purity water using a Tektron Inc., polytron homogenizer. The homogenate was added to a separatory funnel containing 49 ml chloroform: methanol, 2:1, the mixture shaken for 30 seconds, and 8 ml of 0.018 N H$_2$SO$_4$ added. The resulting mixture was shaken for 15 seconds, and the total contents were added to a 15 ml centrifuge bottle, which was spun at 2000 rpm for 20 minutes. The resulting suspension contained two layers separated by a thin white protein disk. The upper aqueous layer was drawn off by suction, and a 20 ml fraction of the bottom layer (chloroform) was evaporated to dryness in a tared 3-g test tube in a water bath at 57°C. Nitrogen gas was used to remove final traces of the solvent. The dry test tube was placed in a dessicator overnight, and the net weight, representing lipids, was determined.
Metabolic work.

**Chemicals and Solutions.** $^{14}$C-chloroform, 2.1 mCi/mmole and 99% minimum radiochemical purity (mrcp); $^{14}$C-dibromochloromethane, 2mCi/mmole and 98% mrcp; and $^{14}$C-bromform, 2.1 mCi/mmole and 99% mrcp were purchased from radiochemical suppliers. Dosing solutions were prepared by mixing the appropriate unlabeled trihalomethane (THM) (all 97% pure) with the $^{14}$C-THM in corn oil (Fisher Laboratory grade): for the rat, 100 mg-THM/ml with a radioactive concentration of 16 uCi/ml, for the mouse 150 mg-THM/ml with a radioactive concentration of 32 uCi/ml.

**Animals and Dosing.** Male Sprague-Dawley rats (Charles River) were fasted for 16 hours prior to dosing. The THM was administered, 100 mg/kg, in a single dose by intragastric intubation. The rats were immediately placed, individually, in glass Roth-type metabolism cages which were equipped with urine-feces separator-collectors cooled to 4°C. Fasting from food was continued throughout the 8 hour sampling period, but water was allowed ad libitum.

The mice, B6C3F1 were given single 150 mg/kg per oral doses of $^{14}$C-THM by intragastric intubation following a 16h fast. Five mice were placed in a single metabolism cage. Other procedures were identical with those used for the rat.

**Sampling of expired air, urine and feces.** Room air was drawn through the chambers by mechanical pumps at a rate of 500 ml/min. A series of
two glass impinger traps was placed between the cages and the pump to collect expired $^{14}$C-THM and $^{14}$CO$_2$ from the air stream. The first trap, for collecting unchanged $^{14}$C-THM contained as a solvent 100 ml of a 9:1 mixture of ACS Grade Xylenes (Fisher Scientific) and scintillation grade 2-methoxyethanol (Eastman). The second trap, for collecting $^{14}$CO$_2$, contained 100 ml of a 5N ethanolamine (Eastman scintillation grade) in scintillation grade 2-methoxyethanol.

The efficiency of the expired air traps for separating and collecting $^{14}$C-THM and $^{14}$CO$_2$ was tested by first evaporating a simulated dose of $^{14}$C-THM (dissolved in methanol) from an open dish in the metabolism cage. Collection of $^{14}$C-THM by the first trap was quantitative at the completion of evaporation, but a small loss, 1.3-5.2%, of $^{14}$C-THM was experienced by continued pumping up to 8 hours. In the second step of the test, $^{14}$CO$_2$ from a calibrated compressed air tank was sparged through fresh traps for 10 minutes. The $^{14}$CO$_2$ was quantitatively trapped by the second trap: there was no $^{14}$CO$_2$ in the first trap. The air pumps were then turned on and room air was drawn through the traps for 8 hours. No loss of $^{14}$CO$_2$ occurred.

Samples were collected from the traps at 0.5, 1.0, 1.5 and 2.0 hours and then at hourly intervals up to 8 hours. During the runs with the rats, 0.5 ml aliquots were taken from the expired air traps at these intervals and assayed for radioactivity by liquid scintillation counting (LSC). The sparging solutions remained in the traps. In the experiments with the mice, fresh solution was placed in the traps at each sampling interval.
Urine and feces were collected at 2, 4 and 8 hours if available. Urines were assayed for $^{14}$C by LSC: 0.5 ml aliquots were added to 12 ml Scintiverse (Fisher Scientific Co.). Negligible amounts of $^{14}$C were found in the feces, so after initial screening feces were discarded.

**Determination of $^{14}$C in selected tissue and organs.** At 8 hours, animals were sacrificed by anesthetizing with Nembutal. After withdrawing a blood sample from the inferior vena cava, selected tissue and organ samples were excised and dissolved in solublizer. The preparation of the solublizer and method for determination of $^{14}$C in tissue samples was that described by Dent and Johnson (1974). Internal standardization with $^{14}$C-toluene was used to determine counting efficiency.

**RESULTS**

As a result of experiments which are reported in the present paper, dose levels were selected for the chronic study now underway. For purposes of comparing the dose levels in the present work and the chronic study with those used in the NCI bioassay (NCI, 1976) calculated intakes of chloroform are given in Table 1. As can be seen the doses obtained by rats and mice at 900 and 1800 mg/liter approximate the doses administered in the NCI bioassay of 90 and 180 mg/Kg in the male rat. The 1800 mg/l dose to the female B6C3Fl mouse approximates the lowest time weighted dose of 238 mg/Kg. (The NCI doses were
administered for 5 days a week by gavage whereas the present study employs continuous exposure increasing the dose levels in the present study relative to the NCI doses).

Included in Table 1 are the number of animals included per exposure group in the chronic study. This design has been chosen to better estimate the tumor incidence at the lower doses. The biological and clinical data presented in this paper are derived from the subchronic study used to design the chronic study or from the interim sacrifices conducted over the first 6 months of the chronic study. In general these measurements have been performed on groups of 10 animals per time interval.

Table 2 gives the average serum chloroform concentrations in rats treated with different concentrations of chloroform in their drinking water. Two trends are noted in the data. First of all there tends to be a consistent increase in the levels of chloroform measured in the serum of the control animals over time. The increase of concentrations over a 180 day period would argue for a serum half-time of several years rather than the 2 hr usually reported (Brown et al. 1974). These data suggest extraneous sources of chemical in serum which are measured as chloroform. Alternate sources of chloroform have not been found to account for this increase (i.e. food and room air) Despite this pattern there appeared to be significant dose-related increases of serum chloroform levels at 30 and 90 days of exposure. The scatter of this data prevented significant relationships with dose to be consistently observed despite the use of early morning samples.
During periodic sacrifices clinical chemistry measurements have been made in rats on 22 parameters and in mice for two. For purposes of this interim report we have chosen to include only those parameters which are referrable to kidney or liver damage.

In Table 3 the effects of chloroform treatment on blood urea nitrogen (BUN) are displayed. As can be seen there appears to be a dose-related increase (evident primarily at 1800 mg/l) of BUN with chloroform treatment. However, this does not appear to be attributable to chloroform since the control group matched for water intake shows the same or even greater increases in BUN at all time points.

Tables 4, 5 and 6 display the results of serum enzyme activities in the rat that would be predicted to increase with liver damage (and to some extent other organs), SGOT, SGPT and LDH respectively. There are no significant changes in SGOT, a transient increase in SGPT at 30 days of exposure at 1800 mg chloroform/l, and a decrease in LDH at 60 days. This decrease in LDH activity has also been observed at the 180 day sacrifice of the chronic study (data not shown).

Serum triglyceride concentrations were decreased by chloroform treatment (Table 7). This was a consistent observation at all 3 sacrifice times at a concentration of 1800 mg chloroform/l and the changes were not reflected in animals matched for drinking water consumption. A similar decrease in serum triglyceride levels was
observed in animals exposed to 1800 mg chloroform/l in the chronic study (data not shown)

Because of the limited availability of serum from the mouse it was not possible to perform extensive clinical measurements. Since the B6C3F1 mouse has been shown to develop hepatocellular carcinomas, SGOT and LDH were the parameters measured in mouse blood (Table 8). As with the rat, the only statistically significant and consistent changes in these enzyme activities were decreases in the normal activity. In both cases the decreases were observed primarily at 1800 and 2700 mg chloroform/l. In the case of LDH the decrease appeared to be complicated by an increase in activity induced by restricted water intake in the matched control. It is not possible to determine the extent to which the increase in activity by restriction of fluid intake may have modified the effect of chloroform at lower doses.

As a result of appearance of increased liver fat in female mice exposed to chloroform in the subchronic test (data not shown), the chronic study was modified to allow liver fat content to be measured in both rats and mice over the entire dose range. Data from the 90 and 180 day sacrifices are shown in Table 9. At both times definite increases in liver fat were observed in female B6C3F1 mice. The lowest effective dose was 400 mg/l at 90 days and 200 mg/l at 180 days. In contrast, no increase in liver fat was observed in male Osborne-Mendel rats at 90 days of exposure; the only significant increase being observed after 180 days of exposure to 1800 mg/l.
Histopathological evidence of fatty infiltration of the liver was observed in the mice at all sacrifice periods of the subchronic study (Table 10). Despite the limited number of animals at each time and dose, a rather clear relationship of this effect with chloroform exposure could be seen. No histopathological evidence of fatty infiltration of the liver has been observed in the male Osborne-Mendel rat.

No other histopathology could be clearly related to chloroform exposure. Particular attention was paid to the development of kidney pathology in the male rat because this site was involved in malignant changes in the NCI bioassay.

The comparative metabolism of trihalomethanes in the mouse and rat are currently under extensive study at the Health Effects Research Laboratory of USEPA in Cincinnati. Table 11 summarizes the overall metabolism of each of the four trihalomethanes commonly found in drinking water, trichloromethane (TCM or chloroform), bromodichloromethane (BDCM), dibromochloromethane (DBCM) and tribromomethane (TBM, bromoform). In general, it can be seen that all of the trihalomethanes are more extensively metabolized in the mouse than in the rat with 4 to 9 times higher proportions of the dose being excreted as expired CO₂. This is paralleled by the appearance of approximately twice the level of metabolites excreted in the urine of the mouse. There is also a relatively higher retention of label in tissues (with the exception of BDCM) in the mouse.

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It is curious to note that the metabolism of chloroform parallels that of bromoform more closely than the mixed trihalomethanes. The mixed trihalomethanes are metabolized to a much greater extent by both species (14 and 18% conversion to CO₂ in the rat and 81 and 72% in the mouse for BDCM and DBCM, respectively). The dose-response characteristics of trihalomethane metabolism and identity of the metabolites are now being studied.

To obtain an estimate of the relative amounts of reactive intermediate available for binding macromolecules our laboratory has been exploring the use of covalent binding carcinogens to hemoglobin that has been demonstrated by Ehrenberg and coworkers (Osterman-Golkar et al., 1976). Experiments with chloroform have demonstrated a linear binding of [¹⁴C]-chloroform to hemoglobin with single doses ranging from 0.012 to 120 mg/kg body weight (Pereira and Chang, 1980). The binding of [¹⁴C]-chloroform to hemoglobin following a dose of 1.2 mg/kg has been compared in 3 strains of mouse and rat. The data so obtained is shown in Table 12. In general it can be seen that the covalent binding of chloroform to hemoglobin is generally greater in the rat than in the mouse. Specifically, the degree of hemoglobin binding in the Osborne-Mendel rat exceeds that observed in the B6C3Fl mouse by a slight but insignificant amount.
DISCUSSION

This preliminary assessment of factors which might influence across-species extrapolation of carcinogenicity data for chloroform can only lead to tentative conclusions. Despite the incomplete nature of the results, some patterns seem to be emerging.

First, clinical parameters which have been developed for the purpose of detecting acute liver damage appear to be of rather limited usefulness in judging liver damage induced by chloroform on a chronic basis. Rather than the increased levels of these enzymes classically found in serum following acute liver injury, the pattern observed with some consistency in the present study was a decrease in these activities. This might be a result of the specific type of injury observed, fatty infiltration of the liver, which occurred with little evidence of gross necrosis.

Despite the failure of the serum enzyme measurements to detect the injury, it is quite clear that the female B6C3F1 is much more sensitive to the development of a fatty liver than the Osborne-Mendel rat. The doses at which this effect is noted are within the range and extend much below those used in the NCI bioassay for chloroform (NCI, 1976) Consequently, it is reasonable to conclude that this effect was present in the study and perhaps even exacerbated by the use of bolus doses resulting from gavage treatment. Similarly, it is
apparent that the Osborne-Mendel rat was much less sensitive to liver injury induced by chloroform than the mouse. It is difficult at this time to determine how the reduction in serum triglyceride levels might be related to the marginal but highly significant increase in liver fat noted in the rat at the highest dose. The coupling of these two effects would suggest a relatively specific interference with lipid metabolism.

In general, these data support the contention that the ability of chloroform to produce liver tumors is not separable from its ability to damage the liver. It is known that other treatments that produce fatty livers, such as choline-deficient diets (Shinozuka, et al. 1978), promote the development of liver tumors. No such relationship could be drawn between chloroform-induced damage to the kidney and tumor development in the Osborne-Mendel rat.

It is premature to judge the contention that a difference in metabolism of chloroform between species can account for different susceptibilities to chloroform carcinogenesis (Reitz et al. 1978). Previously reported differences in the fraction of chloroform metabolized by mouse and rat (Brown et al. 1974) were confirmed in the present work. However, it appears that the levels of activated metabolite which reach hemoglobin in the circulating red cell are essentially the same in the male Osborne-Mendel rat and the B6C3F1 mouse. This latter data may or may not accurately reflect the degree
to which chloroform covalently binds to tissue macromolecules, particularly DNA, and the extent to which it acts as an initiator. On the other hand, the levels of activated intermediate available for such interactions are even more tenuously related to gross measures of metabolism upon which the argument is developed. The extent to which chloroform interaction with DNA might differ between the two species is currently under investigation in our laboratory (Lin and Pereira, personal communication).
References


Table 1. Calculated Doses of Chloroform Corresponding to Levels Administered in Drinking Water to Rats and Mice

<table>
<thead>
<tr>
<th>Chloroform mg/l</th>
<th>n</th>
<th>Calculated dose(^a) mg/kg</th>
<th>n</th>
<th>Calculated dose mg/kg</th>
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<td>330</td>
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<td>151</td>
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<td>263</td>
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<tr>
<td>MO(^b)</td>
<td>50</td>
<td>-</td>
<td>50</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) Calculated from average water consumption/kg body weight in subchronic study

\(^b\) MO = Group matched with 1800 mg/l group for water consumption.
Table 2. Serum Chloroform Levels in Rats Treated with Chloroform in Drinking Water

<table>
<thead>
<tr>
<th>Chloroform mg/l</th>
<th>30 Day</th>
<th>60 Day</th>
<th>90 Day</th>
<th>180 Day</th>
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<tr>
<td>Control</td>
<td>0.60</td>
<td>1.45</td>
<td>6.69</td>
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<td>Matched Control</td>
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<td>600</td>
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</tbody>
</table>

\(^a\)Significantly different from corresponding control by one way ANOVA and t-test at P \(<\ 0.05.

\(^b\)Same as \(^a\) except P \(<\ 0.01.
Table 3. Effect of Chloroform on Blood Urea Nitrogen of the Rat

<table>
<thead>
<tr>
<th>Chloroform mg/l</th>
<th>30 days</th>
<th>60 days</th>
<th>90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20 ± 2</td>
<td>22 ± 2</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>200</td>
<td>22 ± 3</td>
<td>23 ± 2</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>400</td>
<td>23 ± 3c</td>
<td>22 ± 3</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>600</td>
<td>22 ± 4</td>
<td>23 ± 1</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>900</td>
<td>25 ± 10</td>
<td>23 ± 1</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>1800</td>
<td>26 ± 2c</td>
<td>25 ± 3b</td>
<td>25 ± 2c</td>
</tr>
<tr>
<td>Mod</td>
<td>28 ± 10c</td>
<td>26 ± 2c</td>
<td>25 ± 5</td>
</tr>
</tbody>
</table>

\(^a\) mg% I S.D. ten animals

\(^b\) Significantly different from control by ANOVA and t-test P < 0.05

\(^c\) Same as b with P < 0.01
Table 4. Effect of Chloroform on Serum Glutamate Oxalacetic Transaminase in the Rat

<table>
<thead>
<tr>
<th>Chloroform mg/l</th>
<th>30 day</th>
<th>60 day</th>
<th>90 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>174 ± 90</td>
<td>138 ± 48</td>
<td>155 ± 89</td>
</tr>
<tr>
<td>200</td>
<td>202 ± 74</td>
<td>129 ± 28</td>
<td>107 ± 17</td>
</tr>
<tr>
<td>400</td>
<td>144 ± 37</td>
<td>117 ± 24</td>
<td>110 ± 35</td>
</tr>
<tr>
<td>600</td>
<td>161 ± 51</td>
<td>122 ± 37</td>
<td>119 ± 39</td>
</tr>
<tr>
<td>900</td>
<td>151 ± 67</td>
<td>124 ± 46</td>
<td>107 ± 18</td>
</tr>
<tr>
<td>1800</td>
<td>251 ± 125</td>
<td>104 ± 30</td>
<td>153 ± 51</td>
</tr>
<tr>
<td>MO</td>
<td>166 ± 64</td>
<td>142 ± 35</td>
<td>183 ± 81</td>
</tr>
</tbody>
</table>

\[ a_{\text{mu/ml serum ± S.D. ten animals}} \]

\[ b_{\text{MO = Group matched with 1800 mg/l group for water consumption.}} \]
Table 5. Effect of Chloroform on Serum Glutamate Pyruvate Transaminase in the Rat

<table>
<thead>
<tr>
<th>Chloroform mg/l</th>
<th>30 day</th>
<th>60 day</th>
<th>90 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>63 ± 21</td>
<td>65 ± 18</td>
<td>62 ± 7</td>
</tr>
<tr>
<td>200</td>
<td>83 ± 33</td>
<td>66 ± 13</td>
<td>59 ± 7</td>
</tr>
<tr>
<td>400</td>
<td>63 ± 11</td>
<td>64 ± 16</td>
<td>72 ± 33</td>
</tr>
<tr>
<td>600</td>
<td>60 ± 17</td>
<td>66 ± 17</td>
<td>64 ± 14</td>
</tr>
<tr>
<td>900</td>
<td>83 ± 64</td>
<td>65 ± 13</td>
<td>61 ± 16</td>
</tr>
<tr>
<td>1800</td>
<td>112 ± 53(^b)</td>
<td>65 ± 20</td>
<td>84 ± 37</td>
</tr>
<tr>
<td>MO(^c)</td>
<td>62 ± 16</td>
<td>65 ± 11</td>
<td>74 ± 21</td>
</tr>
</tbody>
</table>

\(^a\) mu/ml serum ± S.D. ten animals

\(^b\) Significantly different from control by ANOVA and t-test \(P < 0.05\).

\(^c\) MO = Group matched with 1800 mg/l group for water consumption.
Table 6. Effect of Chloroform Treatment on Serum Lactic Dehydrogenase Activity in the Rat

<table>
<thead>
<tr>
<th>Chloroform mg/l drinking water</th>
<th>Serum LDH Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 day</td>
</tr>
<tr>
<td>0</td>
<td>1593 ± 504&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>200</td>
<td>1651 ± 361</td>
</tr>
<tr>
<td>400</td>
<td>1270 ± 225</td>
</tr>
<tr>
<td>600</td>
<td>1577 ± 303</td>
</tr>
<tr>
<td>900</td>
<td>1275 ± 360</td>
</tr>
<tr>
<td>1800</td>
<td>1616 ± 482</td>
</tr>
<tr>
<td>MO&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1484 ± 287</td>
</tr>
</tbody>
</table>

<sup>a</sup>μm/l ± S.D. ten animals

<sup>b</sup>Significantly different from control P < 0.05 by ANOVA and t-test

<sup>c</sup>Same as b but P < 0.01.

<sup>d</sup>MO = Group matched with 1800 mg/l group for water consumption.
Table 7. Effect of Chloroform on Serum Triglyceride Concentrations in the Rat

<table>
<thead>
<tr>
<th>Chloroform mg/l</th>
<th>30 day</th>
<th>60 day</th>
<th>90 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>87 ± 58</td>
<td>77 ± 26</td>
<td>78 ± 33</td>
</tr>
<tr>
<td>200</td>
<td>80 ± 23</td>
<td>68 ± 16</td>
<td>93 ± 43</td>
</tr>
<tr>
<td>400</td>
<td>96 ± 98</td>
<td>86 ± 21</td>
<td>91 ± 53</td>
</tr>
<tr>
<td>600</td>
<td>60 ± 10</td>
<td>81 ± 30</td>
<td>80 ± 24</td>
</tr>
<tr>
<td>900</td>
<td>313 ± 832</td>
<td>62 ± 25</td>
<td>93 ± 18</td>
</tr>
<tr>
<td>1800</td>
<td>43 ± 9b</td>
<td>36 ± 15c</td>
<td>38 ± 21c</td>
</tr>
<tr>
<td>Mod</td>
<td>123 ± 210</td>
<td>53 ± 13b</td>
<td>84 ± 46</td>
</tr>
</tbody>
</table>

\textit{a} mg\% ± S.D. ten animals

\textit{b} Significantly different from control by ANOVA and t-test P \textless 0.05

\textit{c} Same as \textit{b} except P \textless 0.01.

\textit{d} \textit{MO} = Group matched with 1800 mg/l group for water consumption.
Table 8. Serum Glutamate Oxalacetate Transaminase (SGOT) and Lactic Dehydrogenase (LDH) Activity in Female Mice Receiving Chloroform in Their Drinking Water

<table>
<thead>
<tr>
<th>Chloroform mg/l</th>
<th>SGOT Activity</th>
<th>LDH Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>0</td>
<td>328</td>
<td>623</td>
</tr>
<tr>
<td>200</td>
<td>225</td>
<td>400</td>
</tr>
<tr>
<td>400</td>
<td>233</td>
<td>414</td>
</tr>
<tr>
<td>600</td>
<td>196</td>
<td>257</td>
</tr>
<tr>
<td>900</td>
<td>246</td>
<td>383</td>
</tr>
<tr>
<td>1800</td>
<td>298</td>
<td>136^b</td>
</tr>
<tr>
<td>2700</td>
<td>156^b</td>
<td>619</td>
</tr>
<tr>
<td>Mod^d</td>
<td>522</td>
<td>349</td>
</tr>
</tbody>
</table>

^aAverage values for 10 animals expressed as µu/ml
^bSignificantly different from control by ANOVA and t-test P < 0.05
^cSame as b except P < 0.01.
^dMod = Group matched with 1800 mg/l groups for water consumption.
Table 9. Liver Fat Content in Mice and Rats Treated with Chloroform in Drinking Water

<table>
<thead>
<tr>
<th>Chloroform mg/l</th>
<th>B6C3F1 Mouse</th>
<th>Osborne-Mendel Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 Days</td>
<td>180 Days</td>
</tr>
<tr>
<td>0</td>
<td>3.33</td>
<td>5.82</td>
</tr>
<tr>
<td>200</td>
<td>3.45</td>
<td>7.93&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>400</td>
<td>3.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.77</td>
</tr>
<tr>
<td>900</td>
<td>4.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1800</td>
<td>6.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Average value for 10 animals at each dose and time.

<sup>b</sup>Significant at P<0.05 by ANOVA and t-test.

<sup>c</sup>Significant at P<0.01 by ANOVA and t-test.
Table 10. Histopathological Evidence of Fatty Infiltration of Livers of B6C3F1 Mice

<table>
<thead>
<tr>
<th>Chloroform (mg/l)</th>
<th>30 Day</th>
<th>60 Day</th>
<th>90 Day</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>400</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>600</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>900</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1800</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>2700</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>13</td>
</tr>
</tbody>
</table>

*aOut of 10 animals at each dose group and time point.*

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Table 11. Comparative Distribution of Labelled Carbon Eight Hours Following Oral Administration of $^{14}$C-Trihalomethanes to the Sprague-Dawley Rat\textsuperscript{a} and B6C3F1 Mouse\textsuperscript{b}

<table>
<thead>
<tr>
<th></th>
<th>TCM</th>
<th>BDCM</th>
<th>DBCM</th>
<th>TBM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat</td>
<td>Mouse</td>
<td>Rat</td>
<td>Mouse</td>
</tr>
<tr>
<td>% CO\textsubscript{2} Expired</td>
<td>6.5</td>
<td>49.55</td>
<td>14.3</td>
<td>81.20</td>
</tr>
<tr>
<td>% Unmetabolized Expired</td>
<td>64.8</td>
<td>26.05</td>
<td>41.7</td>
<td>7.18</td>
</tr>
<tr>
<td>% $^{14}$C Urine</td>
<td>2.6</td>
<td>4.91</td>
<td>1.4</td>
<td>2.17</td>
</tr>
<tr>
<td>% $^{14}$C Total Organ</td>
<td>4.3</td>
<td>13.45</td>
<td>5.3</td>
<td>3.18</td>
</tr>
<tr>
<td>Total $^{14}$C Recovery</td>
<td>78.2</td>
<td>94.47</td>
<td>62.7</td>
<td>92.71</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Average not less than 5 animals per group - dose administered 100 mg/kg

\textsuperscript{b}Average of 4 groups of 5 animals each - dose administered 150 mg/kg
Table 12. Chloroform Bound to Hemoglobin in Mice and Rat Strains

<table>
<thead>
<tr>
<th>Animal Strain</th>
<th>Amount Bound pmoles/Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Rat</strong></td>
<td></td>
</tr>
<tr>
<td>a. Osborne-Mendel</td>
<td>109 + 19</td>
</tr>
<tr>
<td>b. Sprague-Dawley</td>
<td>136 + 10</td>
</tr>
<tr>
<td>c. Fisher-344</td>
<td>152 + 14</td>
</tr>
<tr>
<td><strong>2. Mice</strong></td>
<td></td>
</tr>
<tr>
<td>a. Swiss (CFN)</td>
<td>85 + 7</td>
</tr>
<tr>
<td>b. B6C3F1</td>
<td>96 + 8</td>
</tr>
<tr>
<td>c. Swiss (SENCAR)</td>
<td>104 + 10</td>
</tr>
</tbody>
</table>

a - Data of Pereira and Chang, 1980  
b - The values in the table represent the mean ± standard error for 6 animals in each group.
Discussion

Dr. Keefer, NCI: I had always imagined that chloroform was a lot less soluble in water than you indicated, but I have never seen any data. I am wondering whether you had to use any special methods to get it into solution at almost 2 milliliters per liter and, more importantly, whether you have some data showing that the volatilization from the water solution on feeding was not contributing to loss from the water and inhalation exposure as well.

Dr. Bull, EPA: The room air has been monitored, and there is no real indication of that, but more basic to the problem is that the water bottles are changed twice weekly, and the amounts of chloroform monitored on a weekly basis. The levels of chloroform in those water bottles compared to the initial levels were 92 plus or minus about 2%. This was partially attributable to the use of a double balled watering bottle which seems to cut down on the loss to the ambient environment although there is obviously still a small loss to the head space. In addition, there is a very high turnover in the room air which probably accounts for the inability to document any appearance of chloroform in the room air.

Regarding solubility, things which are called non-soluble in the Handbook in Chemistry and Physics are often soluble to several percent. The limit of solubility of chloroform in water is about 0.5% at room temperature. Consequently, no extraneous agents were needed to get chloroform into solution, even at the highest concentrations employed.

Dr. Keefer, NCI: How did you do the measurement of the concentration in water?

Dr. Bull, EPA: By the purge and trap method; standard methodology for looking at trihalomethanes in water.

Dr. Keefer, NCI: I am not sure what that is.

Dr. Bull, EPA: It is a GC-mass spectrometric method.

Dr. Gregory, CPSC: I really object to the last sentence in your abstract that says that such a condition is well known as a promoter of liver tumors in other species as well. I would object to the way it is put. I certainly don't object to the fact that many substances which produce fatty livers are promoters. I object to the indication that it is the condition itself that is the promoter, that is not true.

Dr. Bull, EPA: That would be true, I think. However, maybe it is a little too all inclusive. Conditions other than chemical exposure, for example a choline deficient diet, also produce a fatty liver and promote tumor development. It is difficult to see this procedure acting as an initiator.

Unidentified Speaker: I think what he is trying to say is the fatty liver does not imply cancer.

Dr. Bull, EPA: I did not mean to imply that either.

Dr. Chandler, NIOSH: The observation of a linear increase in hemoglobin alkylation or derivative formation over five orders of magnitude is a very rare
observation, at least in my experiences. Any biochemical phenomenon which would be linear over five orders of magnitude leads one to wonder exactly what is being measured with regard to metabolism. I have three questions: Are you certain that its reactive intermediates are being generated through metabolism that are alkylating hemoglobin or is it a spontaneous reaction between hemoglobin and the trihalomethane? Also, have you characterized the nature of the derivative of hemoglobin? It is established that macrophages can activate many compounds. Is it possible that circulating macrophages could be the source of the reactive metabolism and not the liver or kidney?

Dr. Bull, EPA: That last question in particular is the reason why I think we have to compare the formation of DNA adducts, or at least macromolecular binding of the chloroform in target tissues, with alkylation of hemoglobin. The source of the alkylating group may be all tissues or selectively from some. We are hoping that alkylated hemoglobin will be an integrator of all kinds of tissue, and it is certainly possible that the macrophage might be responsible for at least some of that activity. The critical issue at this point in time is to see if that really accurately reflects the degree to which the material is covalently bound in target tissues.

I think it is pretty clear at this point in time that a covalent binding with chloroform is involved - basically because of the way the product is isolated. However, we have not actually identified the product as of this moment. That is being worked on. Another argument is that probably the half life of the adduct to hemoglobin approximates the half life of the red cell. Therefore, if you were looking at, say, carbon monoxide as a product binding to heme for example, you would not expect that half life.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Wednesday Afternoon, May 7

METHODOLOGY/EXPERIMENTAL MODELS SESSION (CONTINUED)

SESSION CHAIRPERSON

Dr. C. C. Lee
Environmental Protection Agency
Chronic Animal Inhalation Study of Short (<5 µm) Asbestos Fibers

Progress Report

by

Stanley F. Platek
David H. Groth

Division of Biomedical and Behavioral Science
National Institute for Occupational Safety and Health
Taft Laboratories
4676 Columbia Parkway
Cincinnati, Ohio 45226

Contract Number: 210-77-0151

International Research and Development Corporation
Mattawan, Michigan 49071
An animal inhalation study was initiated in 1977 to study the chronic biological effects of inhalation of short chrysotile asbestos fibers. Rats and monkeys were exposed for 7 hours/day, 5 days/week for 18 months to specially prepared chrysotile. Based upon daily chamber measurements, the mean concentration of fibers in the chamber air was less than 1 mg/m^3. By phase contrast and electron microscopy, the ratio of the number of chrysotile fibers/cc <5 µm in length to the fibers >5 µm was established at approximately 265:1. Rats were autopsied for examination 1, 3, 6, 12, and 18 months after initiating exposures. Histopathological examinations of the lung tissue have so far revealed little or no pathological reaction to the inhaled asbestos. Although asbestos fibers could not be seen in lung tissues by light microscopy, they were seen in alveolar macrophages when examined by electron microscopy. Six months after the last exposure date, i.e., 24 months after initiating exposures, the remaining rats will be sacrificed for examination. The monkeys will be maintained and observed for signs of latent pulmonary disease for approximately an additional seven years.
INTRODUCTION

Asbestos has been implicated by numerous investigators as playing a major role in the debilitating human diseases of bronchogenic carcinoma, mesothelioma, and pulmonary fibrosis (1,2,3). Previous studies have focused on the inhalation of asbestos fibers greater than 5 µm in length and largely disregarded the effect of asbestos fibers less than 5 µm in length.

The purpose of this project is to study the relationship of exposure to chrysotile asbestos fibers less than 5 µm long and the development of chronic, asbestos-associated diseases. Chrysotile asbestos was employed in this project because more than 90% of the asbestos used industrially and commercially is chrysotile (4,5).

MATERIALS AND METHODS

Short Fiber Preparation

The chrysotile used in this study was type 7TF1 chrysotile obtained from the Johns-Manville Sales Corporation in Denver, Colorado. Short asbestos fibers were prepared by drying 500 grams of the fibers in an oven at 191°C. After cooling, the chrysotile was placed in a cylindrical ceramic (Burundum) ball mill (7" x 8½") with 120 cylindrical Burundum pellets, each pellet measuring 13/16" x 10/16". The mill was rotated at 73 rpm for 24 hours after which time the chrysotile was removed and again dried in an oven at 191°C for 24 hours. The asbestos was then cooled and stored in tightly secured, double plastic bags. By
this method of preparation, 99.98% of the resulting chrysotile fibers, as viewed and sized by electron microscopy, were less than 0.6 µm in diameter and about 20% were longer than 5 µm.

In addition to the large and short asbestos fibers, the ball-milling process produced amalgamated "balls" of chrysotile fibers which measured up to 10 µm in diameter. Figures 1, 2, and 3 are scanning electron micrographs (SEM) of the ball-milled asbestos showing the various sizes of fibers and "balls."

It has been reported that ball-milling is not a completely satisfactory method for preparing short asbestos fibers for biological studies (6,7), however ball-milling was the best method available for this project in which the production of more than 100 pounds of short asbestos fibers was required.

Animals

The experimental design incorporated 300 male Sprague-Dawley rats and 20 male cynomolgus monkeys. Each animal was individually identified and randomly assigned, half the rats and monkeys to an asbestos exposure chamber and the other half of the rats and monkeys to a control inhalation chamber. Each rat and monkey was individually housed and provided food (Purina Basal Rat Diet and Purina Monkey Chow, respectively) and water ad libitum, except during the hours of inhalation exposure.
All animals were exposed in 12 m³ inhalation chambers for 7 hours/day, 5 days/week for 18 months. At the end of the 18 month exposure period, the surviving rats, both those exposed to chrysotile and the controls, were scheduled for a 6 month post-exposure observation period of which at the time of this report, 5 months have elapsed.

Table 1 shows the serial sacrifice schedule for the exposed and control rats at 1, 3, 6, 12, and 18 months after the initiation of the asbestos exposure. A terminal sacrifice of all remaining rats will be made at month 24.

TABLE 1

RAT SACRIFICE SCHEDULE

<table>
<thead>
<tr>
<th>Sacrifice Interval and No. of Rats Selected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mo. 3 mo. 6 mo. 12 mo. 18 mo. 24 mo.</td>
</tr>
<tr>
<td>Exposed rats 5 15 15 15 15 85</td>
</tr>
<tr>
<td>Control rats 5 15 15 15 15 85</td>
</tr>
</tbody>
</table>

The experimental design designates that all monkeys, 10 control and 10 asbestos exposed, will be maintained and observed for at least seven years after the last exposure day.

Animal Tissue Diagnostic Tests

Five rats from the exposed group and five rats from the control group were sacrificed at the end of one month's exposure. Lung tissue was taken for
scanning electron microscopy examination for asbestos fibers as well as liver, kidney, spleen, and tracheal and mesenteric lymph nodes for histopathological examination. At the 3, 6, 12, and 18 month exposure intervals, 15 rats/group were sacrificed. Of the 15 rats/group, 5 were used for evaluation by scanning electron microscopy and cytochemical determination. The relative amounts of cellular acid phosphatase, beta-glucuronidase, and lactic dehydrogenase were measured to determine the release of non-membrane bound enzymes and the extent of lysosomal exocytosis.

Samples of lung tissue, liver, kidney, spleen, and tracheal and mesenteric lymph nodes were preserved in 3.0% phosphate-buffered glutaraldehyde for scanning electron microscopy examination. Of the remaining 10 rats/group, blood and half of the left lung were analyzed for silicon (Si) by plasma emission spectroscopy. The other half of each left lung was evaluated for relative amounts of hydroxyproline. The right lung of each animal was fixed in 10% formalin and processed with other body tissues for gross and histopathological examination.

Rats to be sacrificed at 24 months will receive the same evaluations previously described for the interim sacrificed animals with 10 rats/group used for silicon analysis and the remainder for histopathological examination.

Exposed and control monkeys will have the complete cytochemical-silicon analysis-histopathological evaluations conducted at the time of their scheduled sacrifice.
Chamber Asbestos Analysis Methods and Results

Conditioned air (humidity and temperature modified) was used to disperse the asbestos into the dilution air of the 12 m³ chamber and to prevent fibers from adhering to one another. The air flow through the chamber was regulated for six air changes per hour. The chrysotile asbestos in the exposure chamber was measured by three methods: gravimetric analysis, fiber length distribution analysis, and by scanning electron microscopy.

The gravimetric analysis consisted of drawing a sample of air from the exposure chamber at 5.3 liters/minute for 60 minutes through a 37 mm diameter fibrous glass filter. The pre-collection filter weight and post-collection filter weight were used to determine total asbestos collection for a determined volume of sampled air. Three samples were collected at evenly spaced intervals during each 7 hour daily exposure and the concentration expressed in mg/m³. The mean concentration of asbestos in the chamber air for the entire study was $0.95 \text{ mg/m}^3 \pm 0.26$ (S.D.).

The NIOSH P and CAM 239 method was used in a modified form to determine the chrysotile fiber length distribution in the exposure chamber (8). The asbestos concentration was not determined by this method. A sample of chamber air was drawn at a flow rate of 5.3 lpm for 90 minutes through a 37 mm diameter, 0.8 µm pore size cellulose ester filter. The 5.3 lpm differed from the 1.7 lpm to 2.5 lpm prescribed in the NIOSH method. The procedures as described in the NIOSH method were only used to clear the cellulose ester filter and to count asbestos fibers longer than 5 µm in length.

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A small wedge of the filter was placed on a microscope slide and the body of the filter was cleared with a reagent containing dimethyl phthalate and diethyl oxalate. The slide was then viewed at a magnification of 400X in a phase contrast microscope and the sizing of fibers accomplished with the use of a Porton graticule and hand counter. Three chamber atmosphere samples were taken at regular intervals during each 7 hour daily exposure. The mean number of chrysotile fibers/cc greater than 5 µm in length for the entire study was 0.79 fibers/cc ± 0.13 (S.D.).

Electron microscopy was used to monitor the number of fibers/cc less than 5 µm in length. On occasion, the >5 µm fibers were also measured by this method. Chamber air was collected in the same manner used for samples drawn for fiber length distribution except that a polycarbonate filter was substituted for the cellulose ester filter. The filter containing the collected sample was mounted on a carbon planchet and viewed through a scanning electron microscope. Photographs were taken at a magnification of 2,000X and the negatives used to print X5,000 enlargements of the field. The asbestos fibers were then counted and measured by hand. Figure 4 shows a typical photo taken of a sample prepared by this method. Short (<5 µm) and long (>5 µm) asbestos fibers can be seen as well as a few of the previously described asbestos "balls." Figure 5 shows a higher magnification (X20,000) of one of these generated asbestos "balls" collected from the asbestos exposure air. By electron microscopy, the number of fibers/cc <5 µm in length was estimated at 210 (a ratio of 265 fibers/cc <5 µm to each fiber >5 µm).
DISCUSSION

At the time of this report, there are less than two months of maintenance and observation to complete before the June, 1980, terminal sacrifice of the rats. The final steps are being taken to arrange the long-term holding of the control and exposed monkeys.

The monthly body weights of both exposed and control rats and monkeys have indicated normal weight gains. Forty-six rats (23 exposed and 23 control) have died or were sacrificed moribund. No pharmacotoxic signs were seen that could be associated with exposure to chrysotile. Lung tissue from serial sacrificed rats through the 18 month sacrifice has revealed little or no pathological reaction to the inhaled asbestos (Figure 6).

Some short asbestos fibers (0.5-1.0 µm) have been seen in the alveolar macrophages by scanning transmission electron microscopy. Due to the relatively small number of macrophages seen in the alveoli, pieces of rat lung 1.0 cc in size were ashed in a low-temperature plasma oven. The ash residue was then suspended in distilled water and filtered on a polycarbonate filter. Scanning electron microscopy examination of this filter revealed a number of short chrysotile fibers and "balls" as seen in Figure 7.

The positive identification of chrysotile asbestos in both alveolar macrophages and in the low-temperature, plasma-ashed rat lung tissue was accomplished by energy dispersive x-ray analysis.
The contractor is compiling the data on the interim rat sacrifice silicon assays and the other cytochemical tests for the final report due in September, 1980. No tests of tissues have been taken on the two groups of monkeys since none have been sacrificed or died thus far during the study.

It is hoped that the results of this study will be of significant value in the continuing assessment and updating of data on a highly hazardous industrial and commercial substance.
REFERENCES


Figure 1. Ball-milled chrysotile (original X400, SEM).

Figure 2. Ball-milled chrysotile showing numerous short asbestos fibers among a few large fibers and "balls" (original X5,000, SEM).

Figure 3. Chrysotile asbestos "balls" composed of numerous short asbestos fibers (original X20,000, SEM).

Figure 4. Inhalation chamber sample of chrysotile fibers (original X5,000, SEM).

Figure 5. Inhalation chamber sample of chrysotile "ball" (original X20,000, SEM).

Figure 6. Terminal bronchus of 12-month chrysotile exposed rat (original X200).

Figure 7. Chrysotile fibers and "balls" from low-temperature ashed asbestos-exposed rat lung (original X5,000, SEM).
Figure 3
Discussion

Dr. O'Conor, NCI: Yes, that is an elegant study. I have two questions. One, you say this is to test the chronic effects, I guess in these animals, but you are sacrificing them all six months after the first exposure, no, I mean after the termination of the exposure.

Mr. Platek, NIOSH: You must realize that the average lifespan of the rat is two years.

Dr. O'Conor, NCI: I was not thinking of the rats. I was thinking of monkeys.

Mr. Platek, NIOSH: Let me explain. I was appointed project director about one year ago. The design was set up by another gentleman at NIOSH. There is talk at this moment of possibly doing pulmonary function tests on these monkeys so the data will not be wasted. Granted the monkeys will live much longer than rats.

Dr. O'Conor, NCI: Have any of the monkeys been sacrificed to date?

Mr. Platek, NIOSH: No, none whatsoever. They will all be sacrificed or are scheduled for sacrifice the 25th of June.

Dr. O'Conor, NCI: Is there any possible consideration of just holding the monkeys rather than sacrificing?

Mr. Platek, NIOSH: I would have to speak with Dr. Groth on that question, but right now there is no consideration.

Dr. O'Conor, NCI: The other point is what do you think will be the implication, let us say that these experiments are negative; what do you think will be the implication in terms of the industry and regulatory action?

Mr. Platek, NIOSH: We were concerned in talking this over, that is, where would a worker be occupationally exposed to purely small asbestos fibers, and we don't know. Brake shoe repair operations might be one of the few places, but as I mentioned before, in any preparation or any exposure, long fibers will be present. However, this project was designed to test a standard of two fibers per cc, greater than 5 micrometers in length. In that standard you don't take into consideration any of these smaller asbestos fibers, and as you can see, our chamber samples here meet that standard and are below it. In fact, they are less than half of the federal standard. We can show that, but you can also see there are multitudes of these small fibers. This is why the project was conducted to determine when only these small fibers are present, do they have any adverse biological effect.
Dr. Burton, NCI: Was there any physical effect on the fibers of superheating them several times compared to fibers that might have been obtained in the original physical condition?

Mr. Platek, NIOSH: I know what you are saying. There have been numerous reports that heating, sonicating, even ball milling of the asbestos can create problems. We have done no tests to my knowledge to test whether the crystal structure of the asbestos was altered. This can be determined by x-ray and electron diffraction analysis. We have within the last year received the proper equipment on our transmission scope that we can do this type of analysis. That has not been done to date, but it will be performed and the results will accompany the final report.

Dr. Cameron, NCI: Just a further comment on the primate aspect. As a rhesus monkey they should have a lifespan--

Mr. Platek, NIOSH: Cynamolgus.

Dr. Cameron, NCI: Cynamolgus. They are still rhesus, the rhesus families have a lifespan in excess of 20 years. So that is minimal. I have a question though, are you aware of the NIEHS asbestos study at a local lab? I don't know the particulars. I wonder if you do?

Mr. Platek, NIOSH: I have been told, in fact, we just learned last week, there is evidently a lab that is doing an asbestos study; an intra-tracheal study of short asbestos fibers. I have no idea how they are doing it, but I have been told that it is a one-dose intra-tracheal injection study. I have no idea of the dose, how they prepared the asbestos, their source was or the size range of the asbestos.

Dr. Lee, EPA: I would like to make a comment. If I am not mistaken, within the EPA at our TP laboratory we have also undertaken a chronic study of the asbestos in rats. I am not too familiar with this project. I wonder if Dr. Waters is at liberty to give us some information? Dr. Waters is not here. As I say, we understand that project is, also, near the end, and that is a two year study for the rats. You may want to get in touch with them. The project is headed by Dr. Coffin.

Dr. Brown, OSHA: Could you elaborate a little bit on the splitting of these particular asbestos fibers?

Mr. Platek, NIOSH: The splitting as far as the process we used?

Dr. Brown, OSHA: Right.

Mr. Platek, NIOSH: We did ball mill these asbestos fibers. I explained the method in which they were dried, ball milled for 24 hours and we dried them again, and they they were shipped to the contractor.
Dr. Brown, OSHA: Specifically what I mean was, was it vertically or horizontally splitting?

Mr. Platek, NIOSH: The ball milling split the asbestos bundles as well as breaking the fibers into shorter lengths. As I mentioned, asbestos "balls" were also created.

Dr. Brown, OSHA: The second question is where are the areas of the greatest deposition?

Mr. Platek, NIOSH: I honestly don't know yet. As I said, or pointed out in the slide that I showed a while back with the terminal bronchus, if you were seeing a lot of asbestos in that lung you would probably expect to see the asbestos fibers near the lymphatics and near the major blood vessels, but we have not seen them. Once again, we are talking about fibers that when viewed by the light microscope are going to be far beyond the range of light microscopy to resolve, and to develop a technique to do it by scanning electron microscopy is another interest of mine in this project.

Dr. Lee, EPA: One more question?

Dr. Hegyeli, NCI: There are studies indicating that the physical size, the diameter and the lengths of the fiber has much more importance in the physiological response than the nature, the chemical nature of the substance, including glass, metal and other fibers. So, my question is what does this study mean physiologically?

Mr. Platek, NIOSH: From what we have seen so far, as in the previous slide that you saw of the electron micrograph of the macrophage containing the asbestos fibers, it would appear the macrophage is doing its job, and it is engulfing the asbestos. There was no adverse effect in that macrophage that we could see, and it looks like they could be clearing themselves as they are supposed to do. That would be of significance in hopefully determining that the short fibers really don't produce the problems by remaining in the lung, and as you stated, it has been pointed out by numerous investigators that the macrophages have difficulty engulfing the long fibers, and therefore you have the influx of fibroblasts, the laying down of fibrin and then your fibrosis sets up. I hope that answers your question.

Dr. Hegyeli, NCI: My question is that in a practical sense you never encounter these type of fibers. You have mixed fibers, and most of them are in the range, and as you indicated with the macrophage, at least there are some scanning electron micrograph studies indicating that if it occurs up inside the cell, it might serve really as a factor.

Mr. Platek, NIOSH: I don't really know how to answer you any further than what I have on that one. I said that all environments with the possible exception of some of the brake shoe removal operations where the asbestos
is under extreme pressures and can be broken down into much smaller fibers, you are going to have large fiber lengths and the smaller ones in all exposures. This was, once again, mainly a project of testing a federal standard and not where will the worker be exposed to these short fibers because as of right now I do not know of a work environment that is strictly small fiber exposure.
MUTAGENICITY TESTING OF SELECTED INDUSTRIAL CHEMICALS

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The mutagenicity of 147 industrial chemicals and structurally related compounds have been studied by the Utah Biomedical Test Laboratory at Salt Lake City, Utah, under the contract with the National Institute for Occupational Safety and Health. The Salmonella typhimurium-microsome plate incorporation test developed by Ames and Co-workers was used as the assay system. The assays were conducted with the tester strains TA 1535, TA 1537, TA 98 and TA 100 in the presence and absence of S-9 prepared from the liver of Aroclor 1254 pretreated Sprague Dawley rats.

The results of these studies indicate that 120 of 147 compounds were not mutagenic to any of the testers tested with or without metabolic activation. Twenty-three compounds were directly mutagenic to one or more tester strains, and the remaining four compounds required metabolic activation for their mutagenic activities.
INTRODUCTION

It is well documented that many synthetic and naturally occurring compounds can interact with the genetic material and cause mutations in somatic and/or germ cells. Induction of mutations in these cells may lead to one or more of the following deleterious effects: Genetic disease, malformation, spontaneous abortion, and cancer. Recent studies have estimated that more than 1000 known genetic disorders and diseases can be related to gene mutations. It has been estimated that approximately 50% of all spontaneous abortions involve chromosomal defects and more than 0.5% of total live births in the United States carry clinically serious chromosomal aberrations. The large number of chemicals created by modern industrial technology may account for this prevalence of genetic diseases and disorders and for the high incidence of cancer noted in humans in recent years.

During the past decade many short-term tests for mutagenesis have been developed. Among these tests, the histidine reverse mutation system of Salmonella typhimurium developed by Ames and co-workers is probably the most sensitive and useful system for mutagenesis testing. In this test system, the use of in vitro metabolic activation has helped detect the mutagenic activity of promutagens. By using different tester strains, mutagenic specificity (base-pair substitution vs. frameshift mutation) of chemicals can be determined. With this test system, McCann et al. have shown that there is an excellent correlation between mutagenicity and carcinogenicity among the 300 compounds studied.

Many industrial workers are routinely exposed to occupation-related chemicals. To protect these workers from any potential mutagenic and
carcinogenic hazards, it is necessary to detect and identify the mutagenic activity of industrial and related chemicals. A contract was, therefore, initiated by NIOSH in 1977 for Utah Biomedical Testing Laboratory to study the mutagenic activity of 147 industrial chemicals and related compounds in the Salmonella/microsomal assay system.

MATERIALS AND METHODS

Bacterial Strains

The bacteria used in this study were *Salmonella typhimurium* strains TA 98, TA 100, TA-1535 and TA 1537. They were provided by Dr. Bruce N. Ames, University of California at Berkeley. Upon receipt, each strain was subjected to the appropriate procedures to confirm its genotype. Strains were reisolated, genotypes tested, and new frozen stocks prepared at bimonthly intervals. Stocks were stored at -80° C, and inocula were prepared fresh for each experiment by subculture in nutrient broth.

Mutagenesis Assay

Most chemicals were tested by the plate incorporation method. Some chemicals were tested by the pre-incubation technique. Details of the methodology of Salmonella/microsome mutagenesis assay have been described by Ames and co-workers³. A brief description of both assay systems is as follows:

1. Plate Incorporation Assay

   Agar plates containing histidine-deficient Vogel Bonner Medium E⁶ and fortified with 2% glucose were prepared and incubated overnight at 37° C prior to use (VBME plates). Aliquots of top agar (0.6% Bacto-agar in 0.5% NaCl) were melted on the day they
were to be used and maintained at 45° C. Each 100 ml aliquot of top agar was supplemented with a final concentration of 0.05 mM histidine and 0.05 mM biotin immediately before use. Five ml of a 16 hour nutrient broth culture of the appropriate Ames' tester strain of *Salmonella typhimurium* was added to the 100 ml aliquot of top agar, and the mixture transferred to sterile, disposable screw capped tubes in 2.0 ml aliquots and held at 45° C. Test materials were added to the tubes, mixed, and the contents of the tube were poured onto the surface of VBME plates. All plates were incubated at 37° C for three days, and the number of colonies was determined using a manual Quebec colony counter.

*S-9* was prepared from livers of male Sprague-Dawley rats (150-200 g) injected with Aroclor 1254 at a dose of 1000/mg/kg five days prior to sacrifice. For tests in the presence of *S-9*, each tube containing top agar, inoculum and test material received 0.5 ml of *S-9* mix containing 0.45 ml of *S-9* base and 0.05 ml of *S-9* fraction. Tube contents were poured onto the surface of VBME plates immediately after addition of the *S-9*.

2. Pre-incubation Assay (as above with the following exceptions)

Test materials were added to sterile, disposable screw capped tubes and inoculated with 0.1 ml of a 16 hour nutrient broth culture of the appropriate Ames' tester strain of *Salmonella typhimurium* (TA 1535, TA 1537, TA 98, or TA 100). The tubes were incubated at 37° C for 30 minutes with shaking. Two ml aliquots of top agar at 45° C were then added to the
tubes, the tubes were mixed, and the contents were poured onto the surface of VBME plates. For tests in the presence of S-9, each tube containing sample plus inoculum received 0.5 ml of S-9 mix prior to the 30 minute incubation at 37° C.

Five different doses of each chemical were tested with and without microsomal activation. The highest concentration used was limited by solubility and toxicity. Five different concentrations of S-9 (10, 20, 30, 40 and 50 µl per plate) were used for each dose in the plate incorporation test while only one concentration of S-9 (50 µl/plate) was used for each dose in the preincubation test. All tests were performed in duplicate and each compound was tested along with positive and negative controls. The positive control compounds used are: Propylene oxide for TA 1535 and TA 100, 9-aminoacridine for TA 1537, and 2-nitrofluorene for TA 98. Ethidium bromide was used as a positive control compound for metabolic activation. All gases and volatile compounds were tested in a sealed jar.

**Chemicals**

Chemicals used in this study, with the exception of platinum related compounds, were obtained from commercial chemical companies. Platinum related compounds were provided by Dr. Dave Groth, NIOSH, Cincinnati, Ohio. Chemicals were dissolved in sterile distilled water (whenever feasible) or in dimethyl sulfoxide immediately before used.
RESULTS

The results are shown in Tables 1 and 2. Among the 147 compounds tested, 120 were not mutagenic to any of the testers either with or without metabolic activation. Twenty-three compounds were directly mutagenic to one or more than one tester. The remaining four compounds (1,2,3-trichloropropane, dimethoxyethylphthalate, 2,4-dimethylaniline and 2,6-dinitrotoluene) required metabolic activation for their mutagenic activities. A compound is classified as mutagenic if it causes a dose related increase in the number of revertants and the increase is more than two times the background level. The ranges of background revertants were 10-50 for TA 1535, 5-25 for TA 1537, 16-90 for TA 98 and 100-350 for TA 100.

Two (nitromethane and monochloroethane) of the 27 mutagenic compounds were mutagenic only for TA 1535 and two other compounds (dimethoxyethylphthalate and n-nitrosoaniline) were mutagenic only for TA 1537. Pt(bipyridyl)Cl$_2$ and 2,4-dimethylaniline were mutagenic only for TA 98 and TA 100, respectively. Several compounds were mutagenic only for one or two testers if tested without in vitro microsomal activation. However, they were mutagenic for more than two testers if tested with S-9 from liver of Aroclor 1254 pretreated rats. Some compounds showed higher mutagenic activity when a low concentration (20 µl/plate) of S-9 was used, whereas other compounds required a higher concentration (50 µl/plate) of S-9 for mutagenic activity.
DISCUSSION

In this study, we found that industrial chemicals such as 1,2,3-trichloropropane, nitromethane, 2-nitropropane, methyl bromide, methylene chloride, monochloroethane, 2,3-dinitrotoluene, 2,5-dinitrotoluene, 2,6-dinitrotoluene, 2,4,5-trinitrotoluene, p-dinitrobenzene, and m-dinitrobenzene are mutagenic for Salmonella typhimurium. More studies need to be conducted in mammalian and other submammalian mutagenesis test systems, and the potential mutagenic and carcinogenic hazards of these compounds for the exposed population need to be determined.

Several interesting phenomena were noted in this study. PtK₂Cl₄ was mutagenic for TA 98 and TA 100 if it was dissolved in water. If dimethyl sulfoxide (DMSO) was used as the solvent, however, negative results were obtained. It seems that DMSO could interact with PtK₂Cl₄ and inhibit or diminish the mutagenic activity of this compound. This result emphasizes the importance of making an effort to dissolve chemicals in aqueous solution for mutagenesis testing.

S-9 from the liver of mammals can activate promutagens to mutagenic metabolites and, in some instances, enhance the mutagenic activity of directly acting mutagens. A concentration of 50 µl S-9 per plate is used by most laboratories for mutagenesis testing. In this study, however, the best mutagenic response of several compounds was found when a lower concentration of S-9 (20 µl/plate) was used. Recently, we have also found that m-aminophenol and the dye, direct blue-15 are mutagenic only when these compounds were tested with S-9 from hamsters.
There appears to be a structure and function relationship among several structurally related chemicals studied. For instance, nitrophenol is mutagenic if hydroxy and nitro groups are in a meta arrangement, but not mutagenic if both groups are in an otho arrangement. Similar results were found with dinitrobenzene. m-dinitrobenzene was mutagenic but o-dinitrobenzene was not.
REFERENCES


<table>
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<tr>
<th>Compounds That Did Not Exhibit Mutagenic Activity for <em>Salmonella Typhimurium</em></th>
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</table>

**Alicyclic and Heterocyclic Compounds:**
- Adenine (1500 µg)\(^a\)
- Dipyridyl (5000 µg)
- Furfuryl alcohol (15 µl)
- Hexachlorocyclopentadiene (0.0002 µl)
- 2-mercaptobenzothiazole (500 µg)
- N-methyldicyclohexylamine (10 µl)

**Aliphatic Amines:**
- sec-butylamine (10 µl)
- 2-dibutylamino ethanol (50 µl)
- 2-diethylamino ethanol (30 µl)
- Ethanolamine (30 µl)
- Hexamethylenediamine (3 µg)
- Hexamethylenetetramine (5 µg)
- n-hexylamine (4.5 µl)
- n-pentylamine (8 µl)
- n-propylamine (10 µl)

**Aliphatic Carboxylic Acids and Other Aliphatic Compounds:**
- Acetone cyanohydrin (0.05 µl)
- Acetonitrile (300 µl)
- Acrolein (0.2 µl)
- Acrylamide (50 µg)
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<td>Diethylenetriamine pentaacetic acid</td>
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<tr>
<td>Tetramethyl succinonitrile</td>
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<td>1,2,4-trimethylbenzene</td>
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**Metals, Metal Salts, and Organometallics:**

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<tr>
<td>Bis (2-pyridinaldoximinato) Pt(II)</td>
<td>300 µg</td>
</tr>
<tr>
<td>Dichloro (2-formimidoyl pyridine) Pt(II)</td>
<td>100 µg</td>
</tr>
<tr>
<td>H(Pt adenine Cl$_3$)</td>
<td>300 µg</td>
</tr>
</tbody>
</table>

**Nitro Aromatics:**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>o-dinitrobenzene</td>
<td>200 µg</td>
</tr>
<tr>
<td>4,6-dinitro-o-cresol</td>
<td>150 µg</td>
</tr>
<tr>
<td>2,4-dinitrophenol</td>
<td>250 µg</td>
</tr>
<tr>
<td>2,6-dinitrophenol</td>
<td>250 µg</td>
</tr>
<tr>
<td>2,3-dinitrotoluene</td>
<td>50 µg</td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>400 µg</td>
</tr>
<tr>
<td>3,4-dinitrotoluene</td>
<td>500 µg</td>
</tr>
<tr>
<td>Metaoxon</td>
<td>2000 µg</td>
</tr>
<tr>
<td>Mononitrobenzene</td>
<td>4 µl</td>
</tr>
<tr>
<td>o-nitrophenol</td>
<td>500 µg</td>
</tr>
<tr>
<td>p-nitrophenol</td>
<td>500 µg</td>
</tr>
<tr>
<td>2,4,6-trinitrophenol</td>
<td>1500 µg</td>
</tr>
<tr>
<td>Solvents:</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Acetic acid (500 µl)</td>
<td></td>
</tr>
<tr>
<td>Acetone (500 µl)</td>
<td></td>
</tr>
<tr>
<td>Benzene (500 µl)</td>
<td></td>
</tr>
<tr>
<td>Cyclohexanone (50 µl)</td>
<td></td>
</tr>
<tr>
<td>N, N-dimethylacetamide (500 µl)</td>
<td></td>
</tr>
<tr>
<td>Dimethylformamide (500 µl)</td>
<td></td>
</tr>
<tr>
<td>Dimethylsulfoxide (500 µl)</td>
<td></td>
</tr>
<tr>
<td>Ethanol (500 µl)</td>
<td></td>
</tr>
<tr>
<td>2-ethoxyethanol (20 µl)</td>
<td></td>
</tr>
<tr>
<td>Glycerol (500 µl)</td>
<td></td>
</tr>
<tr>
<td>2-methoxyethanol (500 µl)</td>
<td></td>
</tr>
<tr>
<td>Phosphoric acid, in H₂O, pH 4 (500 µl)</td>
<td></td>
</tr>
<tr>
<td>Potassium acid phthalate (2%) (500 µl)</td>
<td></td>
</tr>
<tr>
<td>Sodium hydroxide, in H₂O, pH 9.5 (500 µl)</td>
<td></td>
</tr>
<tr>
<td>Sodium phosphate, dibasic (2%) (500 µl)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Miscellaneous:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant 2246 (200 µg)</td>
</tr>
<tr>
<td>Butylene oxide (100 µl)</td>
</tr>
<tr>
<td>Carbon disulphide (100 µl)</td>
</tr>
<tr>
<td>Cyanogen (0.01%)</td>
</tr>
<tr>
<td>Geltrol (20 µl)</td>
</tr>
<tr>
<td>α-(histamine AlBr₃)⁺ (2000 µg)</td>
</tr>
<tr>
<td>Hydrogen sulphide (0.1%)</td>
</tr>
</tbody>
</table>
### TABLE 1 (CONT'D)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropyl isocyanate (0.01 µl)</td>
<td></td>
</tr>
<tr>
<td>Methyl parathion (300 µg)</td>
<td></td>
</tr>
<tr>
<td>Nitrous oxide (90.6%)</td>
<td></td>
</tr>
<tr>
<td>Phenyl isocyanate (0.01 µl)</td>
<td></td>
</tr>
<tr>
<td>Phosgene (0.0001%)</td>
<td></td>
</tr>
<tr>
<td>O-terphenyl (5000 µg)</td>
<td></td>
</tr>
<tr>
<td>p-terphenyl (1500 µg)</td>
<td></td>
</tr>
<tr>
<td>Tert-butyl isocyanate (0.1 µl)</td>
<td></td>
</tr>
<tr>
<td>(Tetrazene A1Br$_2$)$^+$ (500 µg)</td>
<td></td>
</tr>
<tr>
<td>p-toluene sulfonyl isocyanate (2 µl)</td>
<td></td>
</tr>
</tbody>
</table>

*aThe number in parentheses represents the highest concentration of chemical tested in terms of quantity per plate.*
TABLE 2

Compounds with Mutagenic Activity in *Salmonella* *Typhimurium*

<table>
<thead>
<tr>
<th>Compound</th>
<th>S-9a</th>
<th>Dose/Plate</th>
<th>TA 1535</th>
<th>TA 1537</th>
<th>TA 98</th>
<th>TA 100</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alicyclic and Heterocyclic Compounds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vinyl cyclohexene dioxide</td>
<td>+</td>
<td>15 µl</td>
<td>669 (9)</td>
<td>28 (12)</td>
<td>283 (78)</td>
<td>&lt;2000 (226)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>15 µl</td>
<td>647 (11)</td>
<td>-</td>
<td>-</td>
<td>&lt;2000 (285)</td>
</tr>
<tr>
<td><strong>Aliphatic Carboxylic Acids and Other Aliphatic Compounds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethyl carbamoyl chloride</td>
<td>+e</td>
<td>200 µl</td>
<td>82 (16)</td>
<td>21 (9)</td>
<td>157 (59)</td>
<td>896 (183)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>200 µl</td>
<td>107 (43)</td>
<td>-</td>
<td>-</td>
<td>690 (261)</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>+</td>
<td>0.1 %f</td>
<td>&gt;2000 (12)</td>
<td>-</td>
<td>-</td>
<td>&gt;2000 (195)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0.1 %f</td>
<td>592 (12)</td>
<td>-</td>
<td>-</td>
<td>1640 (215)</td>
</tr>
<tr>
<td>2-nitropropane</td>
<td>+</td>
<td>50 µl</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>&gt;4000 (NT)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>50 µl</td>
<td>NT</td>
<td>-</td>
<td>NT</td>
<td>&gt;4000 (141)</td>
</tr>
<tr>
<td>Methyl bromide</td>
<td>+</td>
<td>0.53 %f</td>
<td>124 (8)</td>
<td>-</td>
<td>-</td>
<td>710 (159)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0.53 %f</td>
<td>28 (13)</td>
<td>-</td>
<td>-</td>
<td>700 (151)</td>
</tr>
<tr>
<td>Methylene chloride</td>
<td>+</td>
<td>500 µl</td>
<td>70 (22)</td>
<td>-</td>
<td>632 (58)</td>
<td>&gt;2000 (142)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>500 µl</td>
<td>88 (33)</td>
<td>-</td>
<td>190 (26)</td>
<td>1836 (170)</td>
</tr>
<tr>
<td>Compound</td>
<td>S-9a</td>
<td>Dose/Plate</td>
<td>No. of His⁺ Revertants/Plate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>------</td>
<td>------------</td>
<td>-----------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TA 1535</td>
<td>TA 1537</td>
<td>TA 98</td>
<td>TA 100</td>
</tr>
<tr>
<td>Monochloroethane</td>
<td>+</td>
<td>5000 µl</td>
<td>594 (14)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>5000 µl</td>
<td>159 (18)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitromethane</td>
<td>+</td>
<td>50 µl</td>
<td>146 (19)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>50 µl</td>
<td>105 (36)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1,2,3-Trichloropropane</td>
<td>+</td>
<td>0.5 µl</td>
<td>385 (21)</td>
<td>100 (9)</td>
<td>-</td>
<td>&gt;2000 (219)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0.5 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dimethoxyethylphthalate</td>
<td>+</td>
<td>100 µl</td>
<td>-</td>
<td>34 (8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>100 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2,4-dimethylaniline⁺</td>
<td>+</td>
<td>2 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>446 (194)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>2 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N-nitrosoaniline</td>
<td>+</td>
<td>100 µg</td>
<td>-</td>
<td>75 (14)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>100 µg</td>
<td>-</td>
<td>143 (13)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Metals, their Salts and Organometallics</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Pt(bipyridine)(adenine)]Cl₂</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>40 µg</td>
<td>-</td>
<td>-</td>
<td>511 (40)</td>
<td>543 (221)</td>
</tr>
<tr>
<td>Compound</td>
<td>S-9a</td>
<td>Dose/Plate b</td>
<td>No. of His(^+) Revertants/Plate c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>------</td>
<td>--------------</td>
<td>-------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TA 1535</td>
<td>TA 1537</td>
<td>TA 98</td>
<td>TA 100</td>
<td></td>
</tr>
<tr>
<td>Pt(bipyridyl)Cl(_2)</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>10 µg</td>
<td>-</td>
<td>-</td>
<td>709 (50)</td>
<td>-</td>
</tr>
<tr>
<td>Pt K(_2) Cl(_4)</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>100 µg</td>
<td>-</td>
<td>-</td>
<td>267 (40)</td>
<td>689 (221)</td>
</tr>
<tr>
<td><strong>Nitroaromatics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m-dinitrobenzene</td>
<td>+(^e)</td>
<td>200 µg</td>
<td>-</td>
<td>-</td>
<td>219 (41)</td>
<td>362 (180)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>200 µg</td>
<td>-</td>
<td>-</td>
<td>160 (21)</td>
<td>569 (255)</td>
</tr>
<tr>
<td>p-dinitrobenzene</td>
<td>-(^e)</td>
<td>50 µg</td>
<td>-</td>
<td>-</td>
<td>90 (48)</td>
<td>407 (172)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>50 µg</td>
<td>-</td>
<td>-</td>
<td>505 (44)</td>
<td>565 (209)</td>
</tr>
<tr>
<td>2,5-dinitrophenol</td>
<td>+(^e)</td>
<td>20 µg</td>
<td>-</td>
<td>-</td>
<td>142 (64)</td>
<td>324 (193)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>20 µg</td>
<td>-</td>
<td>-</td>
<td>351 (57)</td>
<td>-</td>
</tr>
<tr>
<td>2,5-dinitrotoluene</td>
<td>+(^e)</td>
<td>200 µg</td>
<td>61 (21)</td>
<td>-</td>
<td>112 (49)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>100 µg</td>
<td>-</td>
<td>-</td>
<td>233 (35)</td>
<td>-</td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
<td>+</td>
<td>2000 µg</td>
<td>-</td>
<td>57 (19)</td>
<td>337 (88)</td>
<td>485 (243)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>2000 µg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Compound</td>
<td>S-9a</td>
<td>Dose/Plate</td>
<td>TA 1535</td>
<td>TA 1537</td>
<td>TA 98</td>
<td>TA 100</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------</td>
<td>------------</td>
<td>---------</td>
<td>---------</td>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>1-nitronaphthalene</td>
<td>+e</td>
<td>100 µg</td>
<td>-</td>
<td>-</td>
<td>82 (38)</td>
<td>1000 (332)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>100 µg</td>
<td>-</td>
<td>-</td>
<td>76 (22)</td>
<td>1080 (300)</td>
</tr>
<tr>
<td>m-nitrophenol</td>
<td>+e</td>
<td>500 µg</td>
<td>36 (18)</td>
<td>-</td>
<td>127 (33)</td>
<td>505 (196)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>500 µg</td>
<td>-</td>
<td>-</td>
<td>205 (20)</td>
<td>-</td>
</tr>
<tr>
<td>Paraoxon</td>
<td>+</td>
<td>4 µl</td>
<td>54 (16)</td>
<td>-</td>
<td>-</td>
<td>536 (198)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>4 µl</td>
<td>71 (35)</td>
<td>-</td>
<td>-</td>
<td>565 (231)</td>
</tr>
<tr>
<td>2,4,5-trinitrotoluene</td>
<td>+e</td>
<td>10 µg</td>
<td>-</td>
<td>-</td>
<td>96 (52)</td>
<td>486 (178)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>10 µg</td>
<td>-</td>
<td>-</td>
<td>279 (44)</td>
<td>384 (209)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propylene oxide</td>
<td>+e</td>
<td>100 µl</td>
<td>168 (16)</td>
<td>-</td>
<td>-</td>
<td>2500 (332)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>100 µl</td>
<td>120 (20)</td>
<td>-</td>
<td>60 (22)</td>
<td>2300 (300)</td>
</tr>
<tr>
<td>Styrene oxide</td>
<td>+</td>
<td>5 µl</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>&gt;4000 (199)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>5 µl</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>&gt;4000 (153)</td>
</tr>
<tr>
<td>Compound</td>
<td>S-9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Dose/Plate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>TA 1535</td>
<td>TA 1537</td>
<td>TA 98</td>
<td>TA 100</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------------</td>
<td>------------------------</td>
<td>---------</td>
<td>---------</td>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>Tetramethyl thiuram disulphide</td>
<td>+</td>
<td>25 µg</td>
<td>43 (12)</td>
<td>-</td>
<td>-</td>
<td>510 (171)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>100 µg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>453 (191)</td>
</tr>
</tbody>
</table>

<sup>a</sup> = Tested with metabolic activation; - = Tested without metabolic activation.

<sup>b</sup> Dose which gave the highest mutagenic response.

<sup>c</sup> Number of revertants is an average of 2 plates; Number of spontaneous revertants is shown in parentheses; NT = not tested; - = Number of revertants is less than 2 times of the background.

<sup>d</sup> Tested by the pre-incubation assay system.

<sup>e</sup> The concentration of S-9 is 20 µl/plate rather than 50 µl/plate. Number of revertants decreased when 50 µl S-9/plate was used.

<sup>f</sup> Percent in air.
### TABLE 3
Summary of the Test Results

<table>
<thead>
<tr>
<th>Types of Compounds Tested</th>
<th>No. of Compounds Tested</th>
<th>No. of Compounds Found to be Mutagenic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without Activation</td>
</tr>
<tr>
<td>Alicyclic and Heterocyclic</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Aliphatic Amines</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Aliphatic Carboxylic Acids and other Aliphatics</td>
<td>38</td>
<td>7</td>
</tr>
<tr>
<td>Aromatic Amines and Aromatic Hydrocarbons</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td>Metals, their Salts and Organometallics</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Nitroaromatics</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>Solvents and Reagents</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>147</td>
<td>23</td>
</tr>
</tbody>
</table>
Discussion

Dr. O'Connor, NCI: I could not read the slide of the list, and you say you will have the list, but could you just tell us were there some metals that were positive?

Dr. Elliott, NIOSH: As you know, if you test the metals like cobalt, nickel and cadmium, they are not positive in the standard Ames' assay. The compounds that were positive in this study were organic platinum compounds. Dr. Groth is interested in beryllium compounds and in the way that they cause cancer. He has synthesized the platinum compounds and then he is going to repeat the same thing with beryllium. We do not have the data on those compounds, but beryllium metal and its salts, beryllium chloride, beryllium nitrate, and beryllium oxide, were negative.

Dr. Lee, EPA: On the same line, I have a question. I noticed that on your list there you did some of the toluene group, and you have about three or four different isomers. Did you check on most of those?

Dr. Elliott, NIOSH: Yes.

Dr. Lee, EPA: The reason is when I was at a Middle West research institute we undertook an extensive mammalian toxicity study for the munitions compounds, the compounds important to the Army, and in one group is trinitrotoluene and dinitrotoluene. Most of the isomers, unfortunately I don't remember which is positive and which is negative and this data has not been in the literature, but it is in the extensive report to the Army.

Dr. Elliott, NIOSH: We have looked at several dinitrotoluenes. The 2,3, 2,5 and 2,6 were negative, and I cannot remember which ones I had up on the slide. It does not make any difference, but--

Dr. Lee, EPA: You had three of them.

Dr. Elliott, NIOSH: The 2,5 and 2,6 dinitrotoluenes were positive along with trinitrotoluene.

Dr. Lee, EPA: One of the derivatives is the nitroaminotoluene, and if I remember that is a positive. I understand that, also, is one of the metabolites, both by bacteria reaction and, also, biological changes in the high species. That is, also, positive.
FIGURE CAPTIONS

Figure 1. Mean daily percent of intervals in which the chop sprayer and gelcoat sprayers 1, 2, and 3 placed molds properly within the spray booths, during baseline and training.

Figure 2. Mean daily work-duration, breathing zone exposures (ppm) for the resin-chop sprayer, rollout person, gelcoat sprayers 1, 2, and 3 and mold repair person, during baseline and training. Means across baseline and training conditions are denoted by (----).

NO DISCUSSION FOLLOWING THIS PAGE

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A Strategy to Validate Work Practices:
An Application to the Reinforced Plastics Industry

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Reprint requests to Department of Human Development, University of Kansas, Lawrence, Kansas, 66045 (Dr. Hopkins).
Most recommendations for work practices appear to be based on common sense rather than an empirical analysis of the value of those practices to reduce exposures. A strategy for validating recommended work practices is presented and applied to a reinforced plastics manufacturing plant. Selected employees were trained to use several work practice behaviors judged likely to reduce their exposures to styrene. Observational data indicated that all of the work practices, with the exception of respirator usage, changed as desired. Indices of personal exposures decreased by up to 74 percent following training for the workers with the greatest exposures and, potentially, the most control over their exposures. The research is presented as a model which could be generally applied to validate work practices and to develop methods by which workers can be trained to participate in their own occupational health protection.
Recommendations of industrial hygiene and occupational medicine commonly include work practices designed to protect people from hazards. Examples may be found in sections on work practices contained in criteria documents published by the National Institute for Occupational Safety and Health (NIOSH)\(^1,2\). The execution of all work practices is some form of human behavior. Although work practices are widely recommended, rarely have researchers attempted to validate them by demonstrated usefulness to reduce hazards. Moreover, when there have been attempts to validate work practices, the research methodology has not provided for conclusive inferences. In a literature search, the authors found 254 publications that included various recommendations for work practices, but only 8 of them included measures of hazards prior to, and after the practices were recommended. Moreover, the recommended work behaviors were not measured in any of the eight papers which reported reduced hazards. Therefore, it can not be inferred that the use of the recommended work practices was responsible for the reduced hazards.

Fitch, Hermann and Hopkins\(^3\) outlined a strategy for applying technologies of behavioral science to safety problems in 1976. Central characteristics of the strategy are that health-endangering and health-promoting behaviors of workers are involved in individual exposure to hazards, and that technology is available to measure the relevant behaviors, to change them in prescribed ways, to maintain those that are acceptable, and to evaluate the effectiveness of the behaviors to reduce the hazards. The present paper extends that strategy to the validation of work practices, particularly to work-practices designed or selected to control exposures.
to toxic substances. By including measurement of use of relevant work practices (behaviors) and measurement of associated hazards, the strategy overcomes the inadequacies of methods employed in previous reports, and provides for the relatively complete validation of recommended work practices. To demonstrate the strategy outlined above and the effectiveness of certain work practices to reduce exposures, workers exposed to styrene in the reinforced plastics industry were chosen for study.

Styrene and reinforced plastics manufacturing were convenient for an initial test of the strategy because many examples of human behavior involvement with exposures result from open, person-performed processes and there are a wide range of measurable exposures which could possibly be reduced.

Human subjects exposed to styrene develop irritation of the mucous membranes, particularly those of the eyes and nose. Exposures at 200-300 ppm produce problems with coordination and balance. Styrene can be absorbed readily through the skin as well as from inhaled air. The 8-hour time-weighted average (TWA) Federal standard for styrene is 100 ppm.

Manufacturing Processes

Manufacturing of reinforced, laminated plastic products, such as those manufactured from styrene-containing resins, typically consists of a series of operations. A mold that has the converse shape of the
desired product is cleaned and waxed and then moved to the gelcoat sprayer who sprays a mixture such as pigmented polyester resin and styrene monomer onto the mold with the compressed air sprayer. The compressed air sprayer is similar to a paint sprayer and is constructed to mix a catalyst such as methyl ethyl ketone peroxide (MEK-p) with a resin such as a resin diluted with styrene as it leaves the gun.

When styrene is used as a diluent-reactant it polymerizes with the resin after application to the mold; during this curing process, the mold is set aside to allow the gelcoat layer to harden. After hardening, the cured gelcoat is given a reinforcing lamination of fibrous-glass. The lamination is applied with a second spray gun that shoots a mixture of chopped fibrous-glass, resin-styrene mixture and catalyst. The operator of this machine is called the chop sprayer.

Immediately after application of the reinforcing lamination, additional reinforcement may be built into the part by the integration of wooden or metal members or woven fibrous-glass mats soaked in a resin-styrene-catalyst mixture. The reinforcement is typically bonded to the part with a light spray of the chopped fibrous-glass mixture.

In the next operation, workers using rollers, much like those employed for painting, roll the newly applied lamination to remove gas bubbles from the mixture and to insure that the resin and fibrous-glass are thoroughly compressed and mixed. These workers are called rollout persons.
The molds and parts are again set aside to cure, the parts are removed from the mold, and the mold is inspected and repaired if necessary. The person who performs this latter operation is called the mold repair person.

Although other finishing operations may be performed, and plants differ with respect to floor plans, engineering controls, storage, and equipment, the above described steps are typical and characterize a major portion of the reinforced-plastics industry.

Methods

Cooperation was secured from Labconco, a producer of reinforced plastic laboratory equipment such as fume hoods and bacteriological glove boxes. In obtaining cooperation of the company, there was an understanding that individual workers would have the option to participate as subjects in the study after being told the general purposes of the research.

Determination of High Exposure Areas and Jobs: Momentary air samples (commonly called grab tube samples) were used to determine styrene concentrations. These samples were taken with a Bendix, Model 400, Gastec pump and Zink styrene detector tubes to identify the plant areas and jobs that involved relatively high exposures. This method sampled only small volumes of air (100 ml) over brief periods of time (less than 30 secs) yielding an estimated accuracy of ±25-35%. The high exposure areas, with momentary concentrations ranging from 110-280 ppm, were the two spray booths in which the gelcoat mixture and the resin-chopped
fibrous-glass mixture were sprayed onto the molds. Styrene was vaporized and aerosolized as a result of being sprayed and due to the heat produced during polymerization. These two processes appeared to be the source of most of the styrene in the plant. The rollout and curing areas of the plant also yielded relatively high momentary concentrations of styrene, ranging from 70-170 ppm. The two spraying jobs and the job in which the resin-fibrous-glass mixture was rolled out involved not only the greatest momentary exposures for workers, but also the greatest total time of relatively high exposure.

Several jobs, such as repairing molds and touching up blemishes in gelcoat surfaces, occasionally introduced relatively small quantities of styrene into the air. Many jobs, such as those involved in finishing operations, and moving parts from one area to another, introduced no or negligible styrene into the plant. Workers in these jobs were exposed to styrene as a result of the ambient concentrations produced by the styrene-introducing processes described above. These momentary ambient concentrations typically ranged from 2-20 ppm.

From the high exposure jobs, three workers, the gelcoat sprayer, the resin-chop sprayer, and one rollout person, were selected for further study. It was assumed that changes in work practices would have the greatest likelihood of affecting the exposures of these workers. To determine if similar work practices would reduce the exposure of a worker who was primarily contacting styrene only in ambient air, the mold repair person was also included in the sample.
Development of Potentially Useful Work Practices: Detailed observations were made of these four selected workers to identify ways in which their on-the-job behaviors might be resulting in styrene exposure. Three general classes of health-promoting work practices emerged: 1) using appropriate personal protection, 2) avoiding high-exposure areas when not necessary for production, and 3) taking advantage of existing engineering controls. Based on these three classes, the following specific work practice behaviors were defined:

1) Gelcoat and Chop Sprayers:
   
a) Using appropriate personal protection
   1) Wearing a respirator when working inside spray booths.
   2) Keeping all skin below the neck, including hands, covered.

b) Avoiding high exposure areas
   1) Staying out of spray booths except when spraying, transferring or arranging parts.

c) Taking advantage of engineering controls
   1) Activating booth exhaust ventilation before spraying.
   2) Keeping doors to spray booths closed while spraying.
   3) Placing molds to be sprayed directly in front of, and close to, exhaust ventilation.
   4) Spraying toward the exhaust ventilation.
   5) Turning molds as necessary to maintain a downwind spray direction.
   6) Minimizing overspray on booth floors and walls.
   7) Not directing spray toward self.
   8) Not directing spray toward others.

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2) Rollout Personnel:
   a) Using appropriate personal protection
      1) Wearing a respirator while in the rollout area or spray booth.
      2) Wearing a respirator when working inside molds.
      3) Keeping all skin below the neck, including hands, covered.
   b) Avoiding high-exposure areas
      1) Performing rollout in the rollout area, not in the spray booth or curing areas.
      2) Avoiding the rollout area when not working.
      3) Not entering the spray booth while the sprayer is in operation.
   c) Taking advantage of engineering controls
      1) Activating floor fans and directing them toward exhaust ventilation before rolling out.
      2) Staying upwind of the part while rolling out.
      3) Turning molds as necessary to maintain an upwind position while rolling out.

3) Mold Repair Personnel:
   a) Using appropriate personal protection
      1) Wearing a respirator when working with uncured resin.
      2) Keeping all skin below the neck, including hands, covered.
   b) Avoiding high exposure areas
      1) Staying out of the mold repair area, if not working, while curing resin is present.
2) Keeping head at least 12 inches away from uncured resin applications on molds.
3) Keeping resin containers covered at all times.

c) Taking advantage of engineering controls
1) Performing repairs within the mold repair area.
2) Activating floor fans and directing them toward exhaust ventilation, before applying resin to molds.
3) Staying upwind of uncured resin applications.
4) Turning molds as necessary to maintain an upwind position to curing resin.

Measurement of Work Practices and Exposures: Data were collected on Tuesdays, Wednesdays, and Thursdays, for seventeen days, on each of the four selected workers. There was greater absenteeism and more time was devoted to scheduling work and getting machinery operating on Mondays, and Fridays were given largely to plant cleanup. Therefore, data were not collected on these days. The gelcoat spraying job underwent two personnel changes during the six-week period. The first gelcoat employee worked on days one through three, the second on days four through eight and the third on days nine through seventeen.

Five classes of data were taken: behavioral data, styrene exposure data taken from work-duration breathing zone air samples, styrene exposure data from eight-hour breathing-zone and area air samples, and mandelic acid levels taken from urine samples collected at the end of the work shifts.
To establish whether training actually produced any changes in employees' work practices, behavioral observations were conducted throughout each day of data collection. For each worker, the specific work practice behaviors were defined in observable terms. Each worker was watched during the entire day by an observer who had been trained to recognize and record instances of these defined behaviors. However, observational data were not continuously collected throughout the work day because there were times during which work practices could not have affected styrene exposures. Observational data were only taken during the times the four employees worked with curing resin. For example, the sprayers were observed whenever they were spraying or working inside the spray booths, but not when they were taking breaks, assisting with jobs such as mold waxing, or participating in cleanup operations. An observer was positioned close enough to a worker to be able to see well, but far enough away to not interfere with the employee's work. Each observer carried a clipboard and stopwatch. The stopwatch was turned on, and recording began, whenever the employee began working, and was turned off, terminating recording, whenever the employee stopped working for longer than one minute. During each 15-second period of the observation, the observer scored any instances of the targeted behaviors on a behavior recording sheet.

The accuracy of observer recording was tested through frequent cross-observer reliability checks. At unannounced times, a second observer, using the same observation procedures and definitions, would make an independent recording of an employee's behavior, simultaneously with the
assigned observer. The two recordings were later compared to determine the accuracy of the observers and the recording procedures. Such observer "reliability checks" were made on an average of 18 percent of all observations, and were conducted during at least 75 percent of the days of the study. Total daily interobserver agreement scores across all observations were 94 percent for observations of the resin-chop sprayer, 98 percent for the rollout person, 97 percent for the gelcoat sprayer and 100 percent for the mold repair person. Additional detail regarding occurrence, nonoccurrence and chance agreement comparisons of the observational data are available from the authors upon request.

To establish whether the recommended work practices had any effect on workers' exposures to styrene vapor, breathing-zone air samples were collected by operating pumps at precisely the same times as the behavioral observations were conducted, that is, only while the employees were actually working with curing resin. This procedure afforded a direct means of assessing the effects of changed work practices on exposure concentrations during the work periods in which work practice behaviors could affect styrene exposures by eliminating those times of the workday when task-specific work practices would not be feasible to help reduce exposures. Operating the pumps only while work with available styrene occurred provided a means to control for effects from variations of production. The amount of time a pump was operated should vary directly with production. Therefore, this measure, unlike an eight-hour sample, should provide an estimate of average exposure relatively free from changes in exposure resulting from production changes.
Several eight-hour breathing-zone samples were collected, beginning with day six, for the resin-chop sprayer and the mold repair person, to provide overall daily TWA's in addition to the work-time exposures. These pumps were worn by the employees throughout the workday, and were turned off only during lunch periods or times when the employees departed the plant.

Eight-hour samples were also taken daily in two areas of the plant which were not directly involved with any of the specific work areas of the participating employees. One pump was located approximately 15 feet outside of the resin-chop spray booth, and the other was located outside the supervisor's office, near the middle of the production area.

Air sampling procedures were supervised by an AIHA-certified industrial hygienist. Air sampling pumps were calibrated daily at a flow rate of 50 cc/min which was monitored regularly and adjusted as necessary. Air samples were drawn into charcoal-filled glass tubes for collection of styrene and analyzed by the Utah Biomedical Laboratory using NIOSH P&CAM method #127 with ethylbenzene as an internal standard. Sample results were adjusted for temperature, humidity and atmospheric pressure.

In order to further evaluate the effectiveness of the program, a 100 ml urine sample was collected from each of the participating employees once daily, during the last 30 minutes of the work shift. Urine samples were treated with 1 ml 6 N HCl and frozen within two hours after
collection and were analyzed for mandelic acid by high pressure liquid chromatography.

**Work Practice Training Procedures:** The above data collection procedures were carried out during several baseline or pre-training days. At the end of baseline, one member of the research staff, functioning as a trainer, met once with each worker for ten to fifteen minutes prior to the beginning of a day's work shift. The trainer explained each of the recommended work practices and how each could help to reduce exposures to styrene. If a worker indicated a lack of understanding of a work practice, the trainer demonstrated it for him or her. The trainer remained with the worker for another ten to fifteen minutes, as work was begun, to give feedback on the use of the work practices and to correct any that were not being properly executed. After this initial training, the trainer visited each worker at unannounced times once or twice each day, for only a minute or two, to provide brief encouragement and feedback on the employee's continued use of the new work procedures.

**Experimental Design:** To provide information on the extent to which changes in data could be attributed to training, as opposed to uncontrolled variables, not all workers were trained at the same time. After baseline data had been collected on all four workers for eight days, training was introduced for two workers, the resin-chop sprayer and the rollout person. Pre-training data collection was continued for the gelcoat sprayer and mold repair person for the next three days, before they, in turn were trained. This experimental design allowed for a number of important comparisons.
Effects of training could be inferred from changes from baseline to post-training data. In addition, the fact that the gelcoat sprayer and mold repair person were not trained until after the other two workers, allows their data to serve as controls for the data of the first-trained workers for three days. For example, if any changes in the data of the first-trained workers should be attributable to uncontrolled variables such as changes in plant policies or weather, these factors might also be reflected in changes in data of the workers who were not yet trained. Finally, the fact that the workers were trained at different times provides a test of the extent to which the training procedures were effective to produce repeatable results at different points in time. This design, called a multiple baseline design, is frequently used in behavioral experiments.\textsuperscript{10,11}

Results

Table 1 displays the percent of 15-second observation intervals in which each of the targeted behaviors occurred during the baseline period and following training. With few exceptions the behaviors changed as desired. The exceptions included several practices which were already occurring at acceptable levels, e.g., the chop-sprayer's spraying toward himself and turning on the booth exhaust. Wearing a respirator while working with resin was not adopted by the chop sprayer even though it was a highly desirable work practice. Training apparently
induced slight increases in the percent of time the chop sprayer, the rollout person, and the gelcoat sprayer were in their work areas but not working. This may have been due to the time required to arrange and turn molds. The extent to which the number of data points, during baseline and after training, deviated from the mean of all data points in the desired direction was compared to the number expected by chance according to a binomial distribution. The probabilities that such results, for each work behavior, would be obtained by chance are presented in the right column of Table 1.

Figure 1 includes graphed data of the percent of observation intervals in which the resin-chop sprayer and the gelcoat sprayers placed molds in the spray booths properly, during baseline and after training. During baseline, the rate of correct mold placement was low for both the chop sprayer and the gelcoat sprayers. When training was given to the chop sprayer on the ninth day of data collection, his rate of correct mold placement increased immediately while that of the gelcoat sprayer remained low until he was trained one week later. Figure 1 provides a representative example of the way in which the data of the gelcoat sprayer and mold repair person served as a control for those of the chop sprayer and rollout person during days nine, ten, and eleven of data collection. In all cases in which the work practices changed as desired, the percent of intervals in which they were occurring remained relatively stable throughout the baseline period before changing with the introduction of training. The fact that training, and the changes of the behaviors, did not occur for the gelcoat sprayer and the mold repair person until a week after they occurred for the chop sprayer.
and rollout person is a good indication that the behavior changes were caused by training rather than by some unmeasured confounding. The fact that the behaviors of the gelcoat sprayer and the mold repair person generally changed, as had the behaviors of the chop sprayer and rollout person one week before, once training began for them, is a good indication that the effects of training are replicable.

The behavioral data, when displayed as in Figure 1, also provide an index of the rapidity with which the various work practices can be induced. It can be seen that the changes in the percent of intervals in which the resin-chop sprayer and gelcoat sprayer placed molds properly in the spray booths occurred almost immediately with the beginning of training. This was true of most of the work practices that changed as desired. Exceptions were the chop sprayer's working with uncovered skin, overspraying and having the booth doors open while working; the rollout person's working outside the rollout area; and the gelcoat sprayer's working with skin uncovered and spraying toward himself. In the cases in which the workers' behaviors changed gradually, anywhere from one week to almost three weeks was required for the full extent of change to occur.

The daily personal styrene exposures for all four subjects, taken during the times they were working as defined above, during baseline and after training, are presented in Figure 2. The exposure of the chop sprayer

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decreased from a mean of 150 ppm during baseline to 96 ppm after training, a decrease of 36 percent; the rollout person from 121 ppm to 70 ppm, a 42 percent decrease; and the gelcoat sprayers from 210 ppm to 91 ppm, a 57 percent decrease. A statistically unreliable eight percent decrease in mean personal exposure occurred for the mold repair person. The significance levels of the reductions in exposure, calculated according to binomial probabilities, were 0.02, 0.01, and 0.02 respectively for the chop sprayer, the rollout person and the gelcoat sprayer.

Decreasing trends occurred in the personal samples of the chop sprayer and the rollout person during baseline. However, the daily exposures during this time very closely correlated with production. The correlation suggests that production was simply introducing less styrene into the plant rather than that these two workers were becoming less exposed to available styrene during baseline. This also suggests that the method of only operating the breathing zone pumps during time worked only partially controls for variations of exposure due to changes in production.

There were immediate changes in exposures, following training, for the two sprayers and the rollout person even though the production schedule was increased. The new levels of personal exposures remained relatively stable throughout the post-training data collection.
The eight-hour personal samples and eight-hour area samples indicate that exposures were well below the Federal standard. The personal samples ranged from a low of 28 to a high of 54 ppm styrene, with a mean of 41 ppm, for the chop sprayer, and from 3.2 to 13, with a mean of 7.5 ppm, for the mold repair person. General area samples ranged from 0.10 to 12 ppm.

Urine mandelic acid levels did not decrease for all workers following training for the recommended work practices. Micrograms of mandelic acid per milligram of creatinine were divided by minutes worked because the amount of available styrene, and consequently the concentration of mandelic acid in the urine is in part dependent on duration of exposure. This index decreased from a mean of .66 during baseline to a mean of .17 after training (binomial \( p < .002 \)) for the gelcoat sprayer and from .46 to .30 (binomial \( p < .03 \)) for the rollout person. There was no change for the mold repair person just as there was no change in his styrene exposure. A slight increase in this index for the chop sprayer may be explained by the decreases in exposure concentration being counteracted by increased exposure times.

Discussion

The training technology was sufficient to induce the desired changes in most of the work practices and these were correlated with 36 to 57 percent reductions in exposures to styrene vapor during the times measured for the three workers who were receiving the greatest breathing zone exposures. That there was little reduction of exposure for the mold repair person was not surprising. A great portion of his exposure appeared to result from styrene introduced by processes in other parts of the plant. Little of his exposure appeared to result from his occasional and brief work with
styrene. Therefore, only a small percentage of his total exposure could be avoided by his own work behaviors with the exception of wearing a respirator.

Mandelic acid levels have been reported to be highly correlated with exposures to styrene,\textsuperscript{13,14,15,16,17} and they have the potential to reflect ingested or percutaneously absorbed styrene as well as that inhaled. Only the data for the gelcoat sprayer and rollout person reflect a consistent change in mandelic acid levels. It is possible that these effects are apparent because of the magnitude of the change in their exposure, and their use of respirators.

The striking reduction in mandelic acid of the gelcoat sprayer likely resulted from his training-induced use of a respirator, the only work practice he, but none of the other workers, adopted during a substantial percentage of time worked with curing resins. This suggests that wearing a respirator may be an important work practice whenever high exposures cannot be reduced by engineering controls or other work practices. In turn, this observation highlights the importance of the difficulty in getting some workers to wear respirators. The chop sprayer declined to wear a respirator at all times and the rollout person generally used one only when working inside a mold.

The training carried out by the senior author was sufficiently simple and straightforward that it could be done by plant personnel. It should be observed that none of the work practices were particularly complex and
all workers probably already had the necessary behaviors in their repertoires. Therefore, training was a matter of prompting the workers to engage in the behaviors. Once the behaviors were occurring, there was, similarly, little difficulty in maintaining them for at least three weeks. There has been general skepticism that workers will reliably engage in protective behaviors over long periods of time. It remains to be seen to what extent this is correct in the present case.

The greatest difficulty in validating work practices by measuring the extent to which they reduce exposures will probably result from the fact that there are many other variables that will contribute to the amount of toxic substances introduced into plant environments. In the present research these variables included such things as the ratio of styrene to resin in the materials supplied to the plant, the amount of catalyst introduced in the spraying operations, the sizes and shapes of the parts being produced, and the weather. These variables will change from day-to-day and will, thereby, produce variations in exposure levels. Such variations in exposures will tend to hide effects that result from changed work behaviors. In addition, whenever reductions of exposures are correlated with changed work practices, there can be questions about whether the reduced exposures result from the work practices or from unknown changes in the many other variables.

In the reported research, the major variation in styrene levels probably resulted from changes in rates of production. Sales demands, breakdowns and numbers of workers present sometimes interacted so that production, and the amount of styrene introduced into the environment, might vary by
a factor of two or three. For the purposes of this experiment, controlling
the rate of production was not reasonable because it would have interfered
with the company's business. Therefore, this factor had to be controlled
by some rational adjustment of data. The adjustment took the form of
keeping sampling times proportional to production and weighting mandelic
acid data by the reciprocal of time worked with curing resin. These
adjustments appear adequate for the exposure data, but partially
successful for the mandelic acid data.

If such measurement problems in estimating exposures can be solved —
and they must be solved to provide empirical bases for all approaches
to reducing exposures to toxic substances — the technology to validate
work practices would appear to be broadly applicable. Once potentially
useful practices are identified, observational definitions and recording
methods can be developed and the reliability of the measurement determined
at least for a large class of practices. This allows for the examination
of the extent to which workers' behaviors change as prescribed. If
appropriate measures of exposure change, as desired, with changes in the
behaviors, and particularly if this correlation is replicated over workers,
the usefulness of the work procedures can be determined. The technology
would appear to be sufficiently flexible to examine the effectiveness of
collections of simultaneously introduced work procedures, as was done in
the present case, or it could be applied to validate a single work practice
such as vacuuming rather than blowing dust which contained asbestos
fibers18,19.
If the technology is applied to collections of work practices, it is impossible to infer, from positive results, that a single work practice contributes to the overall reduction in exposures. However, this may not be a serious loss of information unless some of the work practices are so difficult to implement that including them in the package endangers the acceptance of the other practices.

Analyses of the behavioral and exposure data will allow for determinations of the ease with which work practices are adopted and are useful for different workers within a single plant, the extent to which different practices are adopted and are useful within different plants in a single industry, and the generality of usefulness of work practices over different industries. As work practices are validated, they can be incorporated into training programs. If necessary, motivation programs can be developed to encourage workers to use effective work practices. The research strategy used to validate work practices will also be directly applicable to building an empirical base for worker training and motivation programs. Measurement of worker behaviors and exposures will provide benchmarks against which the effectiveness of training or motivation technologies can be compared and the only means to validate the programs that are developed.

There has been recent emphasis on the importance of human behavior in protecting workers from exposures to toxic substances. The lack of a technology to successfully influence behavior has been noted to be crucial. For example, Dr. Anita Bahn has stated,

"...in general, modification of individual behavior so as to reduce personal hazards is the principal impediment..."
It is our opinion that much of the pessimism about the prospects of influencing behavior has resulted from cases in which there have been failures to produce desired behaviors because: 1) training has amounted to little more than simple communication of information; 2) training methods have not routinely included the extended follow up necessary to alter existing habits; or 3) little attention has been paid to the importance of motivating the person being trained. Training and motivation technology can be made arbitrarily powerful. In some cases, that power can be achieved without undue expense and complexity. In all cases, an appropriate strategy to validate proffered work practices is at hand.
REFERENCES


<table>
<thead>
<tr>
<th>Chop Sprayer</th>
<th>BL</th>
<th>TR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wearing respirator while inside spray booth</td>
<td>0</td>
<td>0</td>
<td>N.S.</td>
</tr>
<tr>
<td>Keeping skin covered</td>
<td>24</td>
<td>76</td>
<td>&lt;.03</td>
</tr>
<tr>
<td>Staying in spray booth when not working</td>
<td>5</td>
<td>12</td>
<td>N.S.</td>
</tr>
<tr>
<td>Activating booth exhaust ventilation</td>
<td>100</td>
<td>100</td>
<td>N.S.</td>
</tr>
<tr>
<td>Keeping booth doors closed while spraying</td>
<td>42</td>
<td>93</td>
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<tr>
<td>Placing molds properly</td>
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<td>93</td>
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<tr>
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<td>99</td>
<td>&lt;.001</td>
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<tr>
<td>Turning molds</td>
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<td>4</td>
<td>&lt;.04</td>
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<tr>
<td>Overspraying unnecessarily</td>
<td>15</td>
<td>4</td>
<td>&lt;.03</td>
</tr>
<tr>
<td>Spraying toward self</td>
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<tr>
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### Rollout Person

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<tr>
<td>Wearing respirator while working with uncured resin</td>
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<td>4</td>
<td>&lt;.01</td>
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<tr>
<td>Working inside molds without respirator</td>
<td>4</td>
<td>.2</td>
<td>&lt;.01</td>
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<tr>
<td>Keeping skin covered</td>
<td>3</td>
<td>98</td>
<td>&lt;.001</td>
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<tr>
<td>Performing rollout in rollout area</td>
<td>4</td>
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<td>&lt;.001</td>
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<tr>
<td>Remaining in rollout area when not working</td>
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<td>3</td>
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<tr>
<td>Exposing self to resin spray</td>
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<tr>
<td>Using floor fans properly</td>
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<td>&lt;.001</td>
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<td>Staying upwind of part while rolling out</td>
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### Gelcoat Sprayer

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<td>Staying in spray booth when not working</td>
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<td>Activating booth exhaust ventilation</td>
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<td>Placing molds properly</td>
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<tr>
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<td>Keeping skin covered</td>
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<td>Remaining in repair areas when not working</td>
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<td>Holding head too close to resin applications</td>
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<td>Leaving resin containers uncovered</td>
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<td>Performing repairs within the repair area</td>
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<tr>
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(TABLE 1 CONTINUED)
Figure 1
Figure 2
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Wednesday Afternoon, May 7

SESSION A
WORKING GROUP ON PROBLEMS, NEEDS AND NEW DIRECTIONS
FOR EPIDEMIOLOGY STUDIES

SESSION CHAIRPERSONS

Dr. Kenneth Bridbord
National Institute for Occupational Safety and Health

Dr. Joseph Fraumeni
National Cancer Institute
Dr. Mason, NCI: I understand that one of the things we would like to talk about is future studies that we would like to have funded through one or another or both of the collaborative arrangements. One would be the funding of follow-up studies in places that we have identified through some of our mapping projects with intermediate studies, whether they be death certificate, hospital record room searches and some analytical case control interview or cohort interview studies in the field. Also, if you will, the potential for using some of these monies to get a better handle on levels of exposure to both qualify and quantify exposures in communities, to pursue some of the more clinical laboratory investigations, and these types of studies.

Dr. Riggan, EPA: I share Dr. Mason's interest. Another area for collaborative studies would be the environmental and occupational health impact from the synthetic fuel program. We need to identify both occupational and environmental cohorts who may be followed over time. A collaborative study involving these agencies may be the only way to establish cohorts with any hope of following them over the time period required for meaningful results.

Dr. Bridbord, NIOSH: I might add that we already have plans for similar studies actually under another collaborative program with EPA in the area of energy. So there are and have been some discussions about developing registries of exposed workers and being in a position to follow those over time.

Dr. Riggan, EPA: I was not aware of the collaborative program of NIOSH and EPA in the area of energy.

Dr. Brown, NIOSH: We have some information on that, a cohort study for coal liquefication and coal gasification. A couple of years ago, we checked into trying to find a cohort study and were fairly unsuccessful in finding a good population.

Dr. Riggan, EPA: This, I think is true today, but it may be very important in a few years. I am interested in estimates of exposure both qualitatively and quantitatively. This is a problem that interferes with environmental studies. While it may be less of a problem with occupational studies, it is a real problem with environmental epidemiology studies.

Dr. Fraumeni, NCI: You are talking now about all studies across-the-board?

Dr. Riggan, EPA: I am talking about epidemiology studies, all studies, where we have a problem, especially with air pollution and environmental exposure, what are the qualitative and quantitative aspects.

Dr. Fraumeni, NCI: How long have the coal gasification plants been in operation?

Dr. Brown, NIOSH: There are some in Europe that have been in operation for a long time, and that is one possible place to try to get a cohort. I think there were some pilot plants started a long time ago in the United States but never any that were put into full-scale capacity with a large enough population.
Dr. Riggan, EPA: Yes, but several are in the planning stage for this country where baseline data could be collected on cohorts. These could be monitored for changes which may occur.

Dr. Brown, NIOSH: I am just saying for a retrospective cohort kind of study it would be very difficult.

Dr. Riggan, EPA: There are some in Poland - Czechoslovakia.

Dr. Brown, NIOSH: I think Germany ran her war machine on coal gasification.

Unidentified Speaker: The biggest ones now are in South Africa; most of their gasoline is produced through coal liquification.

Dr. Bridbord, NIOSH: We sent a team of people to South Africa about two years ago looking at that possibility, and the records there and other complicating factors made it uncertain as to whether you could effectively do that.

Under PL480 there is a cooperative project between EPA and the Department of Energy and NIOSH. I think it is in Yugoslavia, but it may have been Czechoslovakia, an ongoing plant which offers promise for looking at the long-term implications.

In terms of this country the concept would be to basically build a cohort looking at the pilot and demonstration plants and over time, as Dr. Riggan said, to have some baseline data, and thus be able to make some evaluations.

Dr. Sloan, NCI: It will take you a long time though to get results; if you can capture something from those that have been ongoing for a long time, it will be helpful, too.

Dr. Brown, NIOSH: But you could certainly do the industrial hygiene work.

Dr. Riggan, EPA: If we don't start planning now, in 10 or 25 years we will be where we are today.

Dr. Mason, NCI: If I might, since we have sort of gone away from introductions and gotten into topics, one of the things that I believe we would be well advised to take advantage of is the newly formed National Death Index.

We are in a position to set up prospective studies, to collect those 14 data elements that are going to be maintained and taken from every death certificate in the United States from 1979 forward. We are going to be lobbying very strongly to push them back prior to 1979. However, we are currently in the position of ascertaining vital status and obtaining death certificate numbers if we have name, social security number and place of birth and date of birth. I think it behooves us to no longer argue that we do not have the wherewithall to follow large groups of persons. I, for one, would like to propose the registration of all persons resident in the City of Duluth, Minnesota, in 1970, since the question is still unanswered as to whether or not the ingestion of asbestos fibers does, indeed, have a long-term carcinogenic risk. We know what happens when you inhale it. I believe that there does exist a mechanism for collecting the required information from residents of Duluth that would be cost effective. It cannot cost that much to move parallel with the census to get information with regard to individuals who were resident. We need to get information with regard to their duration of residence, and to have the basic data such that perhaps in 1985 and again in 1990 we can ascertain the vital status of this population.
I think that this is the type of project which we need to fund because you are not talking about more than several hundreds of thousands of dollars relative to the total budget which is small, and you are talking about the potential societal impact which is quite large.

Dr. Bridbord, NIOSH: One can make those same arguments when you are talking about any new technology, for that matter, what is coming on board, such as recombinant DNA or various changes in the electronics industry, etc.

Dr. Mason, NCI: Baseline data needs to be collected from persons residing in selected communities which have realized exposures of specific interest. You are going to be looking at levels of exposure. Thus, you are going to have reasonable dose information, and I think that if we put in place a way to efficiently follow these persons, we could make some very strong points with regard to funding for other studies.

Dr. Sloan, NCI: There is merit in following perhaps, through the death index or other ways, the changes that may occur from a major shift from oil to coal in terms of human consequences of acid rain and whatnot; we are apparently going to see a big change in this country in what health effects we may expect.

Dr. Mason, NCI: If we could prioritize the selection of places as a function of some baseline data and baseline risk, yes. I think almost anything that lends itself to a testable hypothesis which is consistent with either a known human response or a suspected human response in relationship to laboratory findings is something which we must pursue. These are the ones that have the potential to affect large numbers of persons in this society.

Dr. Riggan, EPA: I listed coal gasification and liquification as one source of exposure to toxic chemicals which may expand very rapidly in the near future; however, Dr. Mason's proposal is more general.

Dr. Brown, NIOSH: The same sort of thing could be done in the nuclear industry where people want to follow workers in nuclear power plants or areas around them.

Dr. Mason, NCI: If you wanted to do an across-the-board study of every person who has ever worked in a uranium mill in the United States you could do it, and there are some recent changes with regard to the Social Security Administration, independent of any sort of right to access to their data which argues that if a place is no longer doing business then the equivalent to the corporation that the Privacy Act is to an individual no longer applies such that they can release to someone who is interested in doing a study the names of every person who ever worked for that place.

So, you have a way in which to build large rosters of persons, and I think if you look at the massive class action suits that are being filed in the names of miners and millers and if you look at the fact that a number of us have worked in the area of radiation carcinogenesis, it does, indeed, fit in very nicely. I think it is an appropriate utilization of funding from the NCI/NIOSH collaborative agreement. I would like to approach the Social Security Administration and obtain employment rosters for every place that is no longer doing business. This information is sufficient to follow every one of these persons with the National Death Index, and I think that that is a reasonable project for us to consider.
Dr. Blair, NCI: NCI is attempting to do that with the fur and leather industry. There are, however, other possible study approaches using Social Security. One method would be a case-control approach where work histories for each study subject are determined from SSA files. I think NIOSH gets such information from them now, but it is expensive. This might be an appropriate time to launch feasibility studies on particular cancers to evaluate the usefulness of this approach.

Dr. Mason, NCI: I really do think that this is what this collaborative arrangement is all about - to provide a reasonable level of funding to support studies which we are interested in collectively.

Dr. Blair, NCI: The Social Security Administration has data valuable to many governmental organizations. It is important to press this agency and to keep on opening doors to obtain access. There is congressional pressure now, to aid us, and we want to maintain the momentum.

Dr. Mason, NCI: I do believe that if it came from us collectively with a number of specific types of studies that we are interested in we can lobby for it.

Dr. Bridbord, NIOSH: I personally believe that the more studies we could do which are truly collaborative, with people in both institutes working on them, is laudable.

Dr. Mason, NCI: I agree. Let us say there is something else that you would agree to pursue; then it comes down to a policy type of decision. Is it more appropriate or less appropriate for staff members of the Cancer Institute to be involved in that particular type of study?

I argue that if it has the potential for any carcinogenic effect, we should be involved whether or not that is the first priority. There is a study that I would like to pursue now and that is the epidemiology of occupational exposures as they relate to the general health of workers as well as their reproductive outcomes. The effect upon the fetus could be spontaneous abortion, teratogenic or carcinogenic.

Dr. Bridbord, NIOSH: I would second that idea and probably expand it to include reproductive outcomes of working populations, whether those were the result of male or female exposure.

Dr. Mason, NCI: I agree. We are currently negotiating with some of the big unions to get access to sufficient data in order to permit this type of study, and I think we should pursue it.

Dr. Bridbord, NIOSH: Here is an example on the NIOSH side. We are already committed to a major expansion of our own efforts, and it does not make a lot of sense to have independent efforts. We should be sitting down and coordinating and collaborating, and to the extent that it is reasonable, using some of the collaborative money funds. We would probably supplement that activity from our base funds as well.

Dr. Mason, NCI: I have members of my own staff who are ready, willing and capable of working in this particular area, and I would love to have you exchange with me the names of people that I should be talking to on this effort.

Dr. Chu, OSHA: I have a concern here that needs to be addressed in terms of the overview of these studies because certainly now that I am at OSHA there is a
question as to having the resources available. I am in the business of regulating compounds and creating standards. Somewhere along the line, at some juncture, there should be a resource or an input available so that if OSHA was interested in regulating four or five compounds that it have a resource available so that it can say, can we institute a study in these areas? If the activities that you are talking about are more fundamental or if this is a resource where regulatory agencies have some concern like the NTP and get these things done, then the issue should not be, let us collaborate and get something done. It should be a question of going to the regulatory agencies and asking them what kind of priorities they have in terms of the substances that they need studies on. Is this a forum for regulatory agencies that come in? Is it a research forum for a better understanding of collaborative projects? I need to get a clarification here.

Dr. Mason, NCI: The program is an attempt at all things for all people.

Dr. Bridbord, NIOSH: First of all, in terms of the regulatory agency issue, we already did program as part of this conference one of the three workshops to specifically focus on that issue, among others, and that is Workshop C.

The other points to note are that there are already a number of other infrastructures through which these communications are exchanged, and a number of us sit on different groups or at least have sufficient knowledge about those groups that we are aware of that within, actually not just OSHA. It is really the Department of Labor because there are three assistant secretaries involved. There is a whole infrastructure called the NIOSH Planning Group where we do sit down and talk to representatives from all three assistant secretaries.

Dr. Chu, OSHA: I am asking what is the role of this forum towards getting this done? Does it have that kind of responsibility or doesn't it.

Dr. Bridbord, NIOSH: This forum is meant to consider those issues among other things, but the main thrust of this forum is really to build a structure, if possible, that is as much a grassroots collaboration as it is something that is totally driven by the particular needs of the more formal organizational structures.

Certainly the issue of the needs of the regulatory agencies is important. In the case of EPA there is, as you know, the issue of how EPA scientists relate to the needs of the various program offices, and in the reproductive area we talked about the interest between NIOSH and NCI potentially, but there is a whole number of reasons why I would imagine people in the EPA Office of Toxic Substances would be very interested in any collaboration.

Dr. Chu, OSHA: That is a prime example of where a regulatory agency, OSHA, has already gone to court with regard to discrimination in the workplace on this issue in terms of the reproductive effect.

Dr. Mason, NCI: Really what you want to know is whether or not, if in your particular set of circumstances there was a sufficient body of knowledge to argue that there should be a study of this compound, that you should be able to come to a forum such as this and ask whether or not there are sufficient numbers. You have identified it somehow, whether it is through a particular plant or something, but maybe you don't have sufficient numbers to really get your hand on it. Maybe it is an alert clinician response or whatever, and now you want a larger study which is industry wide with regard to that particular exposure and with regard to, in this instance, reproductive outcomes.
Dr. Chu, OSHA: My question is, is this a resource that is available for us to come to with these kinds of questions or is it not a resource. I am trying to simply identify that capability.

Dr. Bridbord, NIOSH: I think the answer to your question is in principle, yes, and that this would be a matter of a case-by-case discussion among the various parties. There are many reasons, both technical, logistical, resource, et cetera, where an answer to a given question may be yes or may be no or it may be let us talk about it next year.

Dr. Mason, NCI: I think we would be most interested in having that type of exchange.

Dr. Bridbord, NIOSH: To the extent that from the OSHA perspective and the NIOSH Planning Group within the Department of Labor and the other formal mechanisms that are already established, those needs do at least surface in these discussions, and a good example would be in the area of reproductive effects.

Part of our desire to move into that area in addition to our own interest and recognition is the driving force out of the Department of Labor that encourages us to recognize the importance of this.

A lot of discussions will come up as to which populations we look at, which exposures are more important, and all the issues that surround why you study one group or another group, but in principle that already is the driving force.

I am more than glad that Dr. Mason raised the point independently because that, from my perspective, was one that I wanted to suggest as at least one of the potential areas where we might somewhat modify our existing situation to at least allow some case-by-case possibilities to move in that direction.

Dr. Sloan, NCI: We are in the position of taking the results of what a good many of you do and trying to apply them in prevention, diagnosis and treatment of patients with cancer, not doing the research in diagnosis and treatment, but applying what is coming out of the research field that is ready for application.

We have a major responsibility for education of practicing physicians and organizing resources in communities and through cancer centers to deliver better care.

One of the areas we have been really concerned about recently is trying to look ahead and estimate the burden that asbestos-related disease is going to represent to this country over the next 20 years.

I don't believe anyone has a very good idea how serious that burden is going to be, how many cases of mesothelioma we are going to have, how many cases of asbestos-related lung cancer, and that there are many populations around the country which have really not been studied so that we don't know what the long-term effects of their exposure would be; brake band workers would be one. I think EPA is going to try to do something about that, but I don't really know.

We have heard a lot in the last couple of years about the hazards of deteriorating asbestos in schools, but we don't have any real evidence that anyone has ever had his health endangered by exposure through a school situation.
Perhaps custodial workers and others who have had more exposure to asbestos through the maintenance work they have to do might be groups that could be studied more specifically.

So, we would like to stimulate or encourage the organization of more epidemiological studies on any kind of cohort that can be identified that would help us assess the burden we have to look forward to and prepare for in the future.

Dr. Mason, NCI: Dr. Sloan, do you think that these talks of collaborative arrangements would be a reasonable forum to consider research in behavior modification? If you think in terms of one of the greatest risks which is cigarette smoking, and if you think in terms of your all but impossible task of communicating to people that they should quit, as well as let us assume that in the next several years we are going to be, you know, really concentrating on nutrition and things like that, we are going to have to go out and start, if you will, preaching to people that they need to change their lifestyles. Do you see that as the type of thing that would come under this collaboration.

Dr. Sloan, NCI: I am not sure because I have not been a part of the discussions of this collaborative group before and I don't know whether behavioral research is part of your area of responsibility or not. The smoking problem or stopping smoking is certainly the one single thing that people could do that would help them more in preventing and slowing the development of asbestos-related disease than anything else. It has no effect on mesothelioma, but there is a major program in smoking abatement organized under Dr. John Pinny in the Surgeon General's Office, and there is a coordinating focus for all of NIH in the Cancer Institute which Dr. Diane Fink is running at the moment, and I don't know how much, in addition to that, is appropriate to carry out under this forum.

Dr. Bridbord, NIOSH: I think the collaborative nature of these programs would by and large have to respect and understand the differing mandates, with the different organizations and not to question the importance of the smoking issue which is certainly a major public health hazard, but I would think that the EPA people would see their mission primarily in terms of the toxic chemicals and the NIOSH mission is primarily focused on the chemical and physical agents in the workplace.

There are many, many examples of where NIOSH recommendations have considered the smoking issue, not only in terms of the interaction but, also, some of the safety and other problems that surround that in the workplace, but I would be reluctant to look at this particular pool of resources as the main focus for that work as opposed to some of the others.

In the cancer control area the argument I would make is that the control technology issue, the control technology assessments and engineering studies in terms of actually translating the information on toxic exposures to a prevention outcome is something that I would suggest we talk about as a possibility, but I think if you are talking about studies that look at work practices and really try to assess how effective they are in reducing personal exposure in the workplace, there is one example.

Any of our epidemiology studies or cross-sectional studies that look at a toxic exposure must consider the other confounding factors.
Dr. Spirtas, NCI: Dr. Sloan are you trying to identify additional occupations which may be beginning to show an asbestos-related health problem. Is that what you are saying?

Dr. Sloan, NCI: If there are some that we have not heard about yet.

Dr. Spirtas, NCI: That is an argument to go ahead with the mesothelioma registry, to find cases of mesothelioma and trace back over time to see where they worked, and if something crops up like custodial workers.

Dr. Sloan, NCI: What mesothelioma registry are you talking about particularly, one that doesn't exit yet or one that does?

Dr. Spirtas, NCI: I am talking about one that is new - in the formative stages.

Dr. Sloan, NCI: Under whose aegis?

Dr. Brown, NIOSH: It is proposed for NIOSH. Did you start it when you were at NIOSH or have something to do with getting that started?

Dr. Spirtas, NCI: I was involved in getting it started. We did not see it from the point of view of research as being a strong research effort because of the ubiquitous nature of asbestos.

We know that asbestos causes lung cancer and mesothelioma, but if there is a reason such as the one that you have just described, there may be workers in occupations who are in need of education or in need of further protection, and we should identify those unknown occupations such as custodial workers, people in school buildings. To my thinking, Dr. Sloan's discussion of intervention strategies strengthens the argument to push ahead.

Dr. Sloan, NCI: There is also the fact that there is some pretty good work going forward now on developing better methods of treating mesothelioma, and there are a few long-term survivors emerging from these trials that may make it very important to be able to identify mesothelioma cases as early as possible. I think we, also, are anxious to know, I think from your death record list, Dr. Mason, that we need to know where in the country they are occurring and what the economic burden is on the community, what the treatment resources are going to be that are required in these areas. The SEER program is showing that there is definitely an increase in the shipyard areas which you might expect.

Dr. Brown, NIOSH: Don't you think that the National Occupational Hazards Survey would be of some use to try to get an estimate of the number of people that have been exposed to asbestos and where they have been exposed. NIOSH is just planning another round of the NOHS project.

Dr. Sloan, NCI: I assume you are going to do that.

Dr. Mason, NCI: What is the timing on the third one?

Dr. Bridbord, NIOSH: It was supposed to start as a substantial activity this summer. We are facing the same issues, I think everybody else is, with perhaps the exception of some parts of EPA, and that is the crunch on the ability to hire people, particularly permanent people. I think that has caused our progress to have been not quite as
active as we would have liked, but there is no question but that we are moving ahead
with the second survey, with some improvement in it, and it will be an important
piece of information, I think, for all of the agencies.

Dr. Sloan, NCI: Asbestos will be a part of that?

Dr. Bridbord, NIOSH: No question about that.

Dr. Spirtas, NCI: I cannot tell you off the top of my head how many places asbestos
was seen in the first survey, but it was a substantial number. In fact, it was not just
in shipyards, but asbestos is also a component in insulation and other products. In my
opinion, one of the valuable things that comes out of an exercise like NOHS is not so
much picking numbers but it is telling you that asbestos was seen in a factory where
you would never have suspected it. Thus, there are reasons to explore the NOHS file
for qualitative information that may be as important as the quantitative information
that comes out of it.

Dr. Sloan, NCI: Could I just ask one more question about asbestos? The problem of
removal of deteriorating asbestos from schools is only a small part of a huge iceberg,
because an infinite number of buildings in this country were treated in the same way,
including some of our most elegant apartment houses and whatnot, and we really have
very little knowledge about the reality of this hazard. Is there any way that through
your collaborative efforts you could address this in any more positive way?

Dr. Bridbord, NIOSH: My guess is that from the occupation perspective it would be
fairly difficult. I think that is a question of the indoor air pollution issue and how
EPA feels about that matter.

Dr. Riggan, EPA: We are concerned and interested in indoor air pollution. Increased
insulation and reduced air exchange in the living areas may create air pollutant levels
consideraably above the environment ambient levels.

Dr. Bridbord, NIOSH: Actually, EPA has some studies of indoor air pollution, I think,
and carbon monoxide? No?

Dr. Riggan, EPA: EPA has had several studies including one at the Lawrence
Berkeley Laboratory in California. At present EPA has a contract with TRC on
estimating individual's total exposure.

Dr. Brown, NIOSH: We do a number of different types of industrywide studies. Most
of our studies historically have been retrospective cohort kinds of studies on cancer.

Dr. Fraumeni, NCI: Could you explain what you mean by industrywide?

Dr. Brown, NIOSH: Industrywide means, if we want to study a certain type of agent,
such as trichloroethylene or asbestos, we go out and we look for a population in an
industry, not a specific industry, but an industry where there is exposure to this agent
and try to generalize the outcome that we see from this exposure industrywide.
Maybe that is the wrong interpretation of industrywide.

Dr. Fraumeni, NCI: Do you always start with a particular agent?

Dr. Brown, NIOSH: No.
Dr. Fraumeni, NCI: For example, would the rubber industry investigations by the University of North Carolina and by Harvard be called industrywide studies?

Dr. Brown, NIOSH: Involving the rubber industry, yes. Our studies start from an agent or from a certain occupational group. One thing that we are getting into lately is the reproductive studies that Dr. Bridbord mentioned, and that is where people should have some collaboration because the analytical methods are not well defined yet. Nobody really knows good ways to analyze all this data that has come in. There are thousands of variables that one has to look at and control for. I think that would be a good topic to have some collaboration on, and I think in general the federal agencies should collaborate more on analytical techniques and be willing to share their computing programs and expertise so that when one agency comes up with a finding, the analytical techniques that were used can be accepted throughout the Federal government.

Dr. Mason, NCI: Yes, that is why I am, personally, very pleased that your life table analysis system is now sitting at the Parklawn computer because I am getting it. You have treated it as a black box for a long time until such time as you got it to a point where you felt more comfortable with the whole thing, and the modifications of that program relative to some other life table programs are something which we need. I mean you allow a person to come in and go out as a function of breaks in exposure. You don't have to make the oversimplifying assumption that once exposed you continue exposure throughout that particular employment which we know is not true but which we have had to make the assumption because the black box that we have does not permit that type of modification. So, I think we are making good strides. Dr. Kreitel uses all of my mapping programs, and he rides free on my interagency agreement with NOAA in order to make maps. There is a growing interest in the sharing of information and I am encouraged.

Dr. Bridbord, NIOSH: I think particularly in the area of reproductive effects, this is going to be one where there will be a number of areas of progress in terms of methodology in the next number of years, and that is an important one to be talking about.

Dr. Mason, NCI: Not only in methodology. It is something which needs to have a clinical laboratory component. It is something where you not only need good industrial monitoring, but you need some very specific measures on the person, male or female, and there are some fundamental questions that need to be addressed with regard to the availability of amniocentesis to working women and how many of them do take advantage of that and whether or not the amniotic fluid would not be a reasonable specimen to look at from the standpoint of certain levels. I, personally, think yes. However, historically amniocentesis had a very strong social class gradient. If you were not better off, you did not avail yourself of it. So whether or not we have made strides along those lines, whether or not 90 percent of women who are working in, let us say the chemical industry or whatever, would indeed avail themselves of this routinely as part of their health program at the company is a question that I think needs to be addressed.

Dr. Bridbord, NIOSH: Or just even doing, in effect, case control studies, just even looking at exposure, getting that technique for various reasons that are already indicated.

Dr. Mason, NCI: As long as you can identify the characteristics of the persons and you can characterize that population or that subpopulation that they come from, then
it is reasonable to look at these particular comparisons, because I do think that it is a
good thing from the standpoint that the fluid can be looked at in a reasonably
complete manner, and it has not been done, not at all uniformly.

Dr. Bridbord, NIOSH: Similarly, just understanding how to measure people's sperm
counts and motility and what all that really means is important.

Dr. Blair, NCI: I have a couple of general categories of projects. One we have
already talked about is maintaining some momentum to gain access to Social Security
files to enhance our research capabilities. It seems that this group might play an
important role because there are several government agencies involved.

Another is making an attempt to build a comparison population of working persons
that can be used in occupational mortality studies. I am sure every agency has
thought about this at various times, but it is difficult to allocate the necessary time
or the resources. There are a number of cohort mortality studies completed where
there were not striking findings. If pooled, these might provide a suitable worker
comparison population which would modify the "healthy worker" problem that arises
from use of the general population for comparison.

Dr. Fraumeni, NCI: There are studies already done on cohorts whose risks were not
exceptional?

Dr. Blair, NCI: The idea is to pool many completed cohorts. You probably would not
want to include insulators because of their high lung cancer rates, but others where
the findings were not so striking could be used.

Dr. Bridbord, NIOSH: We have already started in the area of respiratory disease
where we are developing out of our laboratory in Morgantown a blue collar worker
control population to look at respiratory disease, but I think your point is well taken,
and actually the few data that are available on groups that are, as far as we know,
unexposed might well be confounded by other things, such as alcohol, cigarettes, et
cetera. It would be hard to tease these apart, but they suggest mortality ratios, even
as low as 50 to 60 percent. However, we must always use the United States as a
whole as our control group which would be analogous to trying to at least assess the
quantitative impact of cigarette smoking, looking at a group of smokers and
comparing them with a group that included 30 to 40 percent of smokers. I mean that
is, in effect, what we are doing in all of our occupational studies at this point; in
terms of identifying a high-risk situation, I think you can still do it. Where you are in
the margin you lose something and then in terms of the quantitative impact we may
be understating that by 25 to 50 percent.

Dr. Blair, NCI: Because various agencies already have suitable cohorts available, you
might be able to put something together.

Dr. Brown, NIOSH: I was approached by Stanford Research Institute and they wanted
to do that very thing as a grant.

Dr. Blair, NCI: My guess is it ought to be funded through a contract. It would be a
time-consuming job and that is why it does not get done. Government agencies seem
to be the logical group to initiate the effort.

Dr. Spirtas, NCI: It might not be such a terrible job to utilize the Social Security's 1% CWHS file.
Dr. Blair, NCI: Yes, and it could be that academic institutions would also contribute cohorts.

Dr. Fraumeni, NCI: Are you suggesting that the Social Security file be used for this purpose?

Dr. Spirtas, NCI: Yes. Its population has been followed over time for vital status. I think your two questions may be related.

Dr. Brown, NIOSH: How would that be different than using the US population as opposed to a 1% sample of the US population?

Dr. Mason, NCI: They are people who were identified while working and then followed.

Dr. Bridbord, NIOSH: But you would still like to look at a group that included, for example, perhaps only blue collar workers but who were not in certain types of jobs, or white collar workers.

Dr. Mason, NCI: The ideal set of circumstances would be to have a representative sample of every standard occupational category so that you could lump or split in any way what you wanted in order to get a reasonable group of persons to study. I am hypothesizing this particular outcome, to take this particular group here with interest in a similar age structure, and then I can look to see if there are differences. That is what we want, but until such time as we open up the floodgates, we are going to have to just keep chipping away, and it might well be reasonable to make that a tandem request that this is something which we need, that there is an identifiable need, not just by us, but by you and by anyone who wants to work in this area. We know it can be done, and you are the only resource that can permit this particular thing and just use it that way.

Dr. Fraumeni, NCI: Dr. Blair, would you give us again your first point?

Dr. Blair, NCI: The first one was maintaining the momentum of trying to get access to Social Security files for establishing cohorts from companies out of business or developing a fallback case control capability.

Then there are specific study areas where there is an urgent need for epidemiologic research. One is herbicide exposure and cancer. The Swedish studies need to be replicated in this country, either by a case control approach or by identifying exposed cohorts. Secondly, another group mentioned recently in the New York Times and by Walter Cronkite is farmers. There are several different mortality studies that suggest farmers have high rates for several cancers, leukemia, multiple myeloma, lymphoma, pancreatic cancer, despite a low overall total mortality. Very little is known about what the particular hazards might be, although you can list several hundred chemicals farmers come in contact with. This may be time, to develop studies to pinpoint farm-related hazards.

Dr. Mason, NCI: It is like the grain inspectors hypothesis. If you could really get sufficient numbers of them and access to their records, you could find the response.

Dr. Bridbord, NIOSH: You would probably never be able to identify the chemicals that are responsible.
Dr. Burton, NCI: One study that has never been worked out is the epidemiology of Burkitt's lymphoma in the United States. There have been many case reports and immunologic studies on Burkitt's lymphoma patients and biologic materials. There have been about 350 verified cases of Burkitt's lymphoma in the United States. In the U.S. you don't have the suspect triggering factor which occurs in Africa and New Guinea, that is, malaria, which is said to interact with the Epstein-Barr virus to induce Burkitt's lymphoma in certain individuals.

There have been a number of Burkitt's lymphoma clusters reported, presented as case reports, but I am not aware of any study which attempts to determine any environmental factors which are common to the Burkitt's lymphoma cases in the United States.

Dr. Bridbord, NIOSH: How many of those have occurred?

Dr. Burton, NCI: There are about 350 verified from the American Burkitt's Tumor Registry, based on information provided by Dr. Paul H. Levine of NCI.

Dr. Bridbord, NIOSH: Over what period of time?

Dr. Burton, NCI: Over 10 years.

Dr. Bridbord, NIOSH: I would like to play devil's advocate with you.

Dr. Burton, NCI: The incidence is probably less than 1 per million.

Dr. Bridbord, NIOSH: I would find that hard to argue as a candidate under the collaborative program, particularly from NIOSH's perspective facing so many problems that are just huge in magnitude with many, many people exposed.

Dr. Burton, NCI: Another aspect that has not been studied in detail by the United States Government investigators has been the followup of nitrosamines in the causation of nasopharyngeal carcinoma. Funds for this purpose have been provided to some foreign investigators. Dr. John Ho in Hong Kong has hypothesized that the triggering factors in nasopharyngeal carcinoma among those of Chinese origin are nitrosamines in salted, dried fish.

Another factor is aflatoxin, indeed, the mycotoxins as a group. Mycotoxins have not been carefully studies in relation to human cancer in the United States. Aflatoxins are produced in peanuts and corn in considerable amounts. The Department of Agriculture has long been interested in this. Farmers, of course, are those who handle peanuts and corn and would probably be most exposed. You never see much in the literature about this, not only the aflatoxins but the whole gamut of mycotoxins in the causation of human cancer.

Dr. Bridbord, NIOSH: I suspect of all the things you described, the nitrosamine area is one where I suspect NIOSH will continue to do some follow-up work; and to the extent that we can identify cohorts we will have a decent idea what the exposure might have been. That would be something valuable.

Dr. Burton, NCI: Nobody has ever been interested enough to really pursue that as a subject in itself. It has always been a small part of some large study for some other purpose.
Dr. Brown, NIOSH: We have a cohort that we are studying now in the leather industry where there are some documented exposure to nitrosamines. Dr. Fagen talked about this yesterday. We have a leather plant where they have never used the nitroso compound and a leather plant which has almost exactly the same process except they use a different agent for dehairing the hides which uses nitrosamines.

Dr. Mason, NCI: Do you people have the capability as far as laboratory support if we went out and actually in one of our colon studies or whatever were able to take and do it the same as they did it in Africa and take samples of foodstuffs prior to preparation and subsequent to preparation and get them to a laboratory?

Dr. Riggan, EPA: Dr. Gardner's division in the Health Effects Research Laboratory in Research Triangle Park, NCI, has the capability of quantifying nitrosamines in food. According to my understanding, the Food and Drug Administration has this capability also. The expertise exists in the Division. Administrative details would have to be worked out.

Dr. Burton, NCI: It could be while you are pursuing this you may get on to something as to why nasopharyngeal carcinoma occurs in the other groups, in the other cultural groups.

Dr. Fraumeni, NCI: Dr. Keefer do you have any comments?

Dr. Keefer, NCI: One of the problems with the NPC study, I believe, is that the consensus of analytical chemists does not agree that nitrosamines have been reliably implicated in causation of this tumor. There have been some attempts to confirm their presence in local diets, and some failures to confirm them.

Dr. Burton, NCI: That is what I am saying. Nobody has really put enough money into it as a study in itself with the intention of determining the etiologic relationship of nitrosamines to naso-pharyngeal carcinoma.

Dr. Keefer, NCI: That is not quite true. Terry Gough, one of the best nitrosamines analysts, went to Hong Kong to pick up some samples from John Ho, and I am not even sure what happened to them. It has been a long, sad story.

Dr. Burton, NCI: I know about that study. It was just a small study. They found levels in certain croakers, white croakers and yellow croakers, but nobody ever determined the extent to which croaker fish are eaten in preference to any other kind of fish during one's lifetime. It is all called salted, dried fish. Recently Drs. Huang, Ho. Gough and colleagues have studied volatile nitrosamines in salted, preserved fish, so such studies are continuing, but probably not with NCI support.

Dr. Keefer, NCI: As a matter of fact, in the meantime we had a contract in the Carcinogenesis Program with Mt. Sinai, a small feasibility study to find populations anywhere in the world that could be studied for possible human effects of nitrosamine exposure. That contract was, also, a failure for a lot of procedural reasons; as a consequence, most people in the field of epidemiology seemed to agree that there really was not any population worthy of study because of the small numbers and/or the exposures to a multiplicity of carcinogens, and/or all the other things that you epidemiologists complain about. I should probably admit at this juncture that I don't really belong here in the epidemiology session because I am only a chemist, but I came anyway because I think epidemiology is really where any approach to cancer prevention has to start and end. I have devoted over a decade of my career to the
laboratory study of carcinogenic nitrosamines, and I still have to say that there is no evidence at all that anybody ever got cancer from nitrosamines.

Dr. Bridbord, NIOSH: I think if there is any chance to get some additional information in that regard it will probably be through studies described where one has an opportunity to compare two groups where at least there is a difference in that exposure.

Dr. Sloan, NCI: Isn't there some work going on in the nutrition program where they are trying to relate vitamin C levels to nitrosamine in causation of nasopharyngeal cancer in China?

Dr. Keefer, NCI: Vitamin C is of significance primarily as a blocking agent for nitrosamine formation. It turns out to be quite a good scavenger for nitrite, which is one of the precursors of nitrosamines. You can demonstrate in animal systems that you can block nitrosamine formation in a cancer preventive sense using vitamin C.

I don't know, however, that there is any evidence that vitamin C helps prevent carcinogenesis by preformed nitrosamines, which are present in many of our foods, and in the workplace as many people have mentioned. I don't think there is any evidence that vitamin C can help you in that case.

Dr. Burton, NCI: I thought you might be interested, talking about vitamin C, that in the one intensive study done in Hong Kong on those in their 20's with naso-pharyngeal carcinoma there was a very intensive lifetime food intake history done by Drs. E. N. and M. L. Anderson, anthropologists from the University of California at Riverside. They determined that what was common to every one of the 25 or 30 cases that they studied intensively was that none of them had an intake of vitamin C in their younger days. They did not believe in eating oranges or limes or lemons or anything that had vitamin C in it. They also consumed salted, dried fish from early childhood.

Dr. Fraumeni, NCI: From that perspective, how would you evaluate the role of nitrosamines in human cancer? What would be the best groups to look at - people occupationally exposed to nitrosamines, people exposed to nitrates which are later converted to nitrosamines?

Dr. Keefer, NCI: Let me give a real amateur's answer. People found air concentrations of nitrosomorpholine in one rubber plant in Maryland that approach those which are detectable in small animal studies as being carcinogenic, in other words, 250 micrograms per cubic meter of nitrosomorpholine in the air. These levels may well be sufficiently carcinogenic that you can detect their effects in a small population. But the analytical data are not adequate to launch a major study because after finding 250 ug/m$^3$ that one day on one test, a new ventilation system was installed and the levels were much lower when the analytical equipment was brought back for a confirmation retest. Would such variations have happened in the absence of ventilation changes? How long had those levels been there before they were discovered? We don't have data on these points, so we can't say whether it would be fruitful to study these workers or not.

Dr. Bridbord, NIOSH: The situation where you had a reason to believe that one plant had it and one plant didn't, even if you cannot quantify exactly what that would be over a period of time, such as was described, is about as good a natural opportunity to look at that as any.
Dr. Mason, NCI: As long as you can control for age and have information on other such things. It at least has the potential of eliciting a finding.

Dr. Keefer, NCI: There are several situations that might be worthy of study. Certainly the leather industry is one that I think should be pursued vigorously, both by people with statistical interest and by chemists. I think there is a lot more that needs to be done in the chemistry area, much more systematic study of how exposure levels vary with certain parameters. So far I don't see that there is the massive input of funds that I think is required in that area.

Dr. Bridbord, NIOSH: We want to raise the question that regulatory agencies, I believe, OSHA has been petitioned to set a standard for nitrosamines just for that very reason. It may be extremely difficult to do that, but in terms of the relevance of pursuing this issue to the regulatory needs, that certainly would seem justified.

Dr. Keefer, NCI: Another thing that comes to mind is this diphenylnitrosamine issue, which I don't think we have seen the last of. The conventional wisdom for years has been that it is a non-carcinogen. Everybody "knows" it is not carcinogenic, and everybody is using it in rubber factories in massive amounts.

About one year ago Lijinsky's group came out with a study showing about 50 percent of their rats got bladder tumors from this chemical and after looking back in the literature, Dr. Lijinsky has told me there was only one marginal animal study that the conclusion of non-carcinogenicity was based on. So, people have been exposed tonwise to this particular material, which may well be a powerful carcinogen.

Dr. Burton, NCI: One thing that Dr. Ho emphasizes is that exposure to nitrosamines could begin in early childhood, by chewing and swallowing salted, dried fish. This occurs among southern Chinese, and often among Chinese-American and Chinese-Hawaiians. Such investigations should continue to be pursued.

Dr. Keefer, NCI: Let me throw out one more thing that you may not know about yet. There has been a lot of nitrosamine news in the press but there is one story that is just not in the public press yet which is that 90 percent of the powdered milk prepared in this country contains a small amount of nitrosodimethylamine, the same nitrosamine found in the leather industry. When you mix the milk according to the instructions on the box, the levels come out to less than 1 part per billion, which is five-fold less than what people have been making so much fuss about in beer. But in my house we used to drink a lot of powdered milk and oftentimes people use the powder directly in cooking. We used it in place of whole milk, and I don't know how many American families or families overseas might use milk powder in enough quantity to get significant exposures while their children are in their formative years physically. That is something that we might want to think about preempting the press on and get to work on before it becomes sensational. I don't know that anybody should be alarmed by this, but I personally am.

Dr. Fraumeni, NCI: How does it get in the milk?

Dr. Keefer, NCI: Dr. Scanlan at Oregon State looked for it as an extension of his studies on beer, where nitrosamine contamination had been traced to the malting process in which direct gas flames are used to dry the sprouting grain to make the malt. The gas flames apparently have enough oxides of nitrogen or something in them that they can nitrosate some dimethylnitrosamine precursor. This produces the dimethylnitrosamine in beer.
Similarly, when they spray the milk into drying drums heated with the same kind of gas firing, there are precursors there, too, which lead to dimethylnitrosamine. Certainly that was the hypothesis that Dr. Scanlan used in studying milk in the first place. So, any process in which you have direct gas firing on a foodstuff appears to have that potential, though detectable nitrosamines are not formed in every such situation; some kinds of dried coffee, for example, have not had that contamination. A product is a candidate for nitrosamine contamination if it is gas fired, but it does not necessarily contain a nitrosamine just because it is directly dried.

Dr. Brown, NIOSH: They have taken it out of the beer though, right?

Dr. Keefer, NCI: They can. All they have to do is change the process to indirect heating. Most American companies don't do that, only Coors who has always done it that way can say that they have no detectable nitrosamines in their product. It seems that nobody has changed their process in this country. In Germany they have. In Germany, also, the rubber industry does not now have nitrosomorpholine contamination to the extent we do. They changed their process there too, whether by fiat or what. They have made the changes. In America we don't seem to do that.

Dr. Mason, NCI: And it is actually dimethylnitrosamine in powdered milk?

Dr. Keefer, NCI: Yes. There are a number of other foodstuffs and some other industries that quite probably merit consideration though I don't know whether they are worth studying. I cannot answer that question. I would sure be glad to tell you or anyone else anything I know about the background and analytical backup so that we can get on with preventing cancer.

As a matter of fact, that is really why I am here. I would like to know why the interface between epidemiology and chemistry over the years has been such an abysmal failure. I need some suggestions as to what we can do differently. There are situations out there that statistically just cry out for someone to identify the causative factor in the food or somewhere in the environment to discover that this thing or that causes cancer in people. We have not been able to identify the carcinogens in the "cancer gardens" you have described for us. I don't know what to do, but I came here with that question in mind. I would like to know how we can help solve these very issues you are talking about and particularly how we chemists can change what we are doing.

Dr. Fraumeni, NCI: You talked about analytical techniques to identify nitrosamine in the workplace or in foods or in the environment. Do you have any analytical techniques to measure body burden or other laboratory indices of exposure?

Dr. Keefer, NCI: The analytical methods are there for things like dimethylnitrosamine and nitrosomorpholine, which are the ones that were discovered in massive amounts, relatively speaking. The reason why it might be difficult to do body burden studies is that the compounds are metabolized rapidly, plus there is another reason. New evidence suggests that dimethylnitrosamine may be a normal constituent of human blood, so that we would then be trying to find small levels of exogenously produced material on top of a somewhat smaller background. People have not quantified the blood levels carefully enough yet in their minds to be able to bring themselves to publish it, but it may be that these kinds of levels of dimethylnitrosamine are there in the blood to start off with. So, it would be complex, but the analytical methodology is available, well developed, and well confirmed for those particular compounds.
Dr. Mason, NCI: And not so expensive that it would break the bank to do a reasonable number?

Dr. Keefer, NCI: We have in the contract program at least 10 of the instruments most commonly accepted for doing this kind of thing. We don't have one in our lab, but we have good communications and working relationships with labs who do, and I think we can arrange any analysis.

Dr. Sloan, NCI: I wonder if someone here knows about the program Jeffrey Pearlman is trying to work out in the National Center for Health Statistics. As I understand it the National Center for Health Statistics was ordered to develop a plan which they are due to report to Congress in June that would provide some registration for people who had been exposed to some toxic substance and give us a basis for following them over time. That is not built into your death registry. This is something entirely separate. Do you know about that?

Dr. Mason, NCI: Not really, other than it has been fraught with all sorts of problems. I don't know if they have ever really staffed up to do half of what they wanted. They were in trouble personnel wise over there. It is yet another interface that should be pursued if it can come together. Any reasonably defined identification of persons because of an exposure or an outcome would be desirable. The National Center needs to be doing more of that, but it is my understanding, at least months back, that they were not very happy with its productivity as yet.

Dr. Sloan, NCI: I am sure Mr. Mazzocchi and others have approached some of you regarding the need for some kind of medical surveillance of people known to have been exposed to toxic substances, not all of them carcinogens. For example, the Velsicol Company had workers who were exposed to a great variety of solvents and brominated compounds. It has now gone out of business and Mr. Mazzocchi is very concerned that we have no mechanism, at least within our division, to set up a surveillance program or fund it. These are workers who may turn up with serious disease conditions in the future, particularly because they have been exposed to multiple toxic compounds. Is there any way of doing more to establish a surveillance system for such exposed individuals?

Dr. Bridbord, NIOSH: I think in terms of providing routine medical care, I doubt that either NCI or NIOSH or certainly EPA would be able to provide that to the extent that you are talking about. Demonstration or research projects are another matter, but each of those would have to be looked at on an individual basis to understand the circumstances that surrounded that. I doubt that either organization would routinely be able to take up ongoing medical surveillance just for the sake of that, you know, just in terms of the enormity of what would probably be involved.

On the positive side, NIOSH is making some efforts through the primary care health delivery side of HEW to eventually build up an expertise in occupational medicine that could potentially deal with these situations. We are working, for example, with the National Health Service Corps to establish an initiative in occupational medicine, but these are rather long-term type things. Public Health Service hospitals are another example of trying to get their interest, but the efforts so far have basically been pilot types. We are probably many years away in terms of getting the full-term benefits.

Dr. Sloan, NCI: Wouldn't your ERC's be able to tackle some of those situations, too?
Dr. Bridbord, NIOSH: Not as a routine.

Dr. Sloan, NCI: Not as a routine, but this would be a special study.

Dr. Bridbord, NIOSH: But the ERC's are not provided with money for research per se. They have money for teaching and education. To the extent that a given ERC investigator would want to come in, for example, to NCI or to NIOSH for an investigator-initiated research project, there are mechanisms in place. Also, to the extent that the inhouse people in either organization identify a group at risk and want to do a cross-sectional medical or mortality study that could be done, but the place where the system falls down is in the routine surveillance of populations that don't fall in those other categories.

Dr. Spirtas, NCI: You might want to call Dr. Joyce Sals in NIOSH. She did a study. Her number is 684-3284.

Dr. Mason, NCI: Nobody can afford to do it, and it comes down to a fundamental question as to what you mean by surveillance. Registration is one thing. A routine physical is another thing. You have no guaranty that you even know what test to perform. That is the problem, and the last thing that you want is everybody and his neighbor collecting sputums to look at cytology when some fundamental questions on it are not even answered yet.

Dr. Keefer, NCI: I guess I said my piece, but I will summarize once more by saying that I will do anything I can to help you people identify carcinogens.

Dr. Fraumeni, NCI: I think that would be very helpful. You are one of the few laboratory people at NCI with a solid interest in epidemiology. We would very much like to meet again with you.

Dr. Keefer, NCI: It is just a suggestion that we try to work on the interface between our disciplines. Actually, Mervish first brought up this question over lunch one time many years ago, i.e., can we make a better two-way flow out of this epidemiology-chemistry collaboration.

Dr. Mason, NCI: Operationally, I would like to suggest, since when push comes to shove, if there is not money there, is it really productive, why not in October and then six months later in the fiscal year because of the way these things are funded, we get together on some sort of formal basis? The thing that you threw out with regard to powdered milk was an interesting thing. I cannot for the life of me think of how we can study it right now. I mean it is something that should be taken under advisement, but how do you really identify a group of individuals where you could quantify that use, for what period of time.

I think that these types of things are productive from the standpoint of saying that it is an interesting question. We know what we want to look for, at least have some pretty good ideas about what we want to look for. How do we identify that group of individuals?

Dr. Bridbord, NIOSH: From the perspective of linking the chemistry and the epidemiology, I suppose NIOSH is the place where we have at least been endeavoring to do that for many, many years. I would not profess we always get out medical people and industrial hygiene people working at 100 percent effectiveness, but we certainly recognize that that is extremely important. On the EPA side, also, even
five, six, seven years ago there have been a number of workshops that were just looking at the whole issue of the atmospheric transformation products with fairly sophisticated atmospheric chemistry involved and the need to get the health scientists and chemists together. So, I certainly think it is extremely important.

Dr. Keefer, NCI: I will come anytime you want. As I say, without your help, we cannot answer the question of whether what we are doing in the chemistry laboratory is worth anything in cancer prevention terms.

Dr. Mason, NCI: Obviously, if what we are going to do is go into a real life set of circumstances and take things from people and try to quantify their exposures, and this is independent of the powdered milk thing at the moment, we need laboratory backup, and we, also, will need laboratory tests that are not so expensive that we cannot afford more than 10 of them. That has been the problem with a number of things, that the assays themselves are very, very expensive.

Dr. Keefer, NCI: Chemical assays or some kind of biological work?

Dr. Mason, NCI: Either of them. What I am saying is that we recognize the need for additional laboratories, if we just look at what we are doing in families and familial aggregations of cancer. We are spending a lot of money just for some fairly routine stuff, and to do some additional things, I think we are responsive and would like very much to continue on discussing.

Mr. Steelnack, OSHA: Our basic interest is the OSHA cancer policy. I am here to see exactly where epidemiology is going in the future since we are going to have to be explaining that policy to our own people. One of the big problems I have is that industry keeps coming back to us with what they call negative epidemiology studies. There is no real definition of what it is, but industry can always come back with a study saying that they surveyed their people, and they found that there is no increase in cancer in their industry, and we should not be regulating it at all.

Dr. Bridbord, NIOSH: One has to ask about the quality of the data. Negative studies don't prove safety. They just define potentially limits of risk, even good epidemiology studies.

Mr. Steelnack, OSHA: According to industry there is a difference between exposing an animal to a large amount and getting a small result and to exposing a worker to a small amount and getting no response.

Dr. Bridbord, NIOSH: That is the argument on the first cut, but if you really look at all the dynamics of the absorption, metabolism, retention and excretion you may actually find that the large dose in the experimental animal actually is giving you an equivalent to a much lower exposure in the human being. I think a good example of that is what you find with lead; whereas people were measuring doses for so many years in experimental models based on body weight and food intake, when they finally got around to looking at blood leads, situations where you would think the exposures were orders of magnitude above the human situation were really resulting in blood leads that were not that far different. You have to look at the case-by-case situation.

Dr. Gass, OSHA: I have a little different problem. I am interested in the design of internal and external training programs to educate not only our own inspectors but also workers to risk in industry. I am not an epidemiologist, but I have a couple of questions that I think maybe the group should ponder.
OSHA probably is the United States' largest employer of industrial hygienists, by job title. We probably have 680 or so inspectors out there, covering about 4 percent of the work sites each year. It is not enough, but that is about what our resource level is.

The question to you is since we only have about 23 or maybe 25 percent formally trained industrial hygienists on the work force and those are the ones that have formal epidemiology training, just what should the routine, everyday, OSHA inspector know about epidemiology as he goes through the field, and how could his training program be so arranged as to give the most benefit from data collection and observational points of view? It is a very practical problem.

Dr. Spirtas, NCI: Just one. Dr. Waxweiler from NIOSH and I are going to put on a session at AIHC to try to address this. We talked to Dr. Kelley about this, and I thought originally it was meant to be a seminar, but it is going to include definitional terms and study design, regarding what an industrial hygienist needs to know about epidemiology in order to be setting up programs or evaluating contracts. If you are an industrial hygienist for a company or an inspector for OSHA, our seminar will address what you need to look for and what data is required and then secondly, what should you be doing on your own, and when do you pull in a person trained professionally in epidemiology. We will give some examples of studies to bridge some of the gap between theory and practice. We think it is important to have practicing epidemiologists talk to industrial hygienists and tell them some of the things that we think they should be considering. In addition we hope to describe what sorts of things we do and what words we use.

Dr. Mason, NCI: When is this scheduled?

Dr. Spirtas, NCI: May 21, at the AIHC in Houston.

Dr. Gass, OSHA: You know the problem with that is that we have travel restrictions that are very tight, and I doubt that one-third of our industrial hygienists will get there. Ordinarily we would not send but half our force anyway because we have to cover the shop. You know, industry moves on and so does risk, and our meetings are not that important.

Dr. Blair, NCI: Don't you have regional training programs specifically for government industrial hygienists?

Dr. Gass, OSHA: Yes. That is part of the problem, but that is, also, our problem. We are the ones who formulate that in the national office, and the question still remains, you know, what do you teach internally? You know, you are teaching industry as well in that particular problem.

Dr. Blair, NCI: But couldn't you use the same sort of approach to the regional program and eliminate the travel problem?

Dr. Gass, OSHA: If I had known that ahead of time, I probably would have sent somebody. Are you going to be involved in that?

Dr. Bridbord, NIOSH: Why don't you think about sending a videotape crew even out of your regional office, and then at least you have that on record?
Dr. Gass, OSHA: I don't know. Would they allow that since it is a pay program? It is a tuition program, right?

Dr. Spirtas, NCI: We are involving an industrial hygienist who did his dissertation on a retrospective industrial hygiene study, and another person whose speciality is medical surveillance. So there will be a total of four speakers.

Dr. Gass, OSHA: But you do recognize that as a problem within the association and at large. Very few industrial hygienists that we have on board are really formally trained industrial hygienists. They are really chemists that have cross-trained or they are physicists or engineers.

Dr. Spirtas, NCI: We are willing to make the effort to try to put together a useful program, and there must be some way to work out logistical details.

Dr. Gass, OSHA: We have issues that raise their ugly heads all the time, like I had an industry group come at me because of some training that we were contemplating on carcinogens in the painting industry. Well, the company sent a contingent to have a little talk with me, and some of the pointed questions they asked were where is the epidemiology data that shows that zinc in paints, that lead in paints, that chromium in paints actually cause cancer in any humans? What effect does the vehicle have on the actual industrial hygiene collection? Just what does the method of collection mean? Are there any data points and where are they?

It is hard for me to find that data, and that is one example. Another example is when we prepared training for inspectors in the oil well drilling industry we had a little problem along with the Census Bureau. It seems as though they drill a 10,000 foot hole, tap a natural gas reservoir, cap it, put a Christmas tree on it and move on in about three to four weeks, and by the time we come back with a follow-up citation they are gone and it is the same problem in construction. I was happy to hear some of your points about a national registry, but that still does not tell us what we can do with that epidemiology. Are there any studies of oil field workers and the types of compounds they are exposed to?

I made a list. In our training program we found about 250 toxic substances that they routinely handle. Drillers are at great risk because they are there most of their lives, but mud engineers really do handle a lot of things including asbestos. So, those are the kinds of things that I need to know - where I can find the epidemiology studies listed. I would love to have job titles, agents, hazards, some kind of cross index from the industry so that when I have training problems or for that matter when we are teaching inspectors to go out and do a job, it would be really nice to know the data in advance.

Dr. Bridbord, NIOSH: Have you ever gone to chat with people who do the National Occupational Hazards Survey?

Dr. Gass, OSHA: Yes, I called Dr. Sundin, to discuss these matters. I called the one expert on oil well driving. Are you aware that there are only 14 participating states, and Texas, Louisiana and Oklahoma, who have the most oil wells, don't participate? Where do we get data? We would love to have it for our training programs and for our own professional knowledge.

Dr. Chu, OHSA: OSHA has subpoena powers and so does NIOSH.
Dr. Gass, OSHA: They won't collect it. Louisiana will not even take the data. There is no record in the Louisiana system. They don't collect it, no mechanism for collecting it, no state law. This is someplace where we need to improve, I think.

Dr. Bridbord, NIOSH: A couple of comments. One is we are at least beginning to look at the petroleum industry as part of our work within the institute. I don't know about a full range of operations that includes all the way from the mining, in effect, I mean the drilling in this case, but certainly we are taking a closer look at that generic area.

I suspect the issue of trying to get more efficient recovery of oil which is already in place, particularly as the price of oil has increased, will provide greater incentives to go to secondary means to increase the yield which in previous years would not have been much of an incentive, and one is potentially faced with all sorts of different schemes and mechanisms to increase that yield. I would suspect that as that becomes more generic that is a very valid question to ask.

The third point would be, I am not sure, maybe Dr. Sundin might be able to carry the message back to Cincinnati in NOHS 2, is this at least a group or an industrial situation where we might make some effort to clarify exposure?

Dr. Chu, OSHA: One of the first things that happens in a regulatory program that I am involved in is that in spite of lot of occupational studies on workers, i.e., painters, the problem, from a regulatory sense, is difficult to regulate. It has to be flipped over so that it is substance oriented. We need identification of substances so that we can get at those particular substances. The regulatory group that I am with is caught by the dilemma that when someone comes up with a PMR on dry cleaners, and we cannot identify whether or not it is TCE or carbon tetrachloride, unless we go to some generic classification, we need to have substance orientation.

Consistent with that, I am trying to take some leads from some of the animal data results in the carcinogen bioassays and possible look at whether or not these, by looking backwards and looking at the animal studies that are positive in several species, you can say that here are some candidates, let us look to see whether or not they have some kind of subset that would make a good epidemiological study.

We have identified compounds that are occupational, that are potential occupational carcinogens and in the reviewing of the data we have been able to capture target site information. We are now trying to capture and create a data bank of the chemicals and target sites for those chemicals, full well realizing that in not every single case is there a one-to-one correspondence between animals and humans, but cases where you have a rare tumor in the human situation that will be referenced back to associate chemicals would be a part of this picture.

We would like to get involved in tying together use information with this data bank of chemicals and target sites.

We are now approaching a situation to gather epidemiological tools to be used, computer programs, and I spoke about getting PMR studies programs and maybe getting the life table programs. I would hope these would be available so that at least the tools can be in place, if in fact OSHA decides to get involved in those activities. That is basically the concern that we have; the orientation toward chemical specific entities so that we can regulate. I think of all the concerns that people have, this is probably the greatest frustration.
Dr. Bridbord, NIOSH: I would just comment that there are a number of linkages already with NIOSH. We have had a number of policy meetings with the OSHA directors and the directors of the NIOSH offices and divisions, and there are mechanisms being established to facilitate, at least, in terms of the OSHA-NIOSH relationship, our understanding of what your needs are and we are doing the best we can to meet those needs.

Dr. Brown, NIOSH: As far as the epidemiological study in the single agents, most of the situations that we encounter now are industries where there are multiple agents involved, and the approach that we have been taking lately is to look at a large industry of, say, 10,000 workers, to look at the overall mortality of that plant and then to concentrate on a single cause that is shown to be in excess such as stomach cancer and do a case-control to try and trace that back to a specific agent. That is one way around these multiple agent problems or multiple exposure problems.

The single exposure populations are all used up.

Dr. Chu, OSHA: I don't believe that. When you have 200 bioassay results, as a chemist, I feel there is a driving need for leads that do exist and that it is an interdisciplinary concern and that, as new viewpoints come in, that perhaps it may be a small cohort that has a single compound exposure.

Dr. Brown, NIOSH: I was being facetious, there are some left, but most of the time we do get involved with populations that have multiple exposures, and they are hard to separate out and see where the association lies.

Dr. Sloan, NCI: Is there any group specifically working on interactions between different carcinogens?

Dr. Bridbord, NIOSH: Our own group in toxicology in Cincinnati, I guess as much as anyone else, has at least begun to approach the question, an example being the study we heard this morning. They have looked at the interaction, for example, with high temperatures and certain carcinogens, and I think initially you would really be looking at this primarily through laboratory studies.

Dr. Spirtas, NCI: I think we do have a lot to gain by getting together and talking. Is there anything that we can do as a step in that direction that is not overly bureaucratic and complicated but would create a list of people who call themselves epidemiologists, who want to work with epidemiologists or who have some interest in epidemiologic studies in the various agencies? Is there any way we can easily find out who is in the field?

Dr. Riggan, EPA: Would it be helpful if I compiled a list of individuals with their expertise in the Epidemiology Branch, HERL/RTP? To whom should this list be sent.

Dr. Mason, NCI: Send them to the branch chief. He does not get enough mail.

Dr. Riggan, EPA: I would be glad to share.

Dr. Chu, OSHA: In statistics they have a federal directory of statisticians.

Dr. Mason, NCI: The last thing in the world we need is something like that. If you want to do something along these particular lines and have it be productive, then earmark some of these monies and have a workshop and use the monies to pay travel for individuals to come to discuss, but we don't need another laundry list.
Dr. Bridbord, NIOSH: I think one of the questions that is going to come up is where do you go from here. One of the things that might make some sense is to at least look at the issues of new starts among NCI, EPA and NIOSH in the area of occupational and environmental epidemiology and with particular reference to cancer, but also, allowing at least some flexibility to look at reproductive effects as a minimum start. Also, to ask each of us, as we make our own tentative proposals on program planning, to have the commitment that before each of the organizations makes their signed and sealed approval on that, that each have a representative group of people who come and sit around the table and say that here is what we as collective agencies are planning to do in FY 1981, and then to identify people with mutual interests where we might find that two or three of those studies might duplicate.

We might, after hearing each other's presentations, decide that we left out three or four things that individually we had forgotten about and could begin to establish those mechanisms and be thinking from that perspective.

Dr. Mason, NCI: I agree. We have a lot of internal program money that is committed to these types of studies. It does not have anything to do with this collaboration. I think this should merely be viewed as a mechanism to stimulate additional studies, not as the prime way to fund these types of studies because there is not enough money in the pot.

Dr. Bridbord, NIOSH: I think you both identify other studies that maybe, individually, the agencies had not perceived. The other thing you may do is strengthen those collaborations for example, just identifying another individual who has an interest in that who might be willing to review a protocol or informally work on something. It is just built that way.

Dr. Mason, NCI: Dr. Chu was proposing a directory and I like to argue with him.

Dr. Spritas, NCI: If one did not exist. There is a directory of statisticians, a little yellow book that comes out, but I don't know of a directory of epidemiologists unless SEER is going to put something out.

Dr. Chu, OSHA: I disagree with you because you are working from a position where you are in the catbird seat. Of course, when you are in the catbird seat you don't care what else is out there, but the people who are out there and not in the catbird seat need to know of these activities.

Dr. Mason, NCI: There exists an easy way for you and for anybody else who is not that familiar with what is going on. You make three phone calls. You can get three pieces of stuff in the mail, and you can sit down for yourself and just decide; these are the types of things that they are doing, these are the types of problems that they are addressing, and I think that is true.

Dr. Bridbord, NIOSH: There are a number of assumptions that certain mechanisms don't already exist, and there really are two ways that those things get started.

One is just a scientist with an interest, with some ability to effect the resource allocation decisions and talking to those people and just with the power of the ideas convincing them that there is worthiness in pursuing study A as opposed to study B as opposed to study C, et cetera. Those processes exist within each of our respective organizations, and they exist to some extent across them.
The other mechanism is the more formal organizational policy decision mechanism which is well established, at least in terms of OSHA and NIOSH, and one of the reasons why we specifically wanted OSHA to come here. Dr. Leidel is on two-year detail from NIOSH and is a person who can understand some of those linkages in health that occur, but I just perceive that your assumptions are basically that there really isn't anything when there is something already and we are trying to --

Dr. Chu, OSHA: I guess what I am saying is that I have discovered in the inhouse group at OSHA that the tools, the basic tools like statistical programs, access to computers, these kinds of things are not available which are just fundamental.

Dr. Bridbord, NIOSH: That is a decision for the assistant secretary to make, you know, in terms of whether that person is going to commit their resources to building the internal OSHA research capability versus giving Dr. Gass 20 more people to do work on education, versus adding 30 more compliance officers.

Dr. Gass, OSHA: But you just mixed apples and pears, because the Act does not give us the right to do research. That is your domain, but except in standards development; that is the only place where there is some kind of research done in that sense of the word.

Most of us are compliance people who are pointed and focused toward enforcing the Act and the regulations.

Dr. Bridbord, NIOSH: Right, but to the extent that OSHA does certain kinds of analyses and research associated with the standard setting process, that has been going on for a long time.

Dr. Gass, OSHA: That is an exception to the Act.

Dr. Bridbord, NIOSH: I mean the basic mechanism, at least, in terms of the research, it would be difficult. The mechanism is basically through NIOSH and through the other organizations. I know there are a number of cases where NCI was asked to do studies.

Dr. Spirtas, NCI: I have a couple of small points. I think I have heard of some studies that may be of interest to some of the people here today. On your question about substance versus occupation, I think Sheila Hoar is doing a dissertation on that with Alan Morrison, a computer program that would pick up studies about certain occupations or certain substances, and provide a cross-link between substances. I think in terms of the oil field worker studies, wasn't there a woman from Tulane who come up and gave a seminar?

Dr. Brown, NIOSH: It was people on oil platforms and offshore. That is not in our domain.

Dr. Blot, NCI: I missed the first hour, and maybe I am repeating things that have been said, but in terms of general cooperation between NCI, EPA and NIOSH, I would see three major areas. The first concerns case control studies of certain cancers. These could be conducted in high-risk areas in the country, and focus on tumors that have not been studied yet. For example, there is some indication that there may be an occupational and/or environmental link between herbicide exposure and lymphomas - an hypothesis that could be tested by the case-control study approach. A second case-control study is one that we are planning to do within our own group.
This is a study of colon cancer in retirement areas of the South, which may be of general interest to EPA in that a variety of environmental measures are suspected, particularly to dietary factors. The reason for this selection of Florida is that the rates in certain counties there are quite low even though many people have moved down from the North where rates are high. The colon cancer rates in these retirement areas are the same as they are in other southern areas where there are not retirees even at older ages, whereas you might expect them to be somewhere midway between the low rates in the South and high rates in the North. We have been phasing up now for an interview study contrasting dietary differences and changes on diet, between cases and controls, and also are attempting to build in a laboratory component, and here is where we might possibly call on EPA for assistance. There are certain laboratory measurements of interest, e.g., micronutrients in the blood, vitamin A in particular, with perhaps fecal material analysis for some other constituents.

The second general area concerns cohort studies, particularly of occupational groups. One such cohort study that might be worthwhile would be a follow-up of a cohort of shipyard workers who were employed during World War II. There are people now involved in studying cohorts of shipyard employees. The largest one, by the group at Hopkins, involves employees who began working in the shipyards in the fifties. This study keys on radiation exposures in nuclear yards, but there is some interest among the investigators, if additional money could be provided, to go back in some of the yards and identify workers who began work in the 1930's and 1940's. Job titles are available, and it may be possible to more fully evaluate the role of asbestos and get clues to other shipyard exposures in addition to evaluating the possibility of a radiation hazard.

A third general area of NCI/EPA/NIOSH cooperation would be in utilizing data resources that already exist and linking them up. In particular there are some systems here in the US which would provide useful occupational and/or environmental data if accessible - particularly the Social Security System. If we are not limited to the United States, there are excellent resources available elsewhere, particularly in the Scandinavian countries where perhaps a concerted effort by NCI, NIOSH, and EPA might get some work going. For example, in Sweden there are cancer registry data listing all cancer cases in the country. Census data are taken every five years, and there are rosters of industrial groups that have been specially put together by the Swedish version of OSHA, and there happen to be other rosters. The Swedes are great at making lists of people, and they all have social numbers that can be linked rapidly. There happens to be a roster of all prescription drugs issued in the country. So, there is potential for looking at these together. Some people in the US have talked with people from Sweden about such epidemiologic studies but perhaps a greater stimulation might be beneficial.

Dr. Bridbord, NIOSH: There recently was a US-Swedish conference on occupational safety and health where about 40 representatives from Sweden, including a full spectrum of government, labor and industry representatives, and there is a mechanism being established to facilitate the cooperative studies between the two countries which certainly would include a number of the things you mentioned.

The main collaboration would be Department of Labor to Department of Labor, but NIOSH has already tied into that from a research perspective, and I would imagine that that umbrella could be extended.
Also, the Finns are coming to the United States in October, and they have also had a number of activities and as of now there is the Third Annual NIOSH Scientific Symposium which will be held in Detroit preceding the APHA Meeting which will feature not only the results of some NIOSH work but, also, summaries of the ongoing Finnish work which includes cancer, and a number of other things as well.

Mr. Boeniger, NIOSH: In our primary responsibility to characterize and determine worker exposures we have to deal with biochemists and physicians during morbidity studies, with control technology engineers for controlling and correcting exposure conditions, and most frequently with epidemiologists, among others. I don't like to be redundant, but I would like the idea of trying to exchange directories of some kind of listing of professionals in various organizations. Every once in a while I have been able to extract from someone a telephone directory from a particular group, and it has been indispensable to me, but I know NIOSH has directories of their personnel and how the organizations are broken down, and I imagine many of the other government organizations have similar things. I wonder about the possibility of exchanging them.

In terms of professionals in various areas of research in environmental health and public health, I was wondering if anyone else had heard about an environmental health directory that a New York publisher is coming out with, similar to one that they had perceived in energy technology. It is essentially going to be able to allow one to look under a title area, such as, say, epidemiology or more specifically, printing trades epidemiology, or something, and be able to identify individuals that have done work in that area.

Dr. Bridbord, NIOSH: This just reminded me that NIOSH does contain a system of ongoing research in the occupational health area on computer data base which includes not only the United States but other countries as well. I would not say that we have 100 percent on that system, but it is a reasonable place to start to get lists of names of people who are currently involved.

Dr. Spirtas, NCI: Obviously everyone in HEW enters projects into the Smithsonian Scientific Information Review System. Is that the way it is for OSHA and EPA, also?

Dr. Bridbord, NIOSH: Yes.

Dr. Riggan, EPA: Yes, and on EPA's computers.

Working Group adjourned.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Wednesday Afternoon, May 7

SESSION B

WORKING GROUP ON THE TOXICOLOGY, METHODS DEVELOPMENT,
PROBLEMS AND NEW DIRECTIONS

SESSION CHAIRPERSONS

Dr. Gregory O'Conor
National Cancer Institute

Dr. Norbert Page
Environmental Protection Agency
Dr. O'Conor, NCI: One of the major objectives of this session is to examine the program as it exists and to make it more effective for the future. I think we can begin to talk about the areas that seem most important for joint cooperation and collaboration.

The program is still fairly new. While there were problems at the onset, it is beginning to reach maturity now, and this workshop is a fantastic opportunity to develop the communication and interaction that is difficult when we are all back at our own institutions. I think it is time now to try to identify those specific areas where this type of collaboration can be mutually beneficial and most productive because the funding has been generous, and I hope it will continue that way. However, the funds are limited and I think with the increased interest in the total program we are going to have to prioritize more carefully and identify those areas of research and application research where the benefit is going to be maximum.

Dr. Page, EPA: As I have listened to the papers presented today, it is very obvious that we are only seeing one small part of research in many areas. We have seen or heard results from some of the in vitro testing, some in vivo, and some of the metabolism work which is just a drop in the bucket of all the research going on in these areas. We have to take a perspective look at what is being done in this program in context with that underway in the rest of the research community, identify those areas that are particularly important for the three agencies and, as Dr. O'Conor said, attempt to develop a meaningful program within the constraints of this collaborative effort.

We will start with some of the questions that have been submitted.

Question 1: How would you suggest that a group of workers exposed to suspected carcinogens could be effectively monitored for the presence or absence of early cancer. This question, I guess, is directed toward the immunodiagnostic work, or other markers, in early diagnosis of cancer.

We talked about the possibility of a battery of diagnostic tests, and that seems, certainly, to be a reasonable approach to me, but I am not a clinician. However, knowing what I do of the pitfalls of some of the early diagnostic tests, I think it is an area of great importance; one certainly that NIOSH and OSHA have a great interest in.

Dr. O'Conor, NCI: The question is how would you go about handling a group of workers exposed to suspected carcinogens, and monitoring them for the presence or absence of early cancer. Well, that really is a question for an occupational physician in the workplace, and unfortunately, to my knowledge, there is no ideal way of screening individuals for cancer. It is one of the major problems of cancer prevention or early diagnosis that exists. My response would be that it depends on first of all identifying the suspected carcinogen, and from our knowledge of what organ systems different types of compounds affect, this would determine the type of monitoring activity, be it physical examination and laboratory testing, depending on what you are looking for.
Broad screens in general have not been particularly effective for the detection of cancer in the early phases. So the best approach is that the key to screening and early diagnosis is identification of high-risk groups and then developing selected tests which will try to focus on the particular type of cancer that one might expect in that population group.

Dr. Lowry, NIOSH: I think I asked the question knowing that there really isn't an answer, as you have said, but more or less to place the bottom line of why we are all here in perspective. In other words, all of the basic studies that are done to show a particular compound to be carcinogenic, mutagenic, all of the different testing techniques that have been developed, these are all fine and good, and they need to be done, but at some point in time, you have to take all of this data and try to apply it to the human being.

At this point in time, I would agree with you that the screening techniques, at least those that I am aware of, are not that effective, and there are a lot of false positives and false negatives. In most situations you are dealing with a reasonably healthy population of working people, rather than an acutely ill hospitalized patient who may have lumps and bumps that are rather easily recognized.

Dr. Plotnick, NIOSH: At the present time, and Dr. Lowry knows this better than I because he does a lot of them, these sample analyses, clinical chemistries, et cetera, are required by OSHA guidelines for pre-placement or follow-up medical examinations.

We had a recent discussion at a symposium about testing methods, and it was the consensus of people I consider to be relatively expert in the area that clinical diagnostic testing has not saved one life because of the fact that by the time that these relatively gross changes are picked up in clinical chemistry, it is generally too late to do anything.

Dr. Hygyeli, NCI: I would suggest approaching this from an entirely different angle - from the practical standpoint. My experience is based on two large studies, one in Tyler, Texas, on asbestos-exposed workers and a second one in Louisville, Kentucky on vinyl chloride workers. We also support two new prevention programs in Berkeley, California. As you know, the National Academy of Sciences has recommended that every worker who has been exposed to any kind of carcinogen or suspected carcinogen should be notified. I feel that there has to be a step beyond this since it creates only anguish and does not solve the problem. It is my recommendation that the follow-up of these high risk workers should include more than just notification. For example, our experience with the projects to date shows that unless you have some kind of medical screening for early detection of cancer or very simple medical test(s) to determine damage due to exposure, the educational programs are not accepted by the workers since they can see no reason for or benefit resulting from them. Also, the majority of the workers will not participate unless the educational program is provided on company time. In view of the current limitations in the therapy of occupational cancers it is important that the educational program include emphasis on decreasing the overall risk to cancer. For instance, these workers need to know not only about their exposure to a given occupational carcinogen and the resulting risks, they also need to be educated about risk multipliers such as exposure to other toxic substances and behavioral patterns such as smoking, drinking and nutritional imbalance.
Dr. Turner, EPA: I came from industry and was concerned with safety, health, labeling and regulatory compliance. In my opinion the development of a standard for vinyl chloride by OSHA was accomplished by regulatory action despite the strong resistance by industry because ample evidence for cancer of the liver in plant workers exposed to vinyl chloride existed. Industry is now conforming to these standards and apparently has not been affected from an economic standpoint. Until a good diagnostic test for carcinogenicity is developed which bears a good correlation with the formation of cancers in humans, we should resort to preventive measures similar to those taken for preventive medicine. Cigarettes and saccharin are good examples. By following this approach at least one can avoid problems of defending one's stand and political implications. It is much easier to back off on a cancer suspect than a firm statement of a chemical being a carcinogen when the evidence disproves it.

Dr. O'Conor, NCI: We recognize that research on markers and better diagnostic methods certainly are a high priority, particularly in relation to identification of patients or individuals who might have been exposed to chemical carcinogens. In the meantime we must use the means at hand and put into place those practices which we know can be effective, that is improved practices within the workplace and the design of monitoring systems which are selected on a biological rational basis to survey or monitor the particular type of exposure that we are concerned with.

Dr. Morris, EPA: I like the idea that Dr. Herberman and others are working on in the marker area, but I think we have to appreciate there are other chronic diseases in this process. I realize we have had a lot of emphasis and certainly NCI has focused on the cancer problem. On the other hand, I think at least from our point of view, there should be a more equal distribution of concern, perhaps from all of us, in NIOSH, EPA and certainly the NCI, in looking at other chronic diseases and the potential markers for those.

Question 2: Was inhalation application considered in the study? This is the major route of exposure for workers, lung macrophages, pyrogen products and safe disposal. (Refers to Dr. Lowry's presentation.)

Dr. Lowry, NIOSH: I made an attempt to answer that during the session. Basically inhalation was not considered for two very practical reasons - time and money.

I am not convinced that inhalation is the major or only route of exposure in an environmental situation. I believe Mr. Boeniger may be able to answer the question. Mr. Boeniger do you have any comments on that as to what is considered a major route of exposure?

Mr. Boeniger, NIOSH: We took some environmental samples and tried to ascertain what the respirable and non-respirable fraction of the particulates were and we found that the majority of them were non-respirable.

Therefore, we feel, I guess, that any non-respirable particulates that were inhaled would subsequently be swallowed. So, oral routing of dosing the animals would probably be most efficient and practical in terms of time and money.

Dr. Gregory, CPSC: When the artists and craftspeople first petitioned OSHA and CPSC about the benzidine based dyes, the problem of possible skin absorption was brought up, and DETO felt this was very impossible. When Black 38 was applied to
the skin of rabbits the portion that was labeled with Carbon 14, that is the diphenyl portion, was recovered in the feces and urine up to 90 percent. It was found out later that the experiment was not done correctly, and they needed to put these Elizabethan collars on the rabbits to keep them from licking off the dye, but even when they did this, they found out that there was skin absorption of the amount that 10 percent of the carbon-labeled substance was found in the urine and 5 percent in feces, so that we do know that some portion, at least of the benzidine based dye Black 38, is absorbed through the skin.

Dr. Page, EPA: I don't know that much about these dyes, but it is my understanding they are not that firmly fixed on particles, that they can elute, and regardless of whether the exposure is through the intestinal tract or in the lung, you have a fair amount that is absorbed. Therefore, the issue is really one of systemic exposure, so choosing a route so that you get systemic exposure is a reasonable and practical way to go.

Dr. Lee, EPA: Before I left Kansas City, we did a study on several dyes. Although I am not at liberty to say, the study was performed for a private company. We had a serious discussion concerning the right route of administration. Naturally, the inhalation route is very expensive. Finally, we chose the oral route for two reasons. First, during inhalation a good part of the material will be getting into the GI tract. Second, I understand that this material is mostly used for the copiers, typewriters and so forth. The chance for the operator to get the chemicals on their hand and into their mouth is quite great. For these two reasons, we also chose the oral route to study these dyes.

Dr. Page, EPA: Since we have been talking about inhalation, let us continue with that subject. We have a question pertaining to the chronic inhalation of short asbestos fibers, less than 5 microns.

Question 3: What do you think are the implications for the health and engineering controls for the current OSHA asbestos standard? As a result of your findings, what types of engineering controls would you recommend for the new asbestos standards?

Mr. Platek, NIOSH: Some of that was answered in the talk, but I might comment on another section of it. You said, "What precautions might we take from our findings?" I honestly don't have enough findings to comment on any type of engineering controls. At this point we have not finished the study.

Question 4: How do you assess the potency of a carcinogen? Can we be quantitative?

Dr. Page, EPA: This one, we could spend a lot of time talking about - how to go about addressing potency and the attempts and the failures and the successes, but let us hear a few thoughts on it.

Dr. Saffiotti, NCI: I have my standard reaction to the word "potency" which is, let us not use it. We don't know what it really means to say potency of a carcinogen. There are more and more examples which would show that what might be a measure of the level of effect of a carcinogen in a certain set of conditions becomes a very different type of effect in a different set of conditions.
There is a recent study, for example, of Lisa Prane from Bar Harbor in which she has tested methylcholanthrenes by injection in 8 or 10 strains of mice, inbred strains of mice, at a certain dose and got a level of response different in different strains so that she could rank the responsiveness of these different strains, and then she tried the same experiment with a lower dose of methylcholanthrene and, lo and behold, the rank of response was all different. In fact, it was practically reversed, and this was done several times. It was published in Science last year. So, here you have essentially a reverse order of response, and therefore an implication of different potency, if you wish to use that term, in different biological systems, but it is at a different level depending on the level of carcinogen used. There are many more of these examples that make it very difficult to utilize the concept of potency as if it were an inherent property of the chemical until someday when we will be able perhaps to narrow down that definition to some particular biochemical interaction, such as DNA binding. We know already in DNA binding, for example, that there are all the host factors that control the level of binding and enzymatic activity, penetration, distribution, passage through membranes so that the same carcinogen would bind very differently under these different conditions. Potency is really the measure of an interaction and therefore should not be used as a measure of the property of one of the components of the interaction, and I am just sort of advising everybody to stay away from it. In the IRLG report that we put together last year, we let it sort of sneak through in one case. There was one phrase with the word "potency" in it, but in most other cases we systematically replaced it with the word "sublevel of effect" which then required specifying where and how and under what conditions.

Dr. Gregory, CPSC: Dr. Saffiotti, the level of effect must certainly be detailed. However, I would like to refer to your own work with benzidine, or toluidine, dianisidine, and 2-naphthylamine in hamsters. I am certain that you would not put toluidine or dianisidine in the same category of potency as benzidine or 2-naphthylamine, and yet we do need to make some sort of an evaluation because we do need to know in a very real sense how dangerous toluidine is compared to 2-naphthylamine.

Dr. Saffiotti, NCI: I agree with the obvious general concept behind all this. The difficulty is to be sure that we can make a predictive projection that would cover all the individuals. Are there some individuals that are particularly susceptible to toluidine carcinogens? We simply do not know that.

With epidemiology, in some cases, we will eventually build up a sort of reasonable basis for some general assumptions. Let me give you an example of some of the difficulties in that area. We have always thought of 2-naphthylamine, the alpha naphthylamine, as pretty much being in that category like toluidine, dianisidine and so on in terms of being a much less reactive compound with lower toxicity and lower effect in various biological systems. We have recently done a fair amount of work with aromatic amines in the Ames system, and sure enough alpha-naphthylamine and toluidine and dianisidine were much less effective as mutagens in the system than benzidine, 4-amino-biphenyl, beta-naphthylamine, dichlоро- benzidine and so on.

We have then started doing work on cell transformation with the 3T3 mouse fibroblast system and doing toxicity work in that system, and there is a result that I am still not convinced about. I am still trying to see if there has been some mistake there, but alpha-naphthylamine is much more toxic to these mouse fibroblasts than the beta compound. The first reaction I had when I saw the result was that somebody had mixed the samples, and this has been checked and done over again, and
apparently there is no such simple explanation. We don't know what it is. It may eventually be sorted out.

Dr. Gregory, CPSC: I believe we need to be very careful about whether we are talking about carcinogenicity or cytotoxic effects, because they do not necessarily correlate one with the other, and with respect to - we all know the differences in the extremes, and that is that we certainly don't want to call saccharin the same sort of carcinogen that could be identified with bis(chloromethyl)ether. We know that we have both animal and human data for bis(chloromethyl)ether, that it is, indeed a carcinogen and a very potent one.

My question is whether or not we are satisfied with saying that bis(chloromethyl)-ether is a potent carcinogen and saccharin is a mild carcinogen or do they need to be somewhat more quantitatively ranked.

The National Advisory Board has come out with some of the criteria that are necessary to be taken into account, such as shortened latency period, dose response effect, and whether it is metabolized in the bioassay system the same way that it is metabolized in the human. There were seven criteria, some of them much more important than others. Is it not possible that you could come up with an algorithm whereby you could evaluate the substance with respect to all the data, and this, again, Dr. Saffiotti, I agree with you, we don't have all the answers yet, but on the basis of what data we now have, could we come up with an assessment of potency?

Dr. O'Connor, NCI: I think Bruce Ames is trying to do this, and maybe other people are, too. The question is when you come up with it is it going to be really biologically meaningful, beyond what we already know from one extreme to the other. It would be ideal to be able to prioritize them in a very specific way, but I think what Dr. Saffiotti and others are saying is that we really don't have the type of information today to do this where it would be meaningful in terms of human experience. It might be meaningful in terms of the specific animal experience.

Dr. Saffiotti, NCI: Perhaps in the continuation of our workshop this may be one of the points that we might want to pick up for consideration and recommendation of further work that could be done usefully in collaboration with the three agencies because we may combine really useful information from human studies, monitoring and environmental measurement of exposures, biological responses, and biological models. It may be one of the areas of quantitative studies of carcinogenesis that may be an important one to keep in mind for collaborative research.

Dr. Page, EPA: My own thinking, being in a regulatory agency and, also, having worked in the research environment and now trying to bridge that gap, knowing that in the regulatory agency you have to make estimates, is that you have to come up with your best guess as to the likely impact of exposure to a particular chemical. You cannot use only the innate biological activity, but you have got to look at the exposure from the viewpoint of absorption and target tissue deposition - how the chemical passes into the body and has potential for interacting.

I think that is what Dr. Gregory is alluding to, not simply potency from the viewpoint of the initial biological activity, but the total spectrum of what happens. I think that is what the regulatory agencies have to come up with - a type of weight of evidence assessment.
Dr. Kraybill, NCI: We had discussions on this some years ago, two or three years ago, and I think Dr. Saffiotti was involved. Potency, as he indicates is hard to define. If you want to call it effects, that might fit the bill, but it is conditioned on what species you use and what strain you use. You know, you could use the C3H mouse and get a big response; yet in another strain you may not.

I wonder if it is not more important to develop a classification system such as IARC is using. We, and Dr. Hueper, used to call them strong or weak carcinogens. Well, now, IARC does not do that. They limit it, and in their monographs they say, "carcinogens with sufficient evidence and carcinogens with limited evidence." That sort of classification helps me a lot, and then you might classify chemicals into a group where you have a physical-chemical phenomenon like an irritant effect. That is important to know. Some derive their effect, like say a calcium compound from becoming an irritant to the bladder wall where you get nephrocalcinosis or problems of that sort; and then, last but not least, are those that would fall in the category of promoters. I noticed in a recent article that Boyland published, he labeled saccharin a promoter. That makes all the difference in the world in my opinion. Now that has gone from the classification of a weak carcinogen to a promoter. So, I think a classification scheme might be a step, a first step in that direction.

Dr. O'Conor, NCI: This is as close to a consensus on this subject as I have ever heard. Obviously, it is a terribly important subject, and we have started, and we certainly will come back to it probably today in another forum and certainly tomorrow at the Plenary Session.

Question 5: Was DMSO used in the in vitro carcinogenic system? Were radiolabeled carcinogens used to see the uptake solubility tested? Was liquid aqueous solubility determined?

Dr. Gregory, CPSC: The answers to those questions are yes, no and no. I asked those same questions of Dr. Dunkel and I think she is the best one to answer that. They did use DMSO.

Dr. O'Conor, NCI: Are there advantages and disadvantages in this over the hamster system? So much work has gone into the hamster system, I am wondering how far we should continue to go in terms of development and refinement and validation of a series of these mammalian transformation systems or whether we should really be putting more concentrated attention into a couple of specific ones and get them standardized, and whether we should put more attention into the transformation assays of human epithelial cells? I would like to hear some comment on this.

Dr. Waters, EPA: We have worked with the three leading transformation systems in our laboratory and contract programs. They are the Balb 3T3 system, the C3H 10T ½ system and the Syrian hamster embryo system.

I think it is fair to say that the advantages of the mouse fibroblast systems are that there are continuous cell cultures. They are very reproducible. They have some spontaneous transformation to a greater or lesser extent in certain laboratories. They have a low metabolic activation capability for some compounds. This is a disadvantage, and for this reason the efforts have gone into providing metabolic activation either via co-cultivation with other cell types or with S-9 type metabolic activation systems.
The real advantage of the Syrian hamster embryo system as distinguished from the other two is the fact that you retain in early passage Syrian hamster embryo cells a fair amount of metabolic activation capability.

Theoretically, this should make it more useful as a detection system. However, the other systems may have advantages for mechanistic studies. Their cell cycle kinetics are very well defined; the basic enzymology and so forth is easier to establish because of the fact that they are continuous cells in culture.

The variability of the Syrian hamster embryo cells from preparation to preparation can be overcome to some extent by freezing down large lots of characterized cells. So, these are some of the trade-offs. I think you have advantages and disadvantages depending on what you want to use the system for a screening system or a system for mechanistic studies.

As for your other comment about the use of human cell systems, I think there is a great deal of effort going into this area, both in the use of human fibroblast systems, as well as human epithelial cells in culture. Obviously, if we are going to study carcinogenesis we want to study human epithelial cells, but human fibroblasts may offer a great deal of advantage in terms of their utility.

I think that the human cell work should be very much encouraged, but not to the exclusion of systems such as 10T½, 3T3 and the Syrian hamster embryo system that may be more useful for routine screening and perhaps confirmatory bioassays. I would put them in the latter category, confirmatory bioassays.

Dr. Page, EPA: The Europeans seem very nervous about the possibility of cell transformation systems being introduced in a battery of tests by the OECD. You are familiar with this type of regulatory scheme for testing new chemicals. How would you assess the current state of the art as far as regulatory requirements to have transformation tests performed?

Dr. Waters, EPA: I think that transformation systems have come a long way. I think they are really not readily available in testing laboratories at the present time, and this would be one of the major concerns that anyone would have in terms of regulation requiring the testing in transformation systems as a key component.

There are laboratories that can reproducibly perform these assays. I think the assays themselves can be carried out in a reproducible fashion. There are major difficulties that we are encountering, especially now, and they have to do with, for example, the shortage of serum. This has been a severe problem. One has to characterize the serum to be used in these systems to make sure that it will provide a reproducible system.

This kind of problem has caused difficulty. There have been many laboratories that have tried, for example the Syrian hamster embryo system, and had difficulty with it. There was a period of time when hamsters were apparently infected which produced many difficulties with that particular system. The hamsters seem to have been cleaned up at the present time.

I think it is those kinds of concerns that have warranted, perhaps, some concern over the use of transformation systems in a regulatory context. However, at the same time you have to look at what alternatives exist. If you are moving towards
cacinogenesis as an endpoint, I think perhaps you have the senca system that you could use as an alternative. It takes more time. It may be a little more costly. The question is what else to do, and it is a difficult question right now.

Dr. Page, EPA: In your senca system there are only a few places that really have that going, aren't there?

Dr. Water, EPA: That is right, the same difficulty in some ways, but it is a whole animal system and it does get around some of the problems that in vitro systems inherently have. Maybe Dr. Saffiotti would like to comment. He has been heavily involved with this question.

Dr. Saffiotti, NCI: First of all, I would like to agree very wholeheartedly with Dr. Water's comments. I think that the issue is an open and complex one, and one we need to continue to develop. The methods that have been developed in the last decade or less are beginning to be used in a number of laboratories. Certainly they are not ready to be written into some regulation for any industrial or toxicology lab to apply and use them because even the sophisticated laboratories sometimes have difficulty with these delicate systems.

There are, however, several obvious needs in research development in this area which once again I think are particularly important in the cooperation of three agencies because they complement each other. One is the point that Dr. Waters has mentioned on the increasing difficulties with sera, and the possibility that some systems may be developed to have cell transformation even in chemically defined media or at least with very small amounts of sera present which would minimize some variations due to that.

The other, as Dr. Waters has mentioned, is the problem of epithelial cells and of human cells, human cells both epithelial and connective tissue cells.

I think that in the next decade or so we will probably see, from general research interest in this area which is rapidly expanding, the development of a battery of methods that will come out of different laboratories that will offer reasonably good models for studies of transformation in major different epithelial tissues.

In the last few years we have had progress with the respiratory epithelium in Nettlesheim's group for example. Of course, the liver has been available for some time, the skin system, that is through our laboratory, and Fusinick in Germany has been particularly working on it.

There is work now that begins to extend to kidney, begins to extend to the endometrium, for example, a whole variety of target tissues, colon. Some of those are just beginning and will take years to develop, but I think that one could conceive of a situation, maybe a decade from now, where we will be able to use reasonably standardized cell culture systems to ask questions as to whether certain types of chemicals are particularly interactive with or capable of transformation at selective sites. Obviously, this will have to be paralleled with the experience in in vivo observations and the counterpart of the human tissue in vitro, and we will have a pattern that will evolve of knowing what carcinogens are particularly active in what target tissues and what modifying factors are key factors.
So, I think that this is an area that could be very fertile for collaboration in which we will, in fact, benefit from the interaction that comes from EPA and NIOSH laboratories that see some of the human exposure problems combined with those that we see in NCI and you know, stimulate interest in the whole field.

Dr. Page, EPA: As you probably know, there are a number of schemes that are being proposed now for regulatory use which would have the transformation assays sort of like a second level in a tier scheme. Once you have results in bacterial systems, Ames test, or some other system, this would trigger the possibility of doing a transformation assay more or less from the true positives I would guess. Then based upon results of that you could go on if you have a positive to a longer term test, or else at that point conclude from a negative result, that there is very little potential risk for carcinogenicity and stop at that level.

So, there is, certainly, in the regulatory scene, a big movement to try to use the transformation assays. My concern is like Dr. Waters that we don't push this too fast, but that where there is an available technique, we try to implement it, but do it in a meaningful way.

Unidentified speaker: Is it possible or is there any idea that transformation assays might be useful for detecting possible teratogenic effects?

Dr. Page, EPA: It has been my understanding that the correlation has not been that good between teratogenic effects and positive transformation results. We are considering this kind of a possible screen as to the rationale for whether to require a teratogenic test.

Dr. Gregory, CPSC: I remember that was discussed at the meeting over in Monaco, and I believe that the conclusion was that many of the teratogens were indeed cell transforming. They would transform the cells, but not everything that would transform the cells was indeed a teratogen. We had many transforming types of substances that did not turn out to be teratogenic at all.

Dr. Page, EPA: There are other mechanisms for teratogenic effects that are not genetic in nature or would not respond to a transformation assay. That has been my understanding.

Dr. Waters, EPA: I would like to say that I think most teratologists don't feel that in vitro methods are really applicable, except for limited chemical classes under well-defined and known conditions, and so I think I would accurately reflect the feeling of teratologists by saying that the answer to your original question is no.

Dr. O'Connor, NCI: Again, in a provocative vein, I am glad to hear you say that you are beginning to really talk seriously about the tiered system, using the in vitro test for a screen.

I certainly appreciate what has been said in not pushing them too fast, but I wonder if we can really afford to not push as rapidly as possible, because the in vivo tests, really from simply an economic point of view, are incapable of doing the job that we think probably needs to be done in terms of the number of compounds and the screening that would be ideal.
We hear at the National Toxicology Program that implementation of TSCA will take care of a lot of this, but I wonder do we really think that industry is going to be able to do a better job of the in vitro screening or in vivo testing with a lot more compounds than the government has been able to do. We have had enough trouble, plenty of trouble, trying to grind out in vivo tests at great expense and I understand now that we are only going to get about 40 a year because of budget restrictions from the NTP.

Dr. Bull, EPA: I am not going to do much more than repeat what other people have already said. Basically this problem has been recognized within the EPA at least that people are familiar with the issue within the Office of Toxic Substances and other program officers. Cell transformation assays or any other assays to function at the tier two level have to reject false positives or they really do not serve any function not already met by tier one tests such as the Ames test. With that limitation, I think our inability to say that these systems would rule out false positives and at the same time not produce any false negatives makes their application at this point in time promising but still kind of hypothetical. I think there needs to be a lot of work done before any of these systems, in vitro or in vivo, can be considered fully validated for regulatory purposes.

Dr. O'Connor, NCI: To be argumentative, in terms of the in vivo tests, it is certainly clear whether it is positive or negative that in terms of human application we don't really know whether they are false positives or negatives in many instances.

Dr. Page, EPA: I think it is quite certain that there will be a form of either a battery or a tier scheme come into existence, either on the international scene or within the EPA or other regulatory agencies. We are having difficulty in how to structure this tier scheme. Certainly, a lower scheme is going to be inexpensive tests and I don't think there is much doubt that a bacterial test belongs at that level.

I know Dr. Waters has proposed schemes and Dr. Lee now is working on one which will possibly be put into place in the EPA. The trouble, as I see it, is where to place this transformation assay? What emphasis to put on that particular scheme or that system? It is not so much the bacterial system. I think it is pretty well agreed that it will go into a lower level scheme.

Dr. Waters, EPA: It seems to me that if one is taking a tier testing approach and directing one's attention to carcinogenesis then the appropriate place for the oncogenic transformation assay is in the second tier, among the confirmatory bioassays. I think most people would agree with that.

A lot of people, though, are favoring the combined use of mutagenicity bioassays for gene or point mutation together with oncogenic transformation bioassays, simultaneously testing for mutagenic and potential oncogenic effects.

If one is trying to determine whether a compound is negative, it seems to me this latter approach makes some sense. If you can test for point or gene mutation, primary DNA damage, chromosomal effects and oncogenic transformation, and a compound does not come up positive in one of those assays, I think you have a reasonable assurance, at least in a preliminary fashion, that that chemical is not very biologically active relative to mutagenesis and presumptive carcinogenesis. So, I just wanted to mention that I think you can take the straightforward carcinogenesis tract or the mutagenesis tract, or you can also take a combined approach. A battery of
tests to delineate a presumptive negative response for both mutagenesis and carcinogenesis might be the one that I mentioned.

Dr. Morris, EPA: I think we need to consider these short-term tests in a retrospective sense. We are all talking about their use in a prospective, predictive sense. I think there may be, and I think it needs to be looked at more thoroughly, the use of these short-term tests retrospectively, that is in your bioassay results in the areas of the gray zone, as they are sometimes called.

I think the time that we are detecting strong, positive carcinogens is rapidly passing. We have a pretty good handle on those now. There may be a few sleepers out there we have not pegged down, and we are looking for those obviously, but a lot of the data that we are seeing falls into an equivocal area and, by itself, I cannot decide what the bioassay means. I think that in a retrospective sense, perhaps these additional clues or additional weights of evidence from short test studies may be useful in a retrospective analysis.

Question 6: Was ultrasound treatment of activating cell systems, plus ultra filtration, plus exposure of xeroderma cells to the filtrate considered?

Unidentified Speaker: The idea behind this is if you have a certain chemical which does not get inside the cell you cannot expect any kind of activity. So, it goes to the same solubility and compartmentalization of a certain chemical.

My question was if this particular type of chemical got into the cell, it was metabolized, then if the cells leaches it out into the surrounding area, you mentioned already that you did a preliminary study that through a millipore filter it gets through at least in certain cases. So the question comes up, going back to my early studies that radio labeled material was taken up by the cells. We sonicated, broke up the cells and then ultra filtered it and used the filtrate for further studies.

Dr. Waters, EPA: Those kinds of studies are very useful in the sense that one can track the pathway of the chemical, but I think the question that was asked immediately following my presentation about the half life of certain metabolites in culture is very important there. It would only work if one had a chemical with a metabolite that had a relatively long half life. Some of them are in milliseconds. So, it depends upon whether or not the chemical is sufficiently stable, and I think that it is important to know something about the stability of the chemical that you are trying to test, too. I think that is something that we tend to neglect in a lot of our testing.

Question 7: As useful at the Ames system is with bacterial cells it has some handicaps as to relevance in metabolism extrapolation to in vivo systems. Do we feel we should place more utilization of cell culture, primary or tumor cells?

Dr. Waters, EPA: Is the question either/or?

Dr. Page, EPA: It is the priority we give to the resources. Should we place more resources on methods toward utilization of cell culture rather than Ames type systems of cell culture of the primary cells or tumor cells?

Dr. Waters, EPA: It is always difficult when you are in an audience and you have some people interested in some systems and some in others. I guess the answer that I
would give is that I think all of these systems are important. We are supporting and continuing to support all of them. I think they have different applications, and I think that is the key. At the same time, as I pointed out, in conjunction with the metabolism studies at Michigan State University, it is clear that the metabolism, the S-9 metabolism which we also used right away with cell culture systems is not entirely accurate, if that is the term we want to use in reflecting in vivo metabolism. We know that is the case and yet we still say, let us go ahead and use it because we understand more about it. We have got a larger data base at the present time. I think it would be foolish to make a shift to any other metabolic activation approach for general screening at the present time, simply because we don't have the data base.

With regard to relative funding, I think Dr. Saffiotti made the case that there certainly needs to be a great deal of emphasis on cell culture systems and especially human cell culture systems.

There are some studies being done analogous to the one that was mentioned, Norman Anderson's study at Argonne. Similar work is being done by Dr. Kakunaga at NCI and by Sachs in Israel, that may provide the means to detect changes in specific proteins, specific gene products that occur with cell transformation.

I think that these kinds of studies in simple systems may provide important clues as to what is really involved in transformation, what the gene regulatory mechanism is that controls the transformation process.

This could be extremely important in the basic understanding of carcinogenesis. So, these kinds of things definitely should be supported. They are going to be costly because they are uncertain. I think it is a question of our objectives, if we want to do testing and want to test a large number of compounds, then there are probably no immediate substitutes for the microbial systems, but if we want to become closer to the human situation and we want to learn more about the mechanisms of carcinogenesis per se, then the microbial systems don't help us. So maybe that answers to some extent, diplomatically, your question.

Dr. Bull, EPA: I would like to add to what Dr. Waters said and take it a little bit further. I think of the things that we are struggling with, and I think it was evident in some of the comments that Dr. Saffiotti made earlier, that we have no standard of comparison in the whole area of chemical carcinogenesis. One of the things that has been neglected is research to establish the basic reasons as to why one species responds in a certain way to a chemical carcinogen and another species does not respond or responds in a somewhat different manner. These questions are fundamental to the problem. This is the key reason why we cannot answer questions very clearly when it comes to considerations of potency. I think that same kind of approach suggested by Dr. Waters needs to be taken into the whole animal arena as well.

Dr. O'Connor, NCI: We have another consensus that we don't have the knowledge yet to introduce an effective in vitro all encompassing screen, and we need a lot more research in the mechanism of transformation. We certainly would buy that from the NCI side which brings me to a general question in terms of the philosophy of this particular program.
The NTP is not represented in an official capacity at this particular meeting. Obviously one of the big interests is in in vitro tests and in the consideration of prioritization of compounds in terms of "potency."

This is clearly an area of research that the NTP must be interested in. Can we have some discussion or does anybody have any viewpoints as to how or what emphasis this particular program should give to the subject which is clearly of interest to everybody here, and how this can best be coordinated with the NTP.

At the present most of the funding for that program is coming from the NCI with some from NIEHS, but very little or none so far from EPA, and I don't think any from NIOSH.

Dr. Galbraith, EPA: The problem you mentioned was addressed by Dr. Vilma Hunt in her presentation to the NTP Executive Committee in January 1980.

Dr. O'Conor, NCI: That must have been the meeting I missed.

Dr. Galbraith, EPA: I think the question of which research areas should be concentrated on by the various government research programs is one that weighs heavy on all our minds, particularly in the austere research funding period that we seem to be faced with.

Dr. Hunt addressed three categories of research that the Office of Health Research at EPA is concerned with. However, before reviewing these, I would like to point out that the mandate of the EPA Office of Health Research is to generate, evaluate and continually update the scientific and technological data base necessary to support EPA regulations.

This is what EPA research is all about, i.e., to support regulations that are promulgated as a result of EPA's effort to enforce the Clean Air Act, the Clean Water Act, the Safe Drinking Water Act, FIFRA, TCSA and the Resource Conservation and Recovery Act.

The first category of research (at least in the view of the Office of Health Research) includes tasks that must be addressed by EPA as a result of (1) regulatory requirements and standard setting responsibilities. The second category would be research which EPA must address but collaboration with other programs would prevent a duplication of effort and conserve federal resources, that is collaboration with other programs would result in an extension of our own work, a verification of our own work and an increased understanding of the basic mechanisms that are in operation. The third category would be research which is more appropriate for other agencies to perform.

**Category I - Research which must be addressed by EPA/ORD:**

A. **Methods Development**

Develop accurate and inexpensive screening methods for:

- carcinogenesis
- reproductive effects/teratology
- neurotoxicology

570
cardiopulmonary effects
effects on other target organs

B. Testing of Environmental Agents

Toxicological evaluation of specified agents and complex mixtures to meet regulatory deadlines.

Chemical analysis of environmental media and human tissues to document human exposures.

C. Criteria for Assessment of Test Data

Improve data base for the interpretation of test results derived from animal studies.

Develop data base to extrapolate animal toxicity data to human toxic dose.

Category II - Research which EPA must address but collaboration with other programs would be helpful:

A. Validation of Tests

Validation of proposed methods
Confirmation of test results

B. Fundamental Research in Support of Applied Research

Development of animal models that mimic susceptible human populations.
Characterize mechanisms of chemically induced disease processes.
Development of new sensitive analytical detection devices which identify environmental pollutants.

C. Collaborative Support

Build a health effects data base for a wide range of chemicals.
Interspecies toxicology.

Category III - Vital research which is more appropriate for NCTR or other agencies to perform:

A. Basic Research

Mechanisms of action
Etiology of disease processes

B. Special Testing Conditions
Performance of the rodent carcinogenesis bioassays on potential environmental carcinogens identified by EPA.

Examination of dose-response as applied to the bioeffect levels and presence or absence of a threshold dose phenomenon.

Performance of long-term carcinogenesis studies to serve as reference for validation of short-term in vitro and in vivo methodologies applied to environmental samples by EPA.

Performance of long-term carcinogenesis and toxicity studies involving environmental samples designed to determine risk to people of ambient levels of contaminants. These would be very large scale experiments involving samples selected on the basis of EPA's short term testing program.

The categories are not absolute, and the attempt to categorize research on the part of EPA is provided only to give us a framework in which to discuss our research and to ensure that what we are doing is pertinent to the EPA mandate.

There have only been two interaction bioassays (additive risk) studies, one sponsored by the NCI Bioassay Program, the results of which have not been published because the statistical work is not complete. This study was conducted at SRI International.

The second study is currently in its initial phases at the NCTR. We plan to look at four carcinogens found in drinking water. It will be at least five years before we have the results of that study and we will be fortunate to have them at that time.

The other topic which falls into Dr. Hunt's third category is an understanding of the strengths and limitations of structure/activity relationships for toxicological evaluation. Structure/activity relationships have proven very valuable in the design of new therapeutic agents. There has been an increased desire on the part of regulatory agencies, including EPA, to determine the status of the usefulness of structure/activity relationships in predicting the toxic effects of chemicals.

Dr. O'Conor, NCI: We see evidence that this is an action program between the three agencies, and I guess one of our jobs is to point it out now in more specific directions, but it still leaves the point, as to how do we divide the responsibility for certain types of research between the monies available to this program and the NTP.

Dr. Page, EPA: It seems to me that the uniqueness of this type of a collaborative effort would be the combined energies and expertise in directing the results of the basic research program or basic research findings into application in addressing regulatory needs.

Now the regulatory agencies often do rely on results coming out of the research agencies and often they are a little uncertain as to how good some of these systems are and how to use the systems. We know that some of the systems require additional validation. But a program like this, joining forces of research and regulatory agencies can really bring to bear the type of expertise that is needed to push what I call basic research results on into a mode that a regulatory agency can then utilize.
Dr. Kraybill, NCI: I believe Dr. O’Conor was getting into a deeper subject. I think it is implied in his remark, why we didn’t invite a broader delegation here. We had considered it, but we thought since this was our first effort we should confine it to EPA, NIOSH and NCI, but maybe next year we may broaden our horizons and scope and have other agencies.

The other thing is, I think Dr. Galbraith also defined a problem we must be aware of, that each of us has what we call turf. We have missions and for some of these missions, I am sure between two of the agencies that were mentioned, you cannot tell where one leaves off and another one begins. They are very close, not so much with NCI but I think in the environmental area some of the agencies get pretty close.

Dr. Gregory, CPSC: I think what Dr. Kraybill said is very appropriate. We all tend to have our own turf and Dr. Galbraith talked about how EPA has defined theirs. There needs to be some sort of an interagency coordination. Quite often there is going to be some overlap, especially in areas involving NIOSH and EPA. They are going to both be interested in some of the same things sometimes, and you cannot avoid some of that, but when things are really flagrantly out of control there needs to be some sort of an interagency collaboration so that one group will take the lead and provide the results in one area while the other agency will take the lead and provide the results in another. We will be able to save a lot of money this way and make our overall research much more dollar efficient.

Dr. Rausa, EPA: NIEHS publishes an Annual Report to Congress entitled "Federal Agency Support for Environmental Health Research. The report contains a description, by each agency, of its area of responsibility and program in environmental health research. A copy of the report may be obtained from Dr. Phil Schambra, Office of Interagency Programs, NIEHS.

Question 8: How can regulatory agencies keep from interfering with the development of improved methods for toxicity testing or defining the types of data they need to do their job, that is required protocols for toxicology testing, for example, the Ames testing may get in the way of improved, more accurate, less expensive protocols.

Dr. Page, EPA: This is an interesting question. I know we are trying to deal with development of guidelines and standards at this time, and in the back of our mind we want to be sure we don’t put something down which is going to stymie further development of test method development. So, is there anyone who wants to take a shot at that?

Dr. Waters, EPA: I think maybe you can answer it better than I, but is it not true that with respect to testing protocols under TSCA, for example, you have the option to revise on a yearly basis the protocol? Isn't that true?

Dr. Page, EPA: We have more than one option. The law requires that each year we at least examine the existing standards and determine whether they need to be revised.

Dr. Water, EPA: At least under that legislative mandate, I don’t think it necessarily is a problem. Certainly, I think the scientific community is in favor of flexibility in protocol application.
Dr. Page, EPA: One of the greatest difficulties we have, in fact, is the degree of rigidity versus flexibility in setting our standards.

I think we certainly want to lean toward flexibility, but then on the other hand, in the regulatory agency, you have to have something that is enforceable.

So, you have the other influence of putting something down that in the event you don't get the kind of data you need you have got something to enforce to go back and require additional data. Under TSCA with a testing rule, we are required to put down standards for the testing. The question is to what depth do we go in putting down these standards.

Dr. Waters, EPA: I would just like to use this opportunity to say something about the GENE-TOX program which, if some of you have not heard about it, I think is probably important to mention, and it relates to this question. Understanding the need to define as precisely as possible protocols for testing, one of the efforts that has been supported by the Office of Pesticides and Toxic Substances in EPA has been the so-called GENE-TOX program, the current status of tests in the area of genetic toxicology. One of the charges to the committees (and there are about 200 scientists working on this program with 22 committees on different short-term tests) was to attempt to come to an agreement as to what the current acceptable protocol is for any given test system. The charge did not say that you have to define that protocol such that the system could be used for testing now. We simply wanted an evaluation of where that test system stands. It was left as a judgment for the scientists that are intimately familiar with the test system under evaluation to make. I think that was a very enlightened approach, and I hope that the information that comes out will be used in that fashion. I think it probably will be.

Dr. Kraybill, NCI: I have a practical question to ask Dr. Page since he was with the testing program at one time. You remember that guidelines for testing were developed by NCI, and lo and behold we discover one day that EPA comes in with a guideline. What do you do about situations like this, when you have NIOSH, OSHA, EPA, FDA, and NCI each with their own protocols. I know it is bad to hamstring people and lock them in, but you face the danger of one agency being played off against the other, play A off against B.

Dr. Page, EPA: I will give one quick comment and then I see Dr. Morris wants to address it. You have a very valid concern. This is one of the reasons for the Interagency Regulatory Liaison Group - to attempt to coordinate or harmonize the regulatory directions and activities. Under the Interagency Regulatory Liaison Group there is a test standards and guidelines group. This involves the regulatory agencies, but NCI is participating on the group, along with a couple of other research agencies. Now there are going to be guidelines developed for chronic toxicity. They have already developed a number of them for acute toxic effects, but the guidelines developed for chronic effects, including carcinogenicity, are only now being drafted, and I can mention that it seems that the direction they are probably going in is to stay as close as possible to the recommendations of the International Agency for Research on Cancer. A recent conference was held, about one year ago in Hanover, and certain recommendations have come out of that work group. The Interagency Regulatory Liaison Group is trying to adhere as close as possible to their recommendations.
Dr. Bull, EPA: I just wanted to make one comment, going back to the original question about whether specifying guidelines, specifically guidelines for testing under TSCA negates the ability to develop methodology. I don't think that is the intent of it. I think that is a misunderstanding and to clarify that I will state that I think the development of guidelines is really an attempt to make the contractual agreement with the industry you are requiring to do the test clear, so that they understand what you will accept as positive or negative evidence of safety at a given point in time. This really has nothing to do with retarding methods development, in fact I would see it as a stimulus for methods development. In short, you are asking them to develop the information in such a way that you feel that you are going to have a good chance of understanding the results. Am I making that clear.

Dr. Page, EPA: I think you are basically right, but I can tell you one of the results of this guideline and standards activities is helping to define the uncertain areas, the highly controversial areas so that I think it will lead to additional or new research - to try to resolve some of the prevailing issues. It is likely going to be years before the research can yield the results we would like to have.

Dr. Morris, EPA: I agree with you 100 percent on the activities and the usefulness of the IRLG exercise. I would, also, like to make the group here aware of a document we have developed through another international effort with the Organization for Economic Cooperation and Development that Dr. Page, Dr. D'Aguanno, and I have participated, representing the United States in developing short as well as long-term guidelines including a carcinogenicity guideline. That document is available for review and comment within this country in all of the regional offices of EPA and in our Headquarters Office. We would certainly appreciate comments on them. This was another attempt to try to harmonize at even a 24-nation level our approaches.

Dr. O'Connor, NCI: Everybody is trying to coordinate and harmonize. There is a new program started at WHO, called the International Program for Chemical Safety in which the WHO, the UNEP and ILO and maybe the FAO are all involved, and this is sort of, again, a grand scheme for coordinating information and activities in the area of toxicology and chemical safety throughout the world.

There is one question here that relates to several things, one of which we talked about, and that is the predictability of short-term tests and the suitability of the animal models, but then it goes on to talk about approaching the problem of threshold in a heterogeneous population and interspecies extrapolation. That is a subject, again, that relates to some of the things we have talked about, and it probably has relevance, considerable relevance to the program.

I guess what I want to get back to is the focus to help Dr. Kraybill from the NCI and the others who are responsible for management of this program to ensure that we are really spending these funds in the most useful and meaningful way.

Initially, and to a certain extent it is still going on a little bit, I think the different agencies are using the funds to do projects that they would have done anyway and this is not to say that they are not useful, but I think we really ought to make a concentrated effort in trying to define the program a little more carefully.

Certainly, in epidemiology this is the subject of another session, and we will have the plenary session tomorrow. That clearly is an area where the NTP is minimally involved and where this type of program, I would think, has a very large role to play,
because all three of the agencies have an epidemiology component, and some of the other agencies don't really have that component. I think we can be real leaders in that area if we continue to enhance the degree of cooperation that has been initiated. In this field of toxicology and methodology, though, there really is a lot of overlap with NTP and other organizations, and I think I am not quite sure how to handle that. I guess there is so much to be done that we need not worry too much about overlap, just make sure that we are supporting the best work and the highest quality research.

Dr. Kraybill, NCI: I would like to make some practical comments regarding this. For FY 1980, the funds are just about all committed. For FY 1981 we have almost a full commitment of funds. We had looked upon this sort of effort to get new ideas and stimulus for new projects for the ensuing years. That would be helpful. It would be very difficult, let us say, to cut off a good project. Oh, you could do that, but I think it would be chaos for some of the projects committed for three or four years.

We would like to get good projects and the kind of advice today is very useful to us.

I don't quite see that this collaborative program interfaces or impinges much on the NTP. From what my understanding is, maybe some of the papers we heard on the NIOSH side this morning may have been, but on the NCI/EPA side we are dealing more with mechanisms and surveys, like, for instance, the Gulf Breeze, Florida study. Certainly we are looking into mechanisms and things of this sort that are really not, as I understand it, necessarily the mission of the NTP alone.

Dr. O'Conor, NCI: I guess primarily I am referring to the field of development and validation of short-term tests.

Dr. Kelsey, NCI: We hear a lot about the development of the short-term tests which obviously are going to be very important, and they are getting better and better; particularly one area that has not really been talked about, the area of metabolic activation. However, with regard to the animal tests we don't hear much on how to make the bioassay a more economical model, if that can be done. I don't know what research efforts are being done, but I am sure people here are aware of it, and it would be good to know what is being done in the area of in vivo animal models, in terms of carcinogenesis. I know some people are interested in developing, maybe, a small animal population or a different species and that sort of thing, and it would be good to get some information in those areas.

I would like to hear some discussion about what is being done more with the in vivo models in terms of either streamlining the tests or improving the animal bioassay?

Dr. Bull, EPA: There are several things that can be done, and they are being explored. One in particular that we are interested in in Cincinnati has to do with Dr. Farber's preneoplastic foci which can be found in the liver following treatment with carcinogens. We can develop this system as an initiation promotion assay and that would considerably shorten the time needed to assess carcinogenic potential. Of course, it needs to be validated in the sense that you want to see how predictive it is of actual tumor development and so on and so forth.

The mouse skin system, if you believe that the papilloma development is somehow predictive of the development of a malignant neoplasia, can be used as a short-term assay. Perhaps Dr. Whitmire would like to say something about the Strain A mouse which we are also quite interested in.
Dr. Saffiotti, NCI: In addition to these somewhat new uses of these in vivo systems, particularly for the intermediate range of duration, what I think is really the important area that is still developing is the utilization of in-depth research methodologies for definition of tissue susceptibility, metabolic activation, organotropism and specificity, interactions and so on, to improve our ability to interpret, to evaluate, and to improve the methodologies for in vivo bioassay studies.

The animal bioassay, work which has been sort of codified and started as a large-scale effort about the last 10 years or so, was based on a lot of research on animal models on induction of major forms of cancer reproduced in animal systems by a variety of carcinogens that was done in the 10 or 20 years before. We were able in the early seventies to crystallize some of the animal test protocols that are still largely being used because we had the 10 or 20 years of experience behind us on which to draw. We have now a lot of other information that was not there 10 years ago, especially in mechanisms, metabolic activation specificity, markers for interactions, and markers for binding of carcinogens. That is the kind of work that can be eventually brought to bear on the interpretation of the bioassays, on the design of specific subsets of bioassays. For example, instead of just doing simple general toxicity, one can do and is beginning to see work done that addresses these problems; studies on distribution, binding, interactions, repair, all these things which will qualify the in vivo response.

The other thing is in the development of the in vitro models we still need to draw on the experience of the in vivo models, especially when we are trying to correlate organotropism and target effects in different tissues, epithelial tissues in particular. Again, I see that as an area in which the work being done now and that has been done in the last few years will bear fruit in the next few years in the development of more specific methods and more specific protocols.

So, I would hope that we would continue to work in a collaborative fashion in a way that would be research oriented in that sense and then provide almost a resource from which those who are particularly concerned with developing protocols, including NTP, will draw to develop their additional and more modern protocols.

In terms of our relation to NTP, let me make a sort of general comment on this. I have been expressing this to several of our colleagues in the other institutes and in our own institute. I see a certain amount of polarization. There is NTP going towards the fairly straightforward testing approaches with pretty much existing methods. Some of the areas of major emphasis in the research program of EPA and NIOSH are addressed to very important areas of methodology and data and research on monitoring, on environmental definition of exposure, all these aspects which are somewhat at one extreme of the spectrum.

NCI, in the context of the NIH basic research orientation, and because of the rapid development of very exciting models in the molecular mechanisms of cancer, is putting most of its emphasis on the more basic molecular and more basic research approaches, and there is this area which really links the two which is somewhat, I think, undersupported at the present time. I personally think that the best opportunity we have is this one of an interagency commitment in which we really share a strong interest in this middle area of studies of mechanisms of toxicity, mechanisms of disease related to specific problems that affect human exposure from both sides.
We can, in fact, both contribute specific scientific methodologies and experience from both sides, and this program could well become a unique program and therefore perhaps become unassailable from the fiscal point of view and all the rest of it, if it is, as Dr. O'Conor said, more than just more of what each agency would be doing anyway.

Dr. Whitmire, NCI: I have heard a lot here today regarding our studies at NTP, so I thought I had better stand up.

Dr. O'Conor, NCI: You are still NCI.

Dr. Whitmire, NTP: I appreciate that. Sometimes sitting on the fence gets kind of wobbly.

We are indeed, trying to improve the protocols. We are trying to include parameters which will assist in evaluating toxic signs. If we know that certain aspects of clinical chemistry can assist us in setting the doses for the chronic study, we will include these. Behavioral studies may be included. Any type of study which may give us some indication as to the organ involved may be included. These studies are included in the initial experimental design or may be added if results of the subchronic studies indicate their usefulness. I think you will see more and more improvements in the experimental design because each chemical is assigned to a manager, known as the chemical manager, and they are the ones that are the pivot point between NTP and the other agencies. I plead for a lot of help from the other agencies in letting us know who in their agency is responsible for each chemical. We have more trouble finding out who to contact so that we can take advantage of their expertise in designing the most useful studies than any other aspect of our job. Anything that we can obtain from the other agencies in this area of cooperation would be most helpful, but our job is toxicology, not just carcinogenicity. People are nominating these chemicals now for our use, and when we cannot find out who nominated or why they nominated them we have a difficult time designing the best experimental design, and so this is important.

I might say, also, that we are indeed trying to develop both in vitro and in vivo short-term tests. We have at present two contracts out to verify and determine if two different laboratories can do the "A" strain lung carcinogenicity assay, and when this is done blind, then we will see how much use this test will be. We are also interested in promoter studies and combinations of chemical, but these studies are very difficult to design and until we have an organization, people, and laboratories these will have to wait.

Dr. Page, EPA: We were just wondering what would be a logical follow on. We have got a meeting session tomorrow in which we are going to have to come to grips with a few suggestions as to where this program should be going.

Dr. O'Conor, NCI: Since there are only a few papers tomorrow in the morning, maybe we ought to think about moving pretty quickly into the plenary session and then allowing plenty of time for any general discussion.

Dr. Page, EPA: Unless there are other viewpoints, we will adjourn for today.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

Wednesday Afternoon, May 7

SESSION C

WORKING GROUP ON THE IMPORTANCE OF INTERAGENCY PROGRAMS: DEVELOPMENT OF FUTURE COLLABORATIVE PROGRAMS AND MEETING THE NEEDS OF REGULATORY AGENCIES

SESSION CHAIRPERSONS

Dr. Richard Marland
Environmental Protection Agency

Dr. Nelson Leidel
National Institute for Occupational Safety and Health

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SESSION C - WORKING GROUP ON THE IMPORTANCE OF INTERAGENCY PROGRAMS: DEVELOPMENT OF FUTURE COLLABORATIVE PROGRAMS AND MEETING THE NEEDS OF REGULATORY AGENCIES

Dr. Leidel, NIOSH: The intent of the session is to discuss the importance of interagency programs, development of future collaborative programs and meeting the needs of the regulatory agencies. Probably the last is a very key issue. We do not have a structured format. Hopefully, there will be free-flowing discussion. Are there any particular questions for the first discussion topic?

Dr. Cameron, NCI: I would like to mention a couple of things. Do all of the people here know some of the background? Dr. Herman Kraybill gave some of the opening remarks and he laid the groundwork. Is there anyone that did not hear, who would like some amplification of what he was talking about?

Dr. Leidel, NIOSH: Maybe it would be a good idea to go over it again real quickly because I was not able to make it yesterday.

Dr. Cameron, NCI: I will try to keep it brief. Simply, both of these are congressionally mandated situations. They arose out of the need or the desire of Congress to have the interagency collaboration they felt was missing. Congress felt that there was a non-meshing of gears so far as expertise, and since the National Cancer Act of 1971 passed to the NCI considerable funds, Congress said that the NCI would pass money to other agencies, figuring where money goes, interest will follow.

That is not a bad philosophy, and it has seemed to have worked out. I am the third coordinator for the NIOSH/NCI program so there is a lot of history that I am really not familiar with.

Dr. Kraybill mentioned this morning that initially, it was strictly a transfer of money from NCI to NIOSH, and they really did with it what they wanted. That was in the days of Dr. Finklea who allocated the funds amongst his staff and amongst his own projects. But we are now coming back into an era where there is truly much more collaboration. Within the last year and a half that I have been active in this program there has been a lot more input by NCI. Within the last 2 months, 2 out of the three projects recently authorized were NCI-initiated.

We are not trying to change the ballgame by any means, but I just hope that this type of situation continues, and that NCI will participate in the NIOSH interagency agreement with some new ideas.

The NCI/EPA interagency activity is quite different. Dr. Kraybill has been involved in that since its inception and that has truly been a collaborative effort and all the projects were pretty well split up the middle. Some have come from NCI, and a lot have come from EPA, and it has been a much more organized activity, only because it has been in one man's hands from the beginning and he has done a good job with it.

With that, I will stop. I would mention one thing; there are ceilings on the amount of funding to the extent of $4 million dollars for each program. The funds come out of DCCP/NCI.

I don't know whether Dr. Kraybill mentioned that in the NIOSH agreement since it started in fiscal year 1976, there have been, counting the three we passed this month,
71 projects. One of them is a duplicate. This project, developed last year, was never funded. It just did not get anywhere. They killed it, and then they reactivated it this year at a higher level. Nineteen projects have either been completed or terminated for due reason. So we have about 50 ongoing. In the EPA Agreement - I do not think they have completed any projects - they have something like 30 projects underway.

Mr. Harris, NIOSH: I have been involved with the NIOSH/NCI Interagency agreement for the past 2 months. It has become apparent that most of the projects are concerned with identifying problems. What about resolving some of these problems, resolving some of the issues in terms of, for example, control technology? Most NIOSH/NCI efforts are concerned with basic science in terms of trying to identify the problems, but, what about the other side of the issue, trying to resolve the problems with practical solutions?

Dr. Cameron, NCI: That was alluded to in the third part of the charge to us today: How can we be more responsive to regulatory activities?

Dr. Leidel, NIOSH: Yes. I think, in terms of problem solving. However, the way I think of it, regulation, of course, is one tool, but even when you issue regulations, the companies have to have some mechanism for controlling the exposure, and I think that is what we are getting at. In other words the control technology or control techniques, I would probably envision in the general realm of public health.

Dr. Cameron, NCI: The only ones that come to mind are the projects concerned with protective clothing and respirators. Is that what you had in mind?

Mr. Harris, NIOSH: Yes.

Dr. Cameron, NCI: Then I would certainly agree that there are a few projects in that realm but not too many, certainly not the bulk of them.

Dr. Marland, EPA: May I inquire if any of the projects which have reached you and Dr. Kraybill for consideration have been the result of the Division of Research Grants or, say, Dr. King's extramural program references? Have they referred to any of these?

Dr. Cameron, NCI: I don't believe so. I cannot speak for Dr. Kraybill.

Dr. Marland, EPA: What is the nature, then of the peer review that these are provided with?

Dr. Cameron, NCI: Well, it is basically an ad hoc situation between the senior staffs of the concerned agencies. In fact, Dr. Cooper is Associate Director for Extramural Affairs in our division and he happens to be on the committee we have set up for that purpose. We have Dr. Saffiotti, Dr. Weisburger, Dr. Fraumeni, who fairly well represent the senior people of our division.

Dr. Marland, EPA: So the review is intramural rather than including any personnel outside?

Dr. Cameron, NCI: There is no outside review. In fact, up until last year, anything coming to us from NIOSH was presumed to have gone through their peer review; we were only to consider their proposals on the basis of relevance to cancer and priority. That has changed a bit. There is now a tendency to look at the science, too, which
makes it sort of difficult because obviously the cancer people look at it from slightly different perspectives than NIOSH, and it caused the non-funding of several projects. But I think we can overcome that philosophy problem with a mechanism for presentation, and instead of having a very skimpy written proposal handed to the Cancer Institute, we are now going to go into more of a combined staff review with an oral presentation by the proposers.

Dr. Marland, EPA: Would the principal investigator make the presentation himself or herself?

Dr. Cameron, NCI: We have just done that several times, and it seems to work a lot better than simply sending a piece of paper to us. Dr. Cooper and I are very familiar with that system. We are comfortable with it because that is the way Dr. Saffiotti started the carcinogenesis program, and we were both members of it for a very long time. It is not an adversarial position when you come before the group, but after you get your teeth kicked in the first time, you do your homework the second time.

The one that comes to mind, for instance, that we just had was Dr. Brian Hardin who came with a styrene inhalation study. That was a tricky concept, and we discussed it in the group for about an hour and a half with Dr. Hardin there. It did pass, but I think if he had not been there, it might have been in jeopardy. We had legitimate questions of the rationale for the protocol and some other aspects of the study. I think Dr. Bridbord is getting the same feeling, that it is probably the way we should approach it for our agreement and probably for the NCI/EPA program, I would encourage such a mechanism. It also has the advantage of getting some more interaction between the staff. When our people approve a proposal, they have a chance to talk to the Principle Investigator, to interact with him. I know in one case Dr. Weisburger said, "I like that project and I understand what he is trying to do; I would like to be a co-project officer with him." So there are a lot of spin-offs that I think should be encouraged, but it would require in our case with NIOSH that these people would come in throughout the year and make presentations. Now, I can see one problem. Secretary Harris is clamping down and is essentially telling us that we are now working on a three-quarter year. She does not want anything funded in the last quarter. So essentially you are going to have to fund in the first three quarters, which sort of makes it a skimpy year. Last year, NIOSH met in the summer and got all their projects in line and worked it out staffwise between themselves, and then they come in during the balance of the year with other proposals. This new edict from the Secretary's office will compress that whole process. What I am saying is that the NIOSH people, for new projects, are going to have to start coming in the first of the fiscal year so we can get them approved and start funding them right away. I cannot speak for the EPA agreement, but I would check into it. We just do not have the luxury of 12 months any more to get things approved and funded. They've got to push them in the first of the year. The philosophy of oral presentations by prospective Principle Investigators will make for a busy beginning of the year but I would encourage it.

Dr. Leidel, OSHA: Of course, there is the problem with travel funds constantly being decreased. Every year the cost goes up 10 percent and the amount of travel funds goes down by 10 percent.

Dr. Cameron, NCI: I appreciate that. I think it can be justified on the basis that we've got a $4 million pot, and the only way we can spend it wisely is to have face-to-face discussions. I think some provision has got to be made for it.
Dr. Leidel, OSHA: I do not think there is any basic quarrel in NIOSH that it is a good idea. You've got to have a good idea and the person has to be able to defend it.

Dr. Marland, EPA: What concerned me was my recognition that those projects which came in from the EPA staff had indeed not received an external peer review. They were the proposals that had been received from certain investigators around the country that appeared to be quite cogent and appropriate to the regulatory needs of the agency. I see how you are finessing it. I think that is a very satisfactory way of doing it. I have no qualms about people like Dr. Armstrong or, for that matter, certainly Drs. Fraumeni and Kraybill sitting as peer reviewers of research. I am not questioning that, but I am questioning the absence of any of this. Sometimes the enthusiasm of a non-current bench researcher carries him away when he is maybe 5 years out of step with current practices, current literature. I would not consider myself a satisfactory peer reviewer because I have not kept up in my field.

If I have gained a sense that my fears are put to rest, that is what I was looking for, and you have pretty well done that. I know the stuff coming out of EPA now will have had at least an opportunity and probably will have received a vastly improved review by both extramural and intramural peers than it used to.

Dr. Leidel, OSHA: I would like to pursue Mr. Harris' question. I think it bears on the matter that NIOSH, as I was in it, always prided itself on both having research interests in terms of basic and applied research for problem identification such as in the area of epidemiology. Of course, we have always had a mandate and a role and a very strong interest in developing the solutions to the problems, the applied research; everything, of course, from monitoring methods to control technology, both in the form of engineering controls and personal protective equipments, particularly the area of respirators. In other words, once you have found that a chemical is a suspect carcinogen or it caused a particular health effect, we sense a strong public health role to do something about it and make contributions in that area. I think what he was trying to get at is the question, could we be devoting an increasing share of the funds in the interagency programs to that kind of an effort, which related to OSHA's needs, because when we go forward on a health standard, we both have to demonstrate, of course, that there is a problem, but a very strong input we have to consider is the regulatory analysis, which considers both the feasibility of even doing something about the problem, other than simply putting a respirator on the worker, and then the cost of these controls.

Dr. Cooper, NCI: I think one of the problems in this area is that although there are some NCI activities that are directed toward these goals - for example, how one can accomplish degradation of carcinogenic substances in the laboratory environment - it is a little difficult to see how the Division of Cancer Cause and Prevention of the NCI can contribute much in the way of expertise to the other sorts of control activities that you are talking about. Perhaps other areas of the Institute might be able to contribute but I do not see very much intellectual input that we can provide in this area. Perhaps NCI cannot really collaborate in any real sense in such efforts.

Mr. Harris, NIOSH: You mentioned protective clothing, and one or two others. Areas such as these are meaningful to both agencies. I believe this is what Dr. Leidel is referring to in his statement.

Dr. Cameron, NCI: There is a constraint, albeit minor, that I understand in at least the NIOSH/NCI agreement. There must be a connotation of occupational cancer. NCI funding has to have some relevance to, hopefully, cancer prevention or cancer.
identification. I am not negating the missions of some of you people, with all the other parameters of toxicology, but when our people look at these proposals, they have got to keep that in mind. I am not sure if we can justify NCI partial funding or full funding of subject matter that does not have some relevance to the cancer problem.

Dr. Leidel, OSHA: I think NIOSH would probably look at it from the standpoint that the way you prevent workers in an occupational environment from contracting cancer from a carcinogen is to keep the chemical from reaching the worker, which means either through engineering controls to prevent its release into there workplace or personal protective equipment once it is in the workplace air or the environment.

Dr. Marland, EPA: Is there any reason why such research - if I could use the word "applied" as opposed to a more basic development of the identity of the cause and the solution - should not be the function of the regulatory agency as opposed to a more pure research function that would be the major concern of the NCI?

A prototype that we are beginning to develop, which Mr. Costle last week described in rather strong terms to the Congress which made our group quite happy, is that we were instructed by the Congress some 2 years ago to allocate 15 percent of our research costs to long range, more fundamental, research. In other words, to identify causes and effect relationships, perhaps, or mechanisms, or whatever else would be called a more basic or a fundamental or a longer range project. The other 85 percent therefore, presumably, would be dedicated to the application of some of these principles through a regulatory mode. Now, whether the 15 and 85 is a rational percentage, of course, if a subjective entity and is not necessarily a good or bad figure, but at least it is a recognition that there are two functions involved here, both of which are called research. I think that you were trying to state that the talents that NCI could apply were perhaps more nearly into identifying the basic mechanisms for the NIOSH research group, and then when these are identified, the NIOSH research group can understand how we can apply certain preventive mechanisms to this, which appears to be a rational way to go about it. This is what we are indeed attempting to do at EPA.

Dr. Cameron, NCI: There is another interesting feature that you may not be aware of. The funding from NCI to NIOSH is not necessarily all contractors. They use a varied mix and this impressed me. A project might be either completely staffed and operated in-house in NIOSH, in which case the funds allocated are used internally to pay the salaries of their staff people while they are on the project, or they are a mix. They have staff time allocated and paid for and they go out and get a contractor for support, usually a resource support, and then there are some which are entirely contract operated.

Dr. Leidel, OSHA: Well, of course, the problem as I see it at OSHA is that they are dependent upon the research agencies, and they do not have quite the luxury of the control over what needs to be done.

I know that when I was on the NIOSH end, there was a perpetual problem about OSHA needing both long and short term research and trying to get that into the mechanism. Of course, people come and go and all sorts of things and new ideas keep cropping up in terms of ways of improving communications and getting the long range planning between the two agencies, particularly in light of the fact that we were set up under the same Act.
Mr. Turner, OSHA: Of course, there is the formal mechanism of the NIOSH Planning Group which was set up a couple of years ago to develop the long range needs that OSHA and other Department of Labor agencies could foresee in both safety and health.

I really cannot say -- you or somebody else from NIOSH can probably tell me how that is working from the NIOSH end. NPG has only been effective for a year, so we really do not know what results we are going to get.

Dr. Leidel, OSHA: I could not speak to that. I was not involved in that group when I was at NIOSH. The predecessor groups seemed to work. It goes back to the basic question of how do research projects get proposed, and, of course, there could be two routes. Down at the working group level, that is at the branch level, you can either have the ideas come from the researchers or they can come down from the top. It comes over from OSHA and folks say this is an important problem and it goes down to the division and branch chief and then to the section chief and down to the researcher, who writes up a proposal trying to answer the problem. So these are some of the realities and problems that have to be faced. Of course, how well these working groups pan out many times would depend on the personalities and the kind of people that are sent to them. So, I really cannot answer that.

Dr. Marland, EPA: There is another similar function that is now going on at EPA, and that is the research committees. There are people here who are probably more aware of problems than I am, but it is a research planning function in which the actual research budget for the agency is developed by the research committee, co-chaired by a senior official whose responsibilities are regulatory, and the other co-chairman being a responsible research director. Committees sometimes run 50 persons in size. They are dealing with a budget that runs close to $300 million divided among 13 committees. So, it is a very substantial undertaking, and the agency is pretty well bound by their portion of the resources into various kinds of projects.

This is not an old, enduring function. This is a function which is still evolving. It is in its second year. But it equates to that planning function which you have at OSHA.

Mr. Turner, OSHA: The same kind of thing, yes. This may be too basic a question, but it is one I presume both you and I and other OSHA people would like to ask. What research functions does EPA have and how do they work with their regulatory division?

It was mentioned that NIOSH is our sister agency and does our research for us. Is all your research done within EPA itself?

Dr. Marland, EPA: About one-third. If you are talking about allocation of dollars, about one-third of our research budget is dedicated to intramural research within our own staff. Close to a third is interagency agreements with the Departments of Energy and Agriculture, but a substantial amount of that is with DOE, and another third, but slightly more than a third, is extramural, which ranges from a grants program, which is quite small but is substantially a "hands off" program, to a cooperative agreement activity which is like a grant but is "hands on," through a contract which is hard and fast.

Each of these elements is approximately 100 million, so they are somewhat substantial. In other words, you can influence the course of events with that much of an investment. All of this money is subject to the dictates, if you will, and the very
strong recommendations coming from research committees which are not composed only of researchers. The most influential elements in the research committees are those elements which describe the needs of the regulatory aspects of the agency, which we call program offices instead of sister agency. We use the term "program office" instead of "research office."

The program offices have an absolutely critical and important, an almost dictatorial role, in relation to describing the problem that must be solved. The research people are expected to say, well, if that is the problem, here are our recommended solutions, and here is how we should do this. Ideally, the committees plot that kind of approach and come up with a funding pattern for the entire research budget.

As I say, this is a system which is still evolving. Everyone recognizes that it still has imperfections. It is in its second year, and we are making rather significant changes in it as we go along.

Dr. Murray, EPA: Interestingly, even though you have used the term "program office," it might be just as useful for this group to think of it in terms of client offices. They are the people who receive and use the research results in their regulatory role.

Dr. Marland, EPA: That also describes an attitude which reflects, in a sense, that we are indeed a service agency.

Dr. Cameron, NCI: Is there a list of projects that OSHA has suggested to NIOSH that has been deferred because of funding or staff? Is anybody keeping a tally?

Dr. Leidel, OSHA: Since I, again, personally have not been involved, I really cannot answer that question. I know there have been lists of research needs sent over. I can speak to one small area where I have been involved to a limited degree, and like any list of research concerns, of course, they come all the way from ideas of particular individual's concerns up to very major issues.

Mr. Harris, OSHA: Well, the new cancer policy may help address some of these specific issues as we start identifying some of the materials to be regulated.

Dr. Marland, EPA: Are people troubled within that cancer policy by the allegation that there is a measurable risk associated with a given environment and a form of cancer or maybe total cancer production and risk assessment techniques? Any are of you troubled by the problems associated with risk assessment?

Dr. Byrd, EPA: Well, I would not say I was troubled by them, since I am a consumer of them, but it is interesting to me where things stop. The area of risk assessment technology is one question that is being addressed currently, but it is getting on the table fairly late, and it is, as you describe it, in a two-year evolution.

Some omissions still exist in EPA's research funding strategy. As a matter of fact, I would really be interested in running an experiment within this contract to see how the individuals in this room think that new chemicals are important in relation to existing chemicals. What proportion of the importance of the regulatory actions that we take relates to dealing with the existing problems now and what proportion of importance would they put upon preventing new situations from arising in the future like the case with which we have to deal today, particularly with respect to new chemicals coming on-stream? I would really be curious about what that sort of
allocation of resources would be like or what that allocation of importance would be like.

One of the curious things is that the kinds of algorithms that are devised to deal with chemical problems are technologies which are appropriate to deal with existing chemical problems. They really are not very helpful in trying to cope with how you assess new chemicals which have never existed before. It is a strange kind of omission.

I was examining the research that was presented at the morning session, which deals with the three inputs into our decisionmaking. We have a risk algorithm which says we need to know something about the biological effects, we need to know something about the exposures, which is a concern that has not been here, and we need to know something about the control technologies which are available. Getting an "A" in two out of the three and knowing nothing of the third will not get you a passing grade.

We have to use this algorithm in shorthand for new chemicals. There is no data analysis. It is all a hypothetical world. It is all a future scenario world. I personally am of the opinion that it is possible to do research in how to get at those sorts of things. I do not know that it is appropriate in this particular context, but I can think of some work the NCI's Division of Cancer Treatment is doing that would really be fascinating to translate over into the interagency cooperative research program.

The Division of Cancer Treatment at NCI has been conducting computer assessment of input chemicals. They have a large number of input chemicals in the program and they have to decide how to choose which ones will be bioassayed for anti-tumor effects. DCT has become skilled in assessing chemical structure with respect to biological end point.

Now, given the nature of the kinds of decisions you have to make in regulating new chemicals, an analogous kind of problem arises. I do not think research in this area is coming through the machinery right now. EPA is not interested in addressing new chemicals with respect to their anti-cancer potency, but, for example, with respect to potential carcinogenicity. Any sort of research in an area that will supply us with tools to fulfill these kinds of needs will be extremely valuable. I do not know how people would vote on it. I have my own personal view, which is obviously a biased one, but it is a strange thing that thinking about things that might happen to us in the future, that do not exist right now, is something that tends to slip through the decisionmaking process very frequently in determining research needs.

Dr. Marland, EPA: Do you believe that the area that you are describing, which is extremely close to that which, quite frankly, troubles me when I see rather expensive kinds of decisions being made on the basis of risk assessment technology which is extremely fancy arithmetic — some of the finest statistics and the application of statistical methods that are known are used — but if you look at it simplistically, the existence of 2½ deaths from arsenic-induced cancer each year, is that a good number?

In the first place, are 2½ deaths significant? Yes, if you are one of them, and yes, if you are downwind of a zinc smelting plant. But when the government indicates that we will regulate arsenic on the basis that there will be 2½ lives per year saved, I sure hope that we are right on that 2½ value. You know, if it is 2,500 or if it is a negative and we find that there is a potential error of 10⁶, that 2½ does not look awfully good to me when you figure that the chances are that you are off by an order
of six magnitudes. It could be 25 million, which we know it is not because there are not that many people dying with arsenic-induced cancer. But the research, which could be collaborative, designed to improve methodologies, I would find quite intriguing, at least I would find the results intriguing. I would not find the research intriguing at all because I would not know how to go about it.

You hit a point again that is very much a part of this, and that is exposure. I think that perhaps due to the efforts of NCI, the world has done a better job of identifying health relationships to various substances, particularly cancer health effects, but the rest of the world, meaning probably EPA and NIOSH, have not done an equally good job identifying risks due to exposure and then putting these together to constitute a risk assessment.

You say you are dealing with five equations and three unknowns. It is rather poor arithmetic on which to spend billions of dollars. This is why I would like to see the collaborative program coming into some kind of methodology development or improving the degree of confidence that our risk assessment technology is good. I do not want to fault it. Mind you, I have no intention of faulting it, because I do not have a better idea. If I do not have a better idea, I do not intend to fault something that is in place. But I am worried. It troubles me, because we are spending billions of dollars as a patie in correcting problems where there is a degree of uncertainty in the order of 10^5.

Dr. Cooper, NCI: Well, I think one has to say that the National Cancer Institute is also extremely concerned about both of the issues raised. They ultimately go to the question of risk assessment. Fortunately, or unfortunately, what society is concerned about is the risk assessment in people, not risk assessment in mice. That has created some serious problems in the development of quantitative structure activity relationships. Data bases are extremely weak in this area and if we are talking about human hazard, we are dealing with a very small subset of chemicals which can be clearly identified as members of this class.

Now, when we talk about risk assessment, there is frequently a concept in the back of people's minds that there are a few critical experiments which could be done, which, if done well, would permit the unambiguous extrapolation of animal data to the human situation. Personally, I do not believe that is true. Partially as a result of certain questions from regulatory agencies within the last few months, a group of people were brought together to consider what studies could be done that would facilitate that kind of extrapolation. The kinds of people that were involved were those whom we all recognize as having outstanding expertise in this area.

The consensus of that meeting was that we really are not ready to identify any individual definitive studies that will let us reach that goal. There are general areas of basic research that can be identified that in a 10-year time frame may permit us to get at these kinds of definitive studies, but we cannot identify them now.

As a result of that meeting, there will be a sizable program developed over the two years, largely based on RFA solicitation, requesting the attention of the basic researcher to specific directed areas which may help resolve the problem of differing carcinogenic response among animal systems.

Beyond that I cannot go, but I really believe they are right. There is not any simple set of studies that can be done today that are going to provide us with the answers that the regulators so desperately need. We can get clues, but that is all.
You are talking about arsenic. Let's face it, if you looked just at the animal data and you did not know anything about effects on people, you would say that there is not a cancer risk for man. So, it is really kind of dicey as to how far you want to go in basing your risk estimations for man on single or even several determinations in non-human models.

Dr. Byrd, EPA: This is fascinating. You just gave me half of the paradox that I wanted to demonstrate.

Let me ask you what relative importance you would put as a percentage in the total. How would you spend your time on new versus existing chemicals? Five percent, 10 percent, 20 percent, 2 percent? Just pull a number out.

Dr. Cooper, NCI: I think this could only be determined on the basis of prevalence information, and our prevalence information now says that there are something like 5,000 chemicals that are in major commercial usage; there are something like 500 new chemicals that are introduced each year. I have no hope of catching up with the backlog ever, but I would tend to distribute my resources roughly in a 10-1 ratio.

It may be a lousy answer, but it is the best one I can come up with.

Dr. Byrd, EPA: Nobody can come up with an answer; it is all guesswork. The paradox is this: It is fascinating to me that you would feel that I, as a regulator looking at new chemicals, would only be able to look at proven human carcinogens. In fact, a computer program that tells me whether there is a prediction of carcinogenic risk in a mouse would make me deliriously happy.

I have no problem regulating with the shorthand information, and having to do it now is really based on flipping a coin. You look at chemical structures and you make a guess. A computer program which gives me something better than a random or educated guess, that of all the chemicals out of the universe, this has a higher probability of causing cancer in a mouse, would be a great answer for me.

I construe that kind of computer work as a very basic field of science. I am aware that NCI does research in some areas. I am not aware that it has been plugged into the carcinogenesis program, for example.

Dr. Cooper, NCI: Can I ask a question, because I want to know if I am clear.

Dr. Byrd, EPA: Sure.

Dr. Cooper, NCI: When you say a computer program, are you referring to a data base resource, or are you referring to a de novo calculation of probability?

Dr. Byrd, EPA: I am referring to a de novo calculation of probability.

Dr. Cooper, NCI: That is probably beyond the realm of existing technology.

Dr. Byrd, EPA: Well, why would it be beyond the realm of existing technology for carcinogenic potential when it is not for anti-cancer potential?

Dr. Cooper, NCI: I do not know enough about the anti-cancer area to even make a sensible comment. But in terms of a de novo system for carcinogens you must be prepared to predict metabolic pathways within the system. I think that is the hang-
In terms of direct acting carcinogens, I think you and I could build that tomorrow, in a simplistic sort of way. The system is not going to tell you very much. It is going to say that if the compound has a nitroso group hanging on it, you had better watch it, and that is probably the level of sophistication we would get.

Dr. Byrd, EPA: Sure, but even if you built me one, just for fun and games, that would give me predictions of proximal carcinogens. We have in our employ a number of sophisticated chemists who could predict metabolic patterns, and I could feed those in independently, if I had such a computer program.

Dr. Cooper, NCI: I hope you are right.

Dr. Byrd, EPA: What I am saying is there is an assumption that whatever applies to existing chemicals must also apply to dealing with new chemicals. You made an assumption. I think that it is true that in the world of having to deal with passing regulation for arsenic, that is already out there, you really are confronting a very difficult problem - is it going to cause cancer in humans. But new chemical regulation is a different world. It is qualitatively different. The kinds of decisions, the kinds of thinking that you have to make in order to make regulatory decisions are just fundamentally different.

Dr. Cameron, NCI: Isn't there a structure activity tree?

Dr. Cooper, NCI: Yes. That is probably worth pursuing because we may find an area of commonality that we can really do something with. Quite a number of years ago, the NCI established, as part of a contract at Stanford Research Institute, a chemical hazard ranking index. One side of it was an attempt to develop a structure activity tree which would provide a questioner with a P-value for the probability of carcinogenicity of compounds in the tree. The P-value was basically derived by acquiring the view of experts on what particular structural modifications in a class would mean in terms of that probability. We effectively killed further development of that aspect of the program a few years ago. The basic reason was that the contractor had drifted away from his use of expert groups and begun to introduce P-values which were derived from sources that we felt were questionable, so we abandoned further development. But there is no reason that this aspect could not be further developed. There are some programs in the area of synthetic organic chemistry which allow you to predict reaction products and side reactions, and attempts have been made to plug them into this sort of program in reverse to see if they could predict the metabolic pathway. It is my impression that these efforts have had limited success, but that may well be because we have not invested enough resources. Certainly, it is an area that could be further investigated. It would be of interest to the Cancer Institute. Anything we could do to model the situation better would be enthusiastically greeted.

Dr. Marland, EPA: The tree problem, I think, has already been looked at by EPA, and unless I am mistaken, they have chosen to go the route of more traditional testing for demonstrable carcinogenicity in their Section 5 regulation requiring industry to pretest. Because the affected parties -- which is, of course, industry -- found quite logical and perhaps, from their perspective, quite reasonable objections to classifying a substance toxic on the basis of its structure, there are substantial exceptions to that, and so, as in most technical and even scientific problems, it is not necessarily the science or the technology that deters the solution to the problem. It is the administrative process.
Dr. Cooper, NCI: Well, clearly, what has been suggested would be a major research effort, but it would have only limited value for regulation. Basically, it comes down to the fact that the reason you have said, "X is more likely to be carcinogenic than Y," is because someone, who is an expert in the area, said it was going to be that way, and that is not a sufficient basis for regulation.

Dr. Byrd, EPA: I make those decisions every day.

Dr. Cooper, NCI: Well, I am glad I do not have to do that.

Dr. Byrd, EPA: That is the difference. The difference is, when we go to pull arsenic out of the environment, you are talking about something which is economically very painful. People are using existing compounds because they meet economic needs. You are talking about high cost, you are talking about putting people out of work. When the decision is made to regulate a new chemical, you are talking about something that does not exist right now. The economic cost, except for the company which has come forward with the compound, is extremely low. The decision is most often not to ban it or to let it be made with no restrictions, but rather a decision to require more testing. In actuality, it is not that simple. I am making things sound easy when it is not. What we do is we regulate pending the development of test data, so we have to have a control option available also. Again, my answer is that new chemical regulation is different, and that computer-based predictions would be very useful in a new chemical regulatory environment.

Dr. Cooper, NCI: I am sorry, I feel like we are monopolizing the conversation. I just would be very concerned about trying to regulate on the basis of a de novo sort of calculation for such compounds. This is not a reason to say we should not investigate it and drive the technology further. If you drive it far enough, it may be a very useful system. But right now, I do not think it is.

Dr. Leidel, OSHA: Well, to attempt to answer your question about allocation of resources on new chemicals, keep in mind my tunnel vision of just the occupational environment as opposed to the fact that the chemical, of course, might be released into the general environment via the air, the water, or the land. We look at, of course, the problem of where to focus our resources in terms of, generally, the number of people that might be exposed, which then becomes a function, and the hazard involved, which, of course, depends on the potency of the effect, and sometimes the amount of the chemical produced; but generally, it is the number of people exposed because, of course, there are many industrial chemicals made in this country in billions of pounds to which there are relatively few exposures because they are in closed systems or this sort of thing. And so, it does not bother me personally when I hear about all the new compounds that are being produced each year, because to me usually they are rather limited in the nature of their distribution and poundages. The thing that professionally has frustrated me for many years is the problem we have in attacking the known problems. I feel a sense of frustration from the knowledge we have gained from the NIOSH survey back in 1973-74. The NOHS survey which we are now talking about is NOSH II and it will be discussed tomorrow morning by Mr. Dave Sundin from NIOSH.

Ms. Spadafor, EPA: I agree with you on the need to examine new chemicals carefully because to prevent a hazardous chemical from being made is a first step. Why let the chemical get to market only to later have to go through the regulatory process to try to pull it.
Dr. Marland, EPA: You get a much better bang for your buck.

Ms. Spadafor, EPA: I agree, but it is important to know how the premanufacturing process would assist us. Could you get the information you need to prevent a hazardous chemical from being manufactured? Could you get the information from what is asked for in the notice requirements?

Dr. Byrd, EPA: Well, it is a question of what information comes in. What EPA has a legal right to in premanufacturing notices is very, very skimpy information. What we actually get, in many notices, happily, is a lot better than, legally, we have a right to have. But we have to make decisions in some instances on very skimpy information indeed. Legally, we do not have a right to very much information other than chemical structure, for example, simplified use, and whatever health and safety data is available to the manufacturer, but he does not have to run out and get any data that is not "reasonably ascertainable." Depending on which lawyer you talk to, "reasonably ascertainable" can be construed twelve different ways.

So, it is decisionmaking based on very skimpy information in some circumstances. We confront the decision tree problem again. Industry told EPA before the premanufacturing program came into existence that there was no such thing as a chemical going into manufacture without an acute toxicity test. Then, you know, to turn around and tell us that they would feel unhappy being regulated on the basis of chemical structure sounds a little funny to me.

Dr. Marland, EPA: Kind of skeptical, too.

Dr. Byrd, EPA: Yes.

Dr. Cooper, NCI: But this brings us to another area in terms of old versus new. We heard a paper today on the effects of feeding of a benzidine based dye. Last year, we heard about an NCI study which produced tumors in rodents after 6 months of feeding of this dye. But Dr. Troll published his initial paper in 1971 on the excretion of benzidine in monkeys which were fed such dyes. It was no surprise when we got the results of the NCI study. We knew everything we had to know in terms of what was going to happen if humans were exposed to that dye in 1971. Where have we been ever since?

Ms. Spadafor, EPA: We knew what was going to happen with asbestos in, what, 1940? 1930?

Dr. Cooper, NCI: I am not sure of the specific date at which we had adequate information.

Ms. Spadafor, EPA: Facts about hazards of exposure to asbestos, as far as lung pathology is concerned, go back approximately 40 years.

Dr. Cooper, NCI: Let's accept that as an appropriate date.

Ms. Spadafor, EPA: I don't know what the government did, or if they were equipped to do anything at that time, but when the regulatory agencies were established, little was done.

Dr. Cooper, NCI: Yes, and your argument is clearly quite valid with regard to asbestos, at least since the fifties. Before that, although we had some information, we did not have very much in the way of a strong organization to do anything about
it. In addition, we had a war, and that war involved a lot of shipping, and shipping required a lot of asbestos work, and it just was not seen as being feasible to regulate at that time. I am concerned with why it is that we want to focus our attention on new substances when we already know all we need to know about a lot of old ones, and we are doing nothing about them, or doing very little about them.

Dr. Byrd, EPA: It is a very reasonable question, one which I cannot answer here, but I think that one reason to look at the new ones is simply that you get the most bang for the least bucks in dealing with new chemicals. It is a relatively painless way. Fifteen years from now, our successors will not have to deal with a whole new set of aggravating problems. The benzidine dyes that you mentioned are a fascinating example of the kind of difficult problems that you confront as a regulator in new chemicals. The dyestuff industry will, on demand, make a small batch of a totally new dye. The profit margins are small. The dye industry says, and I have no reason to disbelieve them, that if we require them to do even minimal testing on a new dye lot of that sort, that they will simply choose not to make it. Now, we have a legal mandate exactly not to do that. We are told by Congress that we should not unduly inhibit innovation. It is very hard to justify knocking that guy out of the box on that dye strictly on an exposure basis, because he is not making many pounds. The dye industry has been particularly cooperative with EPA and what we would like to get is testing on selected structures within the benzidine dye class together with an agreement that says that they will agree to abide by a structure activity analysis. The problem is that the research base is deficient; the research base which says how would I tell them which structures to do and how, exactly, would I make a convincing structure activity argument on a new benzidine dye. It is clear to me at this point that not all of them are going to prove to be carcinogens. It is going to be some subset.

Dr. Cameron, NCI: If your structure tree was big enough, strong enough, it would be hard to come out with a new compound that you could not attach to part of the tree and get some idea. SRI is equipped to do that.

Dr. Cooper, NCI: Well, let's not make any arguments for that particular tree. It has a lot of holes and a lot of weaknesses. When you say benzidine dyes, I presume you mean benzidine derived dyes. If you have a modified benzidine structure, you might have serious questions about making a statement that the compound is going to be carcinogenic. But if you have a simple benzidine structure with those two diazo links, I do not think you have any reservation at all today about saying that the compound is going to be carcinogenic. You are perfectly right in terms of the broad class, but once you have gone beyond something like the IARC monographs, you have run out of data base.

Dr. Bellin, EPA: I am sorry, but I think that the dyes are a perfect example of why it does not pay to say the old versus the new, because while we are concentrating on the "old" benzidine family of dyes, we are forgetting about, let's say, thiazine dyes, which have terrible problems, triphenylamine dyes, some of which are suspect. So it would be very convenient and kind of appealing to say, as in hazardous waste, you know, the old hazardous waste or the new hazardous waste, but I do not think that it is going to work that easily, and I do not agree that you can simply say we will get the biggest bang out of a buck by that approach. I think it may well turn out to be somewhat simplistic. I wish I had a better handle on it, but I do not.

Dr. Byrd, EPA: Well, I had not meant to debate that issue. It is just curious to me that the sorts of things that are appropriate towards new chemical regulation tend to
get completely lost in the research priorities. I do not quite understand that, but it seems to work out that way. I can see plenty of things that can be done, and I would anticipate that there are people within the basic research community that would find those problems exciting to pursue.

Dr. Cooper, NCI: What are you thinking about when you talk about whole areas of research that would be useful for the regulator that are falling through the cracks? I am not arguing that that does not exist, but what are some illustrations?

Dr. Byrd, EPA: Well, I am just particularly sensitized to the new chemical program, because that is the area where I work. My problems lie in those areas.

Dr. Marland, EPA: I would be surprised if the research community had the faintest idea about working in that direction. Perhaps the function that the EPA in general, and your office, perhaps in particular, could undertake would be to direct some kind of fundamental research approach in providing you with tools, whether it is a structure tree -- I am scared to death of a structure tree. I am really afraid of it. I would hate to go to court and try and climb the tree, if you will, and be successful. You might get hung on a branch. But really, the reason I brought up this whole thing is that I am personally dissatisfied with the extent to which the research community has addressed the problems of you people who are regulating pesticides, toxic chemicals, and hazardous wastes. The reason I brought up this issue on risk assessment is that we are taking the little bit of known information we have and we are putting this through some extremely fancy statistics and coming up with a number that may appear rational, but at least it confuses people so they do not dare attack it -- well, they do attack it -- but we can put all kinds of hand blessings on these things and give this figure some kind of authority which it really does not possess. Of course, the basic problem is a lack of functional knowledge about what we are talking about, and that comes only from research. That is why I tried to stimulate you, since I was aware, not of your particular relationship, but I knew that you were concerned with the toxic substances program, and I am very much aware of your lack of research. Our job is to try and provide it but we are not giving you any. By the way, you are not asking for any, either, but that is a small point.

Ms. Spadafor, EPA: We ought to be discussing developing future cooperative programs. I do not see how we are fulfilling our purpose at this meeting - discussing the development of interagency programs - unless we consider the problem of coordinating our activities in risk assessment. I was involved in the Interagency Regulatory Liaison Group, Regulatory Development Work Group for OSHA (Occupational Safety and Health Administration), and I am presently a member of the asbestos work group for EPA. I notice that there is a serious lack of communication among the IRLG member agencies. The working group discovered that two research projects being done by two different agencies were identical. There should be a greater sharing of information and conservation of federal funds. Better communication on what projects are being undertaken by each agency is needed so we do not have duplication of research.

Dr. Marland, EPA: Some people would say that two researchers working on the same topic is not redundancy.

Dr. Plotnick, NIOSH: On this duplication, I think you will see less of that in the future, and what you probably saw was completion or soon completion of two identical studies that were started three or four years ago in most cases, not more recent.
Dr. Spadafor, EPA: Not in this case.

Dr. Plotnick, NIOSH: Well, it depends upon which agencies, but generally, or at least with the NTP program and some of these collaborative programs now, the duplication has been cut down considerably.

Dr. Marland, EPA: We have a new mechanism in EPA now on our grants research that, prior to our funding any grant, the National Institutes of Health and the National Science Foundation, the Department of Agriculture and the Department of Energy are canvassed if the grant we are talking about is appropriate to those areas, to see whether or not they are funding that or a similar project. Interestingly, we have found that we have changed our intentions to fund or not fund based on communications with some of those agencies, because the same investigators, of course, are sending their proposals to all of these groups, therefore, it is a very important communication that we make. That has begun within the last 3 months because that is how old our grants program is. It is 3 months old.

Dr. Leidel, OSHA: Well, just out of curiosity, do you happen to know, Dr. Plotnick, how NIOSH avoids this? I assume that there are some sorts of lists of research being done that go into common data bases, and you plug in the chemical you are interested in and you see who is working on it or what has been in the reported literature.

Dr. Plotnick, NIOSH: It is not that mechanized. It is still a manual search system. Generally, the compounds go into the National Toxicology Program (NTP), at least, but that is only a portion of NIOSH's total toxicology budget. But those go in and obviously are compared with all the other projects going in from the other agencies, and if there is duplication, it is going to be noted and some modification will be recommended, and generally, being the smallest, we are probably the ones that will have to modify ours. But that is not the important thing. I think that that is something that really bothered me when I started here 5 years ago because I saw two or three studies that were identical. EPA was doing one, NCI was doing one, NIOSH was doing one. If the routes were different, let's say, and there were different reasons for support of regulatory action based upon different routes and they were not otherwise duplicated and they would in the end, support each other if they came out with essentially the same or similar results, then I would see nothing wrong with them. But I agree with you that some of it was absolutely duplicative.

Dr. Plotnick, NIOSH: Without being critical of EPA, Congress put all kinds of responsibilities on one single agency which has to act as a research agency and a regulatory agency, and again, this is the only time that, without being facetious, I will say that Congress in its wisdom separated research and regulation with NIOSH and OSHA, and I think it works better. You know, there is a lot of cooperation but there is a total separation, administratively. Our research is not dictated by somebody's idea of what standards should be set. We look at priorities on our own. We obviously get suggestions from the interagency group, but it does not absolutely dictate our work and our research, whereas, I think in other areas it does.

Dr. Marland, EPA: We get a little schizophrenic in EPA, but I think that I would rather have the relationship with the regulators that we have in R&D than you folks have in NIOSH. So, I guess that each one of us likes the activity in which he is engaged.
Dr. Plotnick, NIOSH: Were you making some comments earlier about the fact that some things were difficult to support in rulemaking hearings because of the way that the research is supported?

Dr. Marland, EPA: No, because the basic scientific evidence that the regulation is justified sometimes is sparse.

Dr. Plotnick, NIOSH: We can give you examples where there is more than sufficient justification for regulation, and yet it is overturned.

Dr. Marland, EPA: Well, of course. Dr. Bellin pointed that out effectively. That is because the system on which government operates and in which the economic system of the country operates is at play in whether or not a regulatory action can indeed become effective. I am not talking about that.

Dr. Bellin, EPA: What I am talking about is that combination of statement of problem associated with a quantification of that problem and the science that permits you to do that. In other words, this man's decision to regulate or not to regulate has to be based on something that has to be a scientific fact, and I allege that we are not doing a terribly good job at providing that scientific fact, whether it is a new chemical or an old chemical or anything else. Now, however well you document the facts, you then go into a series of procedures where scientists no longer are in command of what happens, but as long as we are in command of what happens, providing science and a recommendation to the regulator, I think that we are not terribly proud of the extent to which we have good science going into our regulatory actions. This is where I had hoped our collaborative program could bring a stronger influence into an expression of confidence on the part of such as the NCI and NIOSH, who are better known and established and have reputations for good scientific integrity. If our research can be pointed in that direction, I think that we would get a better utility out of a collaboration than if we all find that there are really some worthwhile projects here that we ought to solve. What I am trying to say is, focus on those issues that make our regulatory life miserable, which is lack of good science, and good scientists saying that it is good science.

Dr. Plotnick, NIOSH: No, I disagree. I am not sure that it is a lack of good sciences as much as it is a lack of understanding on the part of the scientist who has to support this in an advocacy manner, and a scientist is not to be an advocate.

Dr. Marland, EPA: Oh, that is very much a part of it.

Dr. Plotnick, NIOSH: But I know it is not the quality of science or the lack of data many times; it is professional judgement in interpretation of the data. You turn it over to somebody who is writing a regulation, who may or may not have a great deal of scientific background, and then, all of a sudden, he says a hearing is going to be held on this day, we need you, and you, and you to support us at a rulemaking hearing. I can tell you that there is absolutely no briefing or understanding of the rulemaking on the record and the Administrative Procedure Act and what the purpose of the advocates for the government and those opposing the government is and how to come across in the right way without losing your perspective. I think it is more of that than it is of quality of science.

Dr. Marland, EPA: I had originated my comments on the need to address science to risk-making quantification, risk assessment quantification.
Dr. Plotnick, NIOSH: I have less difficulty than you with indicating that a given compound is very highly suspect as a potential human carcinogen and it should be regulated as such once there has been a determination in several species, let us say, that this compound is a carcinogen. If we have supporting solid evidence in animals and possibly, at least in our case, we have very often at least got some suggestive epidemiologic evidence to show that it is producing an effect in man because man is our own experimental animal in a lot of these places.

Dr. Marland, EPA: I am talking about benzene, I am talking about arsenic, I am talking about those real tough ones where we have what any scientist will say is a good scientific basis for regulating, and yet you are not regulating, you are not regulating effectively because we do not have credibility, we do not have the ability to translate a series of mouse deaths into something that will convince a jury. Now, I allege that that is a scientific effort that calls for that.

Dr. Plotnick, NIOSH: Okey, but the benzene standard was not being challenged on scientific basis as much as it was on a cost benefit-analysis.

Dr. Marland, EPA: Your description of risk did not warrant the economic cost.

Dr. Plotnick, NIOSH: Within the terms of the Occupational Safety and Health Act, there are differences, because it says, "so that no worker will suffer impaired health or decreased life expectancy as a result of his work experience." The Clean Air and Clean Water Act are not quite as specific, and yet the court decided that that requires a cost-benefit analysis and that the Secretary had not done it. I still say it is a lack of good interface between the lawyers on the line and the scientists in presenting the data, and I am both. I am a lawyer as well. I have been involved in rulemaking hearings just as a scientist, and the other side is not that potent, even the high priced Washington lawyers. They are not that good and effective. It is just that we are not that good in presenting our side because we do not have the training.

Dr. Leidel, OSHA: I will second Dr. Plotnick on that one.

Dr. Plotnick, NIOSH: Dr. Leidel and I were involved in one case where we ran all over the people on acrylonitril. We had solid evidence. We had all gone over how our presentation would be made. You know, we were not faking any data or anything else. It was our interpretation. But we know what information we had. We did not have to look at our notes. We could answer any questions and we could respond to cross-examination. If you are well prepared -- you know, most of the time I would bet you that you bring your experts in to testify the same day that the hearing is being held. They do not know anything else about it other than maybe they have prepared a written statement. They have no idea of what to anticipate. I do not think that is science.

Dr. Leidel, OSHA: There has been poor communication in the past.

Dr. Marland, EPA: Risk assessment is not science.

Dr. Plotnick, NIOSH: I agree.

Dr. Marland, EPA: Risk assessment is the application of science to the regulatory process, and I say we have provided very, very poor scientific basis for risk assessment. It is not that as a scientist I say that acrylonitril is good or bad. I do not have to convince myself. I think that there is a scientific component to the
calculation which convinced even an attorney from Fargo, North Dakota, who does not even have to be a high priced Washington lawyer, but you have to convince him that the simple arithmetic which takes your confidence of toxicity and translates that into the risk of a person in Fargo, North Dakota, that has to be convincing evidence and we have not done that. I allege that that is not a job for an attorney. That is not a job for anyone but a scientist, and this is what I have been trying to drive at, that I have not seen the dedication of a single research dollar from the Cancer Institute or from NIOSH or from EPA, a single research dollar, the improvement of the technique of presenting scientific data in the form of hazard of that environmental element. There is not any research being done on this, and I just do not know why because it is a most important stumbling block, and that is the basis for my complaint.

Dr. Rivkin, EPA: So you see the problem, then, perhaps, as presenting information, packaging it?

Dr. Marland, EPA: I think it is more fundamental than that. A scientist talking with an attorney is a difficult thing in itself, but putting it in writing is even more difficult. Getting a convincing story presented in writing where you have calculations that are simple and clear and understandable by an intelligent person, this is not being done.

Dr. Leidel, OSHA: I will agree with you to a point, but to me there are two things we have to talk about, one is the convincing argument, but in many cases I have found nothing will convince industry. You can come up with a convincing model, but then the second thing is, is it a "defensible model," and that to me is the real tough part in the regulatory arena as I have experienced it. The problem is that you can go out on any subject in this country, and, for a price, get some expert witness to come in and say the government's model or the government research was just full of hot air, and here are all the weak points. That is what is so disturbing to me on this advocacy thing. People say it would sure be great if we did not have it, and OSHA has caused all the problems, and, if we would just be nice to industry, we would not have this. Whether we like it or not, we are never going to go back to the good old days, as I personally see it. We are just going to have to recognize that it is here. It reminds me of the situation in NIOSH with some of our epidemiologists who would get involved in very controversial issues and find their scientific credibility attacked and all this sort of thing by other, company-paid, scientists simply because the companies realize that the easiest way to cut off new government regulation is to go to the roots of the problem, which is the research, and attack the credibility of the research. What happened to our scientists, of course, is they were just not prepared for this kind of advocacy science. The thing that disturbs me, and I agree, we have essentially nothing in the area of risk assessment, is how good are those estimates and what is the credibility of the data that gets into them. I think what has happened, as I interpret the position of the government regulators that say we cannot get into the area because we cannot put a price on human life, is maybe to avoid getting dragged into that whole arena.

Dr. Marland, EPA: The cancer policy calls for you to do it.

Dr. Bellin, EPA: You cannot avoid it. The executive order says every regulation we have to do involves that kind of thing.

Dr. Marland, EPA: Yes, absolutely, every one.
Dr. Leidel, OSHA: Well, it will be interesting because I think that probably what I would anticipate, then, is in the decades of the Eighties they would test chemicals and the companies would come in and say how good are your estimates and then attack the credibility of the data that went into them. Once they force you into that position, which they have, of course, then they will find something else to go at.

Dr. Plotnick, NIOSH: To give you an idea of this, the Office of the Solicitor in Dallas sent me a copy of an opinion from an administrative law judge in an OSHA situation where OSHA's expert witnesses were not nearly as good as the ones brought in by, in this case, Texaco. The administrative law judge, in the findings of fact, said, "Benzene is a leukemogen; benzene is not a carcinogen." OSHA was attacking it for its carcinogenicity, but he made that finding because they did such a poor job of presenting their side.

Dr. Cameron, NCI: I think that is just something the regulatory agencies have to concede to industry that, by and large, we will never have on the government side an expert who can match in depth the people brought in by industry, just because of their long association with a process or a compound or a class of compounds. I am sympathetic to the regulatory agencies. I do not think they have the staff to afford that specialization. I have seen it in the bioassay program when we brought in industrial people to help us in the chemical selection process and/or the protocol development. It is uncanny how much they know. When you talked about the dye people, that is a good example. You come up against a man who has spent 40 years with one class of dyes. There is just nothing you can debate with him about. He knows every reference, every test. He knows every company in the country that is using it, how they are using it, probably how much they are using, and they just tear you up. I think, if you get into an adversarial relationship with them, you are doomed to failure.

Dr. Holland, EPA: Everything we regulate is adversarial. As I say, we are coming in with pretty poor tools. I want to improve our tools.

Dr. Cameron, NCI: We in NCI have been fairly fortunate. They understand we are a research operation. They do not like the way some of our research findings are used, but that is another experience. We are going to have to coordinate that a little better. It is not the subject here.

Dr. Plotnick, NIOSH: OSHA taps you all the time for expert witnesses.

Dr. Leidel, OSHA: I would disagree to the extent that I do not think we always have to concede. I think there are areas where, obviously, industry is up on us. I helped out OSHA when I was at NIOSH as an expert witness many times, and I think the cases the government loses are just due to the tremendous workload that they have. The government will not give the resources to the regulatory agency for the regional solicitor's offices. Many times a typical attorney will be carrying 60 cases. The average attorney that I work with carries 60 cases.

Dr. Cameron, NCI: No, I was not questioning their competency. I am talking about the aspect of diffusion. You cannot carry 60 case briefs at one time. It is impossible.

Dr. Leidel, OSHA: Where the government is shorted is on time and resources, not, I do not think necessarily, the technical expertise. That is the impression I have gotten. Many times it is tough to get the right expert witness, especially a fellow that works
as a consultant to companies, say, designing equipment. He is not going to ruin his professional career by coming in and testifying for the government, and that has become more and more a problem.

Dr. Plotnick, NIOSH: All right, but you agree that when we support regulatory agencies in rulemaking, we overwhelm the other side with people, we take six or eight people. There will be epidemiologists, statisticians, industrial hygienists, toxicologists, chemists, etc.

Dr. Leidel, OSHA: Well, the resources are available. It is just a matter of coordinating the efforts in putting together a good case. I think that is an area where we can always improve in.

Dr. Cameron, NCI: It seems that with some of the regulatory agencies there is an eagerness to move ahead very rapidly, perhaps too rapidly, when looking at other agencies, they may be notorious for missing milestones, but it does not seem to bother them, and probably they avoid a lot of problems by doing that. They just take their time. The major case that comes to mind was Reserpine. We agonized over that and we gave out the results, and the report was delayed 10 months by industry for a pathology review. Finally, when it became a known carcinogen, it was given to an FDA advisory board, and they said, "Thank you. We hear you, but we are not going to do anything about that drug just yet."

Dr. Marland, EPA: I guess maybe the courts have gotten tired of trying to get that agency to move. However, EPA is still trying to react quickly.

Dr. Plotnick, NIOSH: EPA has a different constituency that is filing the actions compared to FDA. The drug companies are ultraconservative because they do not want to get burnt the next time they submit something, either. I presume, just about with anybody, you remember when somebody has challenged you and pushed you a little bit, and New Drug Applications are slow enough as it is, and you don't want it to take you an extra three years.

Dr. Marland, EPA: You can figure that 30 days after EPA has set a deadline there will be a lawsuit instituted by one of the environmental groups requiring compliance with a statute, and the judge, of course, is inclined to say, well, the statute says you shall report, you have not reported here, you are thirty days late. You tell me when you will make a report, and EPA may say it will be 5 years from now. Forget it. When? Is it 60 days or 90 days? Take your choice. These deadlines are not welcomed by EPA staff, I can assure you. They are thrust on us and I think conscientious people will do the very best they can to do a reasonable job of complying with them. That is what EPA is struggling to do.

Dr. Plotnick, NIOSH: Remember, you recommend the legislation that actually sets those standards, too.

Dr. Marland, EPA: I do not want to allege that that is a correct statement.

Dr. Plotnick, NIOSH: No, I think in some cases EPA obviously recommends what should be done and what time.

Dr. Marland, EPA: I do not say that that is correct, but what may be true is that EPA does not protest enough that they are wrong, that the deadlines are wrong.
Dr. Plotnick, NIOSH: I did not say that EPA invites the short time. They should make a rational projection of how long it is going to take and indicate during the committee hearings the period of time; give us a little more legislative leeway. You know, just a little foresight.

Dr. Marland, EPA: I agree.

Dr. Cameron, NCI: You did not solve my problem. Where are we going next year with projects. I think I got a glimmering of one, that structure activity tree.

Dr. Marland, EPA: That is very, very close to what I see is badly needed, and that is to give the regulatory agencies some help.

Dr. Cooper, NCI: It seems to me that we are not addressing in this meeting all of the interagency agreements between EPA, NIOSH, AND NCI. That suggests to me that when we say NCI/EPA/NIOSH collaborative program, that "collaborative" must mean something other than just things that are of mutual interest. That is what I was trying to get at when the first question came up about why don't we do more in terms of physical, primary prevention kinds of activities. I felt that NCI does not have a great deal of expertise in this area. Perhaps it would be useful to try and understand what we mean by a collaborative program. It does not mean everything we do under an interagency agreement.

Mr. Harris, NIOSH: NCI has an awareness of problems, and together with the people who know how to solve them is where the collaboration begins. And together with the problem solvers, such as the engineers and other technologists, the problems can be solved.

Dr. Cooper, NCI: You are describing, it seems to me, an interagency agreement.

Mr. Harris, NIOSH: A collaborative effort.

Dr. Cooper, NCI: But we are not talking about all interagency agreements; all the things in which two agencies have mutual interest. We are now talking about a subset which we have defined as a collaborative program. I am not at all sure what that word "collaborative" is meant to imply, and I feel we ought to think about that.

Dr. Cameron, NCI: I think I would define it as meaning a blending of expertise.

Dr. Cooper, NCI: I think that is the case, too, but that implies that there ought to be expertise on both sides in areas undertaken under this collaborative program.

Mr. Harris, NIOSH: Let me give you an example. The synthetic fuel technologies are in the early stages of development. If the engineers work together with the health professionals and the biologists, we may be able to understand how to solve many of the problems. This is what we are talking about here. It is an intermeshing of different types of expertise to work together in a common bond.

Dr. Cameron, NCI: You are giving me a hypothetical case when you are talking about DOE and synthetic fuels, and so on. It is obvious there is going to be a lot of toxicology done on the products, the by-products, the combination. There will be bioassays attempted by DOE. They should not place contract one for a bioassay until they have consulted with our group, or NCI, specifically in bioassay. We have had our experiences and our problems. We know all the mistakes you can make with a
bioassay. We should be asked to freely offer our advice. Dr. Cooper and I were offended, candidly, at that monkey experimental design. NIOSH should not fund any bioassay until there is an input. Our people should not attempt any epidemiological studies in the work place until they have talked to you people at NIOSH.

Dr. Cooper, NCI: That is a fiat.

Dr. Cameron, NCI: That is just the way it has got to be.

Dr. Cooper, NCI: Maybe that is a good working definition of collaborative program, — one in which neither of the agencies has sufficient expertise to undertake the task alone. In that case we ought to consider as candidates for the collaborative program only those activities in which the expertise of both the agencies must come together to do the job. That is fair enough, and I am not holding a brief for any definition. I am just suggesting that we ought to agree on what is suitable for this collaborative program.

Dr. Leidel, OSHA: I am just wondering, I kind of balk at the idea of bureaucracies and review groups, but it reminds me of a problem we had in NIOSH that in the early seventies we had studies done with, say, poor statistical protocol, both in experimental design and then the techniques used to analyze the data and draw the conclusions. Well, out of that, some of our people got sufficiently embarrassed so that they set up within the Statistical Services Branch what they called SPRG, Statistical Program Review Group, and basically it comes down, you know, after all these fancy acronyms, that all interagency agreements and research contracts and in-house research programs have to go through this group and be reviewed by some statisticians. Theoretically, that could occur here. Anything that involved epidemiology could go through an industrial hygiene review group that we could provide scientists from NIOSH on, or anything that involved bioassays, we could set up a bioassay group and that sort of thing. That is just tentative thinking.

Mr. Harris, NIOSH: We could maybe start off the meeting tomorrow with some of these ideas.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Thursday Morning, May 8

CONCURRENT SESSION I:
RADIATION CARCINOGENESIS

SESSION CHAIRPERSON
Dr. Wayne Galbraith
Environmental Protection Agency
Laboratory and Field Trial Evaluations of the Cost/Effectiveness of Two Types of Personal Ultraviolet B Dosimeters

Dr. Arthur J. Sober, Massachusetts General Hospital
Dr. George Goldsmith, Boston College

Project Officers:
Dr. Thomas W. Orme, NCI
Dr. Herbert Wiser, EPA

I am glad to see Dr. Joseph Scotto in the audience because the first talk I am presenting may be of interest to him. I am presenting this material for Dr. Herbert Wiser, the EPA Project Officer. The subject matter deals with the development of a personal UV dosimeter which may be useful ultimately in epidemiology studies.

The key personnel in this project who are doing both the thinking and the experimenting are Dr. Arthur J. Sober in the Department of Dermatology, Massachusetts General Hospital, and Dr. George Goldsmith in the Department of Physics at Boston College. The project is supported by an EPA grant funded through the NCI/EPA Collaborative Program. The material I will present has been abstracted from progress reports submitted.

A major source of uncertainty in the correlation of skin cancer incidence and exposure to solar ultraviolet radiation is the difficulty of relating the actual exposure received by individuals to the measured incident solar radiation at given geographical locations. Geographically fixed meters record maximal incident radiation and seasonal variation. Although data obtained from fixed meters have contributed significantly to our understanding of the relationship between skin cancer incidence and UV, we still lack data about variation in UV exposure related to lifestyle and, in particular, about the relationship of pulses of exposure, which an individual might receive in the summer during a trip to the beach, to overall individual exposure.

Two types of UV dosimeters are being developed: an electronic meter based upon the principles of the Robertson-Berger meter and a film badge based on photographic film or photochromic materials. Such instruments have many potential uses. A few of the questions which these dosimeters could be used to answer are the following:

1. Which activities are associated with greatest UVB doses? Conceivably some activities may be more dangerous per unit time than others. For example, beach activities involving UVB intensified by reflection from sand may result in much greater exposure doses of UVB than golfing on grass. Measurements with a personal dosimeter could lead to a rational evaluation of the hazards associated with various types of recreational activity.

2. How much acute UVB in comparison to total annual dose does an individual receive on a vacation to a sunny climate? Conceivably a substantial fraction of the total annual dose may be experienced in a relatively short period of time. A vacation UVB to annual UVB exposure ratio could be calculated.
3. How much UVB penetrates clothing? From earlier studies a tentative conclusion has been drawn that about 20 percent of incident UVB penetrates white clothing. Since the most frequent site of occurrence for melanoma in the males is the back, the use of UVB meters beneath clothing could help quantify the dose through clothing and evaluate a potential solar role for melanoma arising in these so-called covered sites.

4. What are the dose differentials by anatomic site? For example, basal cell carcinomas are frequent in sun-exposed areas; yet, the vast majority are on the face and neck rather than hands. Do these areas differ intrinsically in their response to UVB? Or does the dose differ at these sites?

5. Which occupations are associated with highest UVB exposure?

Clearly, the ability to quantitate UVB exposure in the above examples would allow classification of exposure by type of activity, recreational and/or occupational, which in turn, would lead to some quantitative estimate of environmental hazard for each type of exposure.

Before any such device can be employed for extensive short or long term studies, each type of device must be carefully studied to determine that it actually measures the desired UVB wavelengths under a wide range of situations. The following must be determined:

- UVB spectral sensitivity.
- Quantitative abilities under high and low flux rates.
- Positional sensitivity.
- Temperature and climate sensitivity.
- Shock resistance.
- Reproducibility.
- Response to non-UVB wavelengths.
- Cost per unit.

Dr. Sober has received prototype personal UV dosimeters from the Boston College group and is beginning field evaluations to determine whether they will be useful in large scale personnel studies. Some judgment has to be made as to the feasibility of producing large quantities of personal dosimeters and conducting large scale clinical trials. Two dosimeters are now ready for clinical evaluation. One is an electronic monitor with a spectral response similar to the Robertson-Berger meter, employing a magnesium-tungstenate fluor as a sensor. This has been developed by Dr. Goldsmith and Dr. Davidson of Photometrics, Incorporated in Lexington, Massachusetts.

The second device is a UV sensitive film badge similar to the polysulfone film badges which have been previously employed.

Trials will be of two types: laboratory evaluations and field evaluations. All laboratory testing will be completed on both types of units before field trials will be undertaken. Laboratory evaluation will consist of the following testing procedures. For each type of dosimeter, spectral response, spatial response, reciprocity with the badges and linearity with the electronic system, dynamic range, and variation due to temperature and stability will be determined.
The field trials will consist of the following testing procedures to be conducted during two different seasons. In early March, the low intensity solar radiation series of experiments was to have begun. This summer Dr. Sober's group will conduct a set of experiments under conditions in Boston which supposedly will mimic high intensity solar radiation. Phase I will consist of static outdoor testing for reproducibility, specificity, climate, overload. Phase II will consist of dynamic outdoor testing to determine the effect of motion, inclination, and responses to graded increases in solar exposure, dynamic range, and temperature.

In the NCI/EPA annual report, which has just come out (yellow book) the physics of these devices is described more fully.

At this time, it might be worthwhile to mention some of the expected advantages and disadvantages of both types of dosimeters. The electronic meter will probably be quantitatively more precise, be reusable and will have a better spectral match to the DNA action spectrum than the film badges. It will also have a response that is roughly similar to the Robertson-Berger meter so that comparison with geographical data already available will be facilitated. Its disadvantages are higher costs, which will limit the number of devices available, and a slight deviation from the DNA action spectrum.

The film badges, for their part, are inexpensive, easily produced and easy to quantify with a densitometer. There are problems with reciprocity in the film badges and there is a major deviation from the DNA action spectrum.

As I said, this project is at an early stage with respect to the evaluation of the devices. Dr. Sober sent me a photograph of the electronic meter (Figure 1). It is about half the size of a package of cigarettes. It looks like a very convenient device. I am still not convinced that it is going to be the type of device you can ask people to carry around for long periods of time, but I think there are problems for which Dr. Sober will be able to enlist the help of Bostonians, student population probably, and to look at the specifics of how useful these devices can be. I expect that by the end of the year Dr. Sober will provide some useful data on the practicality of using these meters in any large population project where personal monitoring would be preferred to geographical monitoring.
DISCUSSION

DR SCOTTO: You mentioned that the personal dosimeters are going to be measured simultaneously with the global measurements in a particular location. Is that true? In other words, are they also going to have Robertson-Berger meters measuring the global response in Boston? Because I did not know that there was a meter there.

DR. ORME: That is not what I implied. I implied that the spectrum of the electronic dosimeter is specifically designed to match that of the RB meter. I do not know whether they are going to set up an RB meter in Boston. That might be a good idea.

DR. SCOTTO: My suggestion would be to either set one up there or go to a place where we do have the other measurements. At the same time of the day when you are measuring the individual, you are getting the global measurement. In this way, I would know how to evaluate the global responses that we have been getting.

DR. ORME: That is a very good suggestion. I think Dr. Sober is committed to evaluate these meters in the Boston area.

DR. SCOTTO: Then you should get one of the other meters. Because how are you going to quantify it? As you said, you know how much is reaching the earth's surface, but you do not know how much is reaching the individual's skin.

I do not think you mentioned the price, although you said the electronic meter was more expensive than the film badge. Also what is the size of the electronic device?

DR. ORME: I do not have specific numbers on price. Figure 1 gives the size of the device in relationship to a package of cigarettes.

DR. SCOTTO: When I was talking with Davidson, who is developing this, I thought the meter was to be no larger than a 25¢ coin as originally planned. So, it got bigger and it was in the neighborhood of $500 or $600 back then.

DR. ORME: I do not know how much it will cost.

DR. SCOTTO: So the differential in cost in terms of $500 or $600 for one of these units as opposed to the film badge is to be considered.

DR. ORME: Yes, there is appreciable difference expected.

DR. KELSEY: How are they going to wear this thing? It is like a cigarette pack. They are going to wear it at different times of the year and when they go to the beach? How are they going to wear it?

DR. SCOTTO: That is part of the problem, if I might answer that. I was going to mention that they wanted us to be available to field test these in an epidemiological way, as we do the incidence surveys. All of these things have to be worked out. Should you put it on the shoulder? Should you wear it on your lapel? What is convenient and
what is practical? This all goes into the problem of which device to select. Nobody has really answered that.

DR. ORME: I was thinking of one particular problem that you alluded to. To indicate the relative exposure that you get on the forehead and on the back of the neck as opposed to the hand one must actually place the meter on those positions. I think Dr. Sober is going to have to hire people to run around with the meters on to measure the exposure received by skin. How to relate this type of exposure to that received by cancer patients is an important question. It is not going to be a direct correlation, but I think that it is possible to enlist people to obtain exposure data when they are playing tennis, lying on the beach and so forth. That does not have to be done over long periods of time.
I will begin this presentation by explaining that both projects listed in the program refer to the same basic mission - that is, to provide epidemiologic information relative to the potential human health effects of stratospheric ozone depletion. The NCI/EPA program provided support in two waves. The first was for a small amount of funds ($60,000) to supplement our initial, short term project entitled, Special Skin Cancer Epidemiologic Studies. The second, also a small amount ($200,000) was to initialize the long-term effort, the National Nonmelanoma Skin Cancer Study.

At the opening session, Dr. Kraybill reviewed the brief history of the NCI/EPA program. I believe it was around 1978 when funding was actually provided under this cooperative effort. But just before this program materialized the EPA and NCI were already engaged in an interagency collaborative agreement on skin cancer epidemiology. The NCI was asked to utilize its ongoing Surveillance, Epidemiology and End Results Program, usually referred to as the SEER Program, to obtain information, as soon as possible, which would reduce the degree of uncertainty in the dose-response estimates of UV related skin cancer in our country. It was recognized that the SEER locations were not necessarily the best or only places where these studies should be done, and that to monitor the trends in skin cancer incidence as well as ozone depletion, a longer term project was needed. In addition NCI was asked to prepare for field studies which would provide new measurements of solar radiation exposure utilizing personal dosimeters, which were currently being developed by the EPA. The project presently labeled the "National Nonmelanoma Skin Cancer Study", is essentially an extension of the Special SEER study. To start us off on this
long-term effort, funding was provided to initiate studies in two new locations, San Diego, California and the combined states of New Hampshire-Vermont. The data collection phase in San Diego is just being completed, and the New Hampshire-Vermont study has just gotten underway this winter. This presentation will now deal with the progress, early findings and first analysis of the current surveys just being completed.

Slide 1

The first slide shows the locations where incidence data and UV-B measurements were obtained. Before looking at the preliminary report, a brief review of the recent history of events leading to the urgent need for skin cancer data may put this project into proper perspective. As an adjunct to NCI's Third National Cancer Survey, 1969-1971, which provided incidence data on all cancers, except nonmelanoma skin cancer, a special survey of skin cancer was conducted during the later part of 1971 and the early part of 1972. Four locations were able to participate in this study: Dallas-Ft. Worth, San Francisco-Oakland, Iowa, and Minneapolis-St. Paul. In 1973 while we were editing and reviewing the results from this study, the Department of Transportation was becoming quite concerned about the potential danger to the protective stratospheric ozone layer which may result from the excessive use of supersonic aircraft (the SST's). The DOT developed a multifaceted research program called the Climatic Impact Assessment Program (CIAP) to study the effects of the nitrogen oxides which were being emitted as exhaust gases from the SST's. Ozone depletion results in increases of solar ultraviolet radiation reaching the earth's surface, and consequently potentially greater risk for skin cancer among humans. In addition to the incidence data for these four locations, NCI collected and reported to the CIAP Program measurements of solar ultraviolet radiation reaching the earth's surface at these and other locations in the United States. By 1975, other man-made pollutants,
chlorofluoromethane gases (CFM's) which we know as "freons" used in aerosol spray cans and as refrigerants in air conditioners were discovered to be potentially much more devastating to the ozone layer than the nitrogen oxides. Soon afterward federal regulatory agencies were in great need of information on both the biological effects to plants and animals as well as the human health effects of ozone depletion. The CIAP Program had only begun to scratch the surface.

The epidemiologic information which the NCI provided from its early surveys supported the hypothesis that UV may cause skin cancer and that greater amounts of UV exposure which result from ozone depletion may lead to increased risk to skin cancer. However, most researchers agreed that much more information was needed. Not only more geographic locations but also more epidemiologic information on host factors (such as skin color and ethnicity) and environmental factors (such as lifestyle and outdoor exposure habits) would be needed to estimate the potential hazards of increased doses of solar ultraviolet radiation with greater precision. In the mid 1970's it was estimated that an eventual ozone depletion of 7 percent may be expected to occur sometime in the 21st century. Today, National Academy of Science sources indicate that a 16.5 percent ozone depletion may be expected from the continued release of chlorofluoromethanes at 1977 levels. It was also noted that a one percent decrease in ozone translates to a two percent, or a twofold increase, in solar ultraviolet radiation reaching the earth's surface. This is usually denoted as the physical amplification factor. And this factor may be greater than 2 for relative decreases in ozone greater than 10 percent.

Turning back to the map which displays the locations where UV and incidence data are available, in addition to the locations depicted on this map, we will include New Hampshire/Vermont, representing the Northeast, and San Diego, California, representing the Southwest Pacific Coast.
The next slide shows a schematic diagram of the electromagnetic spectrum. We are most concerned with the invisible solar ultraviolet, called UV-B. Stratospheric ozone shields the earth from high intensity wavelengths shorter than 290 nm. However, UV-B between 290 nm and 320 nm, which does reach the earth's surface in small amounts, is known to cause skin cancer in experimental animals and erythema, or sunburn, in man and is suspected of causing skin cancer in man.

Measurements of the amount of UV-B reaching the earth's surface are provided by Robertson-Berger meters. A count of 400 to 440 units of UV-B will produce a reddening of the skin in a typical, untanned Caucasian. The next slide shows that, in general, as latitude decreases, UV-B increases.

The next slide shows the added SEER locations where new estimates of annual amounts of UV-B were obtained. The open circles represent the original 10 locations obtained in 1974. The new 1977-78 UV locations are depicted by the asterisk (*) in the graph. It can be seen that the relationship between UV and latitude remains, as we have seen before. In addition to latitude dependence, we should consider altitude and sky cover as well. That is why some of the locations may not fall in line.

We will now turn to the epidemiological information on our recently collected studies dealing with basal cell and squamous cell skin cancers from these eight locations. The eight locations are in the order of increasing latitudes: New Orleans; Atlanta; Albuquerque, New Mexico; San Francisco/Oakland; Salt Lake City, Utah; Detroit; Minneapolis-St. Paul; and Seattle.

This slide shows the dramatic difference in the latitude dependence of skin cancer morbidity compared to all other cancers. Incidence rates for the White race only are given, since this disease is rare in other race groups. The broken line indicates a limited amount of variability in cancer risk by geographic location for "all other cancers" combined. The solid line shows that as latitude decreases, skin cancer incidence increases.
The next slide ranks the age-adjusted skin cancer incidence rates by sex and geographic area according to recent estimates of the annual amounts of UV-B reaching the specified locations. In Utah the Robertson-Berger meter was placed at Salt Lake City, and in New Mexico it was placed at Albuquerque. The Salt Lake City rates appear to be comparable to those for Utah State as a whole. In Albuquerque an additional adjustment was made for ethnic group. The "Anglo" rates for Albuquerque refer to Caucasians other than Latin. It should be noted that Albuquerque, while not the southernmost point in the survey, had the highest UV-B index. It is clear that the risk for males is approximately twice that for females. Utilizing these new rates we now estimate that as many as 400,000 Caucasians will develop new skin cancers each year in the United States. Compared with data from the earlier NCI survey, incidence rates appear to have increased by 15 to 20 percent over a six year period.

The next two slides show the age-specific incidence rates by geographic area for males and females. In the southern locales, the male rates appear to diverge from the female rates and show increased risk as early as age 30 (see Albuquerque, Anglo). In the Northern and Central regions (next slide) the male rates begin to depart from the female rates by age 45. This difference in age-specific risk by geographic area should be remembered when applying mathematical models to these data.

The next slide shows age-specific incidence by grouped anatomical site, for all geographic areas combined. Basal cell and squamous cell cancers occur most frequently on the face, head and neck. Exposed areas of the body account for about 80 percent of the malignant lesions for both men and women. The incidence for lower extremities among females is equal to or greater than that observed for males.

The next two slides summarize the most important findings to date. All available information on the annual UV-B levels, and the age-adjusted skin
cancer incidence rates are graphically displayed. The solid squares represent the results from the most recent 8-area survey and the empty squares represent results from the earlier 4-area survey. Two locations, Minneapolis-St. Paul and San Francisco-Oakland, were involved in both surveys. The UV-B indices for the 10 locations vary from a low of 101 for Seattle to a high of 197 for Albuquerque. The incidence rates for males vary from a low of 172 for Detroit to a high of 752 for Albuquerque Anglos.

An exponential, or log-linear model, was applied to the data to estimate the change in skin cancer risk due to small relative increases in ultraviolet radiation. In locales of relatively low insolation a 1 percent increase in UV-B (290nm-320nm) may result in about 1½ percent increase in skin cancer incidence (e.g., Seattle, White males); while in locales of relatively high insolation levels, skin cancer incidence may be expected to increase by more than 2 percent if UV-B levels are increased by 1 percent (e.g., Albuquerque, Anglo males). Estimates for females were somewhat (next slide) lower than those for males. At this juncture the results appear to be consistent with earlier NCI estimates of the biological amplification factor (roughly 2 to 1). The degree of uncertainty in the estimates, however, has substantially been reduced. Should these relationships hold, a one percent decrease in ozone may result in an eventual four percent increase in skin cancer incidence. A preliminary report on the nonmelanoma studies will be available for distribution, perhaps by next week. Please leave your name and address if you would like a copy.

**Interview Studies**

In addition to the incidence studies, we conducted telephone interview surveys designed to obtain information on host factors and environmental factors which may be associated with skin cancer incidence. The information obtained from these studies will soon be incorporated into the incidence and UV exposure analyses. This should further decrease the degree of uncertainty in the dose/response estimates.
The next slide shows the instrument which was used. Individuals received a copy of the questionnaire in the mail, prior to responding to the telephone interview. In the patient sample, 500 patients were computer-selected for interview. Before any contact was made, the dermatologist or attending physician granted permission to make contact with the patient. The patient's free and informed consent was obtained prior to conducting the interview. In the general population sample, at least 500 Caucasian households in each location were selected through the telephone random-digit-dialing technique. Adults 20 years of age and over were selected for interviews in these households. The instrument was mailed to cooperating households and again, free and informed consent was obtained prior to conducting the telephone interview.

The next slide shows the number of individuals responding to the telephone interview. The overall general population response rate was between 75 and 80 percent. The patient response rates vary widely among geographic areas. In fact, the success of the patient surveys in San Francisco and New Orleans remain questionable. In New Orleans, physician cooperation was the big problem, only a 50 percent response rate was obtained. It should be mentioned, however, that once contact was made with the patient, the response rate was well over 90 percent. As you can see, there are over 10,000 interviews to evaluate.

The next series of slides will highlight preliminary findings for several host and environmental factors which have historically been associated with skin cancer morbidity. This slide (14) shows the proportions of respondents who claimed to have "fair" complexions. As expected, the patient group had a greater proportion of "fair complexioned" individuals than the general population group. Also, women apparently admitted to be more "fair" than men. We were concerned that this type of question may produce only a subjective response, and we therefore attempted to provide a more objective measure of determining
skin color by developing a skin complexion chart, which you noticed on the bottom of the instrument.

Slide 15 The next slide shows the proportions of respondents who matched the inside of their upper arms to the lighter colored skin swatches, color numbers 7 through 10. It is the inside of the upper arm which is usually untanned. Here again, it appears that the women may indeed be the fairer sex. At each location, the female proportion with light skin matches was greater than the male proportion.

Slides 16-17 The next two slides show the response to questions on eye color and hair color. Blue eyes and blond or red hair predominate among the patient groups for both sexes.

Slides 18-20 The next three slides deal with ancestry or ethnic categories. More Scottish (18) and Irish (19) people are found among the patient groups, as expected. Responses to Scandinavian ancestry were somewhat surprising. In Minneapolis-St. Paul, where the concentration of Scandinavian decents is high, the proportions of Scandinavians were lower in the patient group for both sexes.

Slide 21 The next slide shows the proportions of individuals who held outdoor jobs. The differences in proportions are clearly in the expected direction, except for New Orleans females.

Slide 22 Finally, the last slide shows the proportions of individuals who are able to develop a deep tan. There is no question that the patient group cannot tan as easily as the general population group.

To summarize our progress to date, we are winding down on the data collection phases of this project and we are beginning to get into the thick of the analyses. We plan to provide two monographs displaying complete details and descriptions of the data probably by the end of this fiscal year. It has taken us a great deal of time to edit the information which we have received. Unlike some of the other studies that go on in the National Cancer Institute, we had the
responsibility for all of the editing procedures and developing the programs for the analysis, doing the resolution checks and actually working with the physical documents and making all kinds of comparisons by hand as well as by computer. It is very time consuming and we are glad to be getting out of this phase and getting into the thick of the analysis.

With respect to future research, more information is needed on personal dosimetry measurements, as Dr. Orme has already mentioned. But perhaps even more importantly, we should look to epidemiologic studies of skin melanoma. Most of the general relationships relative to UV-B exposure and skin cancer are also found for skin melanoma. But skin melanoma is a much more serious skin malignancy than the nonmelanomas. The nonmelanomas are 95 to 99 percent curable, whereas the malignant melanomas have a survival rate equal to that which is found for breast cancer (about 70%). The process by which UV may be involved in either the induction or promotion of skin melanoma is complex. Some of the reasons, which Dr. Orme also mentioned, are the distribution of the anatomical sites on skin melanoma patients, the trunk in the males, for example. We strongly suggest that if this long term effort is to continue, that we get the skin melanoma studies under way very soon.
THE ELECTROMAGNETIC SPECTRUM

X-rays and gamma rays

Ultraviolet

Extreme XUV

Far FUV

Middle MUV

Near NUV

Visible

Infrared and radio

UV-C

UV-B

UV-A

Violet

Blue

Green

Yellow

Orange

Red

Infrared

Wavelength in Nanometers
FIGURE 2.1. ANNUAL UV COUNT BY LATITUDE

Mauna Loa
El Paso
Albuquerque
Tallahassee
Fort Worth
Oakland
Des Moines
Philadelphia
Bismarck
Minneapolis
AGE ADJUSTED INCIDENCE (U.S. 1970) AMONG WHITES BY LATITUDE

- NONMELANOMA SKIN CANCER (1977-78)
- ALL OTHER MALIGNANCY (1973-76)
AGE-ADJUSTED (1970 U.S.) INCIDENCE RATE/100,000 POPULATION/YEAR

NONMELANOMA SKIN CANCER INCIDENCE AMONG WHITES BY GEOGRAPHIC REGION AND SEX, 1977-78

Seattle

Minneapolis-St. Paul

Detroit

Utah (State)

Salt Lake City, Utah

San Francisco-Oakland

Atlanta

New Orleans

New Mexico (State)

Albuquerque, New Mexico Anglo

All Survey Areas
AGE-SPECIFIC NONMELANOMA SKIN CANCER INCIDENCE AMONG WHITES BY REGIONS OF THE UNITED STATES

SOUTHERN REGION (LATITUDES 30-35 DEGREES NORTH)

AGGEGROUP

RATE/1000/YEAR

ATLANTA

NEW ORLEANS

ALBUQUERQUE

AGE GROUP

MALES

FEMALES
AGE-SPECIFIC NONMELANOMA SKIN CANCER INCIDENCE AMONG WHITES BY REGIONS OF THE UNITED STATES

NORTHERN REGION (LATITUDES 40-50 DEGREES NORTH)

SEATTLE

MINNEAPOLIS ST. PAUL

DETOUR

MID REGION (LATITUDES 35-40 DEGREES NORTH)

SALT LAKE CITY

SAN FRANCISCO-OAKLAND
TRENDS IN ANNUAL AGE-SPECIFIC, NONMELANOMA SKIN CANCER RATES AMONG WHITE MALES

TRENDS IN ANNUAL AGE-SPECIFIC, NONMELANOMA SKIN CANCER RATES AMONG WHITE FEMALES
NONMELANOMA SKIN CANCER INCIDENCE AMONG WHITE MALES IN THE UNITED STATES BY GEOGRAPHIC AREA AND UV-B EXPOSURE

Model: \[ \ln R_{ij} = \alpha_i + \beta_j (UV_j) + \epsilon_{ij} \]
(Assumes common slope)

AGE-ADJUSTED (1970 U.S.) RATE PER 100,000/YR.

SOLAR ULTRAVIOLET RADIATION INDEX (ANNUAL UV-B COUNT X 10^{-4})
NONMELANOMA SKIN CANCER INCIDENCE AMONG WHITE FEMALES IN THE UNITED STATES BY GEOGRAPHIC AREA AND UV-B EXPOSURE

Model:  \[ \ln R_{ij} = \alpha_i + \beta (UV_j) + \epsilon_{ij} \]
(Assumes common slope)

WHITE FEMALES 1977-78
WHITE FEMALES 1971-72

SOLAR ULTRAVIOLET RADIATION INDEX (ANNUAL UV-B COUNT X 10^-4)
COMMUNITY HEALTH SURVEY – TELEPHONE QUESTIONNAIRE

When the Survey's interviewer telephones, the following questions will be asked:

I'm going to ask some questions about the amount of time you have spent outdoors during the summer.

1. In your early adult life (20's and 30's) during a typical summer week, how many hours per week did you spend outdoors during daylight hours on weekdays? What about during your 40's and 50's? What about since you have been 60?

2. In your early adult life (20's and 30's) during a typical summer week, how many hours per week did you spend outdoors during daylight hours on weekends? What about during your 40's and 50's? What about since you have been 60?

3. How many weeks per year do you usually vacation?

4. How many hours per week do you usually spend in the sun when you are on vacation?

5. Since age 20, during a typical summer, did you sunbathe frequently, occasionally, rarely or never?

6. When you are out in the sun do you use suntan lotions frequently, occasionally, rarely or never? What about sun screens? What about protective clothing such as long sleeve shirts or hats?

Now the next two questions will deal with your reaction to the sun without the use of suntan lotions.

7. In the summer, once you have already been in the sun several times, what reaction will your skin have the next time you go out in the sun for two or more hours on a bright day? Would you say you get no reaction, some redness only, a burn, or a painful burn?

8. After repeated sun exposures, for example, a two-week vacation outdoors, what kind of a tan will you have: Will you have practically none, a light tan, an average tan or a deep tan?

9. Do you use a sun lamp frequently, occasionally, rarely or never?

10. Have you ever worked with or been routinely exposed to oils, coal tar, pitch, radiation or radiation therapy, industrial chemicals, dusts, fumes, or arsenic? If yes, to which one(s) of these were you exposed?

11. Have you ever been treated by a doctor for any of the following skin conditions?
   - Dry skin
   - Eczema
   - Oily skin
   - Psoriasis
   - Acne or pimples
   - Warts
   - Moles/birthmarks
   - Hives
   - Unusual loss of hair

12. What is the color of your eyes?

13. Do you have freckles?

14. What was your natural hair color when you were 15 years old?

Thinking back over your working lifetime:

15. What is the occupation in which you were employed the longest? In what kind of business or industry was that? For how long? Were you outdoors on this job frequently, occasionally, rarely, or never? How many hours was that per week?

Now I would like to ask you about any jobs you have held for more than one year at a time, since age 20, that required you to be outdoors for two or more hours per day.

16. Would you start by telling me about those jobs you had during your 20's? How many years did you hold that job? How many hours per day were you outdoors on that job?

17. Have you lived in this State most of your lifetime? If no, where did you live most of your lifetime?

18. In what countries were your four grandparents born?

19. To which of the following ancestral groups do you consider yourself to belong? You may answer more than one:
   - English/Welsh
   - Scot
   - German
   - Irish
   - Scandinavian
   - Polish
   - Russian
   - Other Slavic
   - French
   - Italian
   - Spanish
   - Mexican
   - Other
   - Greek
   - American Indian
   - Asian
   - African
   - Middle Eastern
   - Other

20. Please look at the color chart on the bottom of the questionnaire and tell me which color matches your skin complexion best. Match the chart against the inside of your upper arm, (the portion that is not exposed to the sun). Please give me the number above the color. How closely does your choice match your skin color? (exactly, fairly closely, not very closely) Is the color chart lighter or darker? What do you consider your complexion to be? (fair, medium, dark)

SKIN COMPLEXION CHART

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630
### No. of Individuals Responding to Telephone Questionnaire

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<td><strong>Total</strong></td>
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### SKIN CANCER EPIDEMIOLOGY - White Males * All Ages

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### SKIN CANCER EPIDEMIOLOGY - White Females * All Ages

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### WHITE MALES

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### SKIN CANCER EPIDEMIOLOGY - White Females * All Ages

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640
Discussion

Dr. Kelsey, NCI: Have you included any data on people who use sunscreens, for example?

Mr. Scotto, NCI: Yes. We ask the question whether they use a sunscreen, as you may have seen in the slide. We have gotten very little information on that. I do not think that the general population understood what a sunscreen was, but the patient group, as expected, had a higher proportion. They did admit to using or even knowing about sunscreens.

Dr. Cameron, NCI: I had two questions, but I think you have already answered the first one just in the last few moments. The reason for rationale for breaking out the melanoma from the other skin cancers is the fact that it does not necessarily appear in the exposed portions of the skin, is that correct?

Mr. Scotto, NCI: The reason for breaking out?

Dr. Cameron, NCI: Yes, for separating the melanomas from other skin cancer.

Mr. Scotto, NCI: One reason for separating these studies is that melanoma is a malignancy which is routinely reported to the SEER program, which the NCI also conducts and monitors. But SEER does not uniformly collect incidence information on non-melanoma. The reason for this is that the basal cell and squamous cell carcinomas of the skin are usually treated in the physician's office or as an out-patient. We have to canvass doctor's office to access their records, a more tedious kind of study. The information on the other malignancies is pretty much complete and available in the hospital chart records. Another reason is, as I indicated earlier, that the process by which UV relates to either the induction or the promotion of skin melanoma appears to be different from the skin cancer. I think Dr. Orme mentioned that the reasons why we want to get at personal dosimetry information is because we want to measure something about a short-period, and to see if we could measure the effects of various modes of exposure. Mathematical models applied to the various skin malignancy data indicate that the process involving UV may be different for skin melanoma and skin cancer.

Dr. Kelsey, NCI: My second question is has anybody approached the reason for the difference or variance in physician cooperation?

Mr. Scotto, NCI: There is usually a variance of physician cooperation in most studies. Epidemiological studies are usually difficult in the South, where the tendency has been to not get involved with federal projects. Our contractors in each of the locations were local universities, health groups and cancer registries. That was the beauty of attaching to an existing program. The SEER program had already established the cooperation from the medical community. Physicians are not reluctant to provide medical records. However, obtaining permission to contact the patient for additional epidemiological information was difficult in some locations.

Dr. Orme, NCI: I was not aware that the personal dosimeter was tied into your program. I think that is a major incentive to prod the Boston group.

Mr. Scotto, NCI: We have been waiting. The information on the personal dosimeter was supposed to come to us eventually. Drs. Forziatti and DeFabo, who had earlier
represented the EPA on this NCI/EPA project, had hoped that we could set up some field tests for personal dosimeters. I have talked to Dr. Davidson and the people who are developing the personal dosimeters and one of the reasons we were getting into the new locations was not only to obtain more needed epidemiologic information from northern and southern locations and to explore some of the leads on these epidemiological factors, but also to be able and ready to conduct the field studies. From what you said, it sounds like when Boston is finished developing and evaluating the physical measuring device, we will probably be out of funds and out of the new locations where studies have recently been implemented.

Dr. Orme, NCI: Right. That is what I am asking.

Mr. Scotto, NCI: We are going to run out of funds by the end of this year.

Dr. Orme, NCI: Would the film badge type of thing, even in the developmental stage, be useful to you now?

Mr. Scotto, NCI: Yes. I would recommend that whatever you do on it, first of all you should, before we do anything as you indicated, make sure we make all the laboratory tests to see what kind of variability we are stuck with and to see how useful such a thing would be, before we conduct field studies. I suggest and recommend that you do these in locations where we already have epidemiological information on skin cancer and where we already have UV measurements such as from the Robertson-Berger meter, especially if you are going to use the personal dosimeter device which was calibrated to the R-B meter.

Dr. Orme, NCI: Well, I am more optimistic about a continuation of this than perhaps you are at this stage.

Mr. Scotto, NCI: Right now, by the way, is a good time. The study is going on in New Hampshire/Vermont, which is real close to Boston.

Dr. Orme, NCI: Well, I will definitely get back to Herb Wiser about this to see if we can coordinate it a little more closely. The other question I had was, you mentioned that the incidence rate has gone up from 300,000 in an earlier estimate to 400,000. Now, I was not sure that you were suggesting that that was real change in incidence or is that an improvement in your methodology? Are you saying that that is actually correlated with real decreases in ozone?

Mr. Scotto, NCI: No, I cannot say that that is correlated with real decreases in ozone. With respect to the measurements of the ultraviolet radiation reaching the earth's surface over time, we hardly see any trends during the short period we have been obtaining measurements. So, I cannot say that there has been a substantial, or any notable, increase in UV, or decrease in ozone. The estimate of the biological amplification factor is better because of the added locations. After making adjustments for the time of the year in which the studies were conducted in San Francisco and Minneapolis-St. Paul the indications are that there has been a 15 to 20% increase in skin cancer over the six year period from 1972 to 1978. These increases are mainly observed for basal cell carcinomas of the skin. Hardly any increase was noted for squamous cell carcinomas.

Dr. Orme, NCI: If in fact the Robertson-Berger meters over this same period are giving us generally a steady reading, I am just wondering whether we should take into consideration the possibility of a chemical UV interaction in some of these areas.
Mr. Scotto, NCI: I thought some of you were doing that.

Dr. Orme, NCI: We are doing it experimentally.

Mr. Scotto, NCI: I have not gotten that far into the human studies.

Dr. Orme, NCI: The third question I had was the relationship between susceptibility to skin cancer and fair skin, which we have toyed with in a lot of ways. This is obviously an over-simplification of things. I was just going to point out some of these things. We have looked at a number of different strains of albino mice, for instance, and measured the susceptibility. These were hairless albino mice and they still showed a wide spectrum of ranges of susceptibility. So there are obviously many factors contributing to that variation.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Thursday Morning, May 8

CONCURRENT SESSION I (CONTINUED)
RADIATION CARCINOGENESIS

SESSION CHAIRPERSON

Dr. Thomas P. Cameron
National Cancer Institute
Hairless Mice for Carcinogenesis Studies

Thomas W. Orme
National Cancer Institute

Key Personnel:
Dr. P. Donald Forbes, Temple University Skin and Cancer Hospital

Project Officers:
Dr. Thomas W. Orme, NCI
Dr. Herbert Wiser, EPA

First, I am going to talk about non-haired mice in general and work sponsored by the NCI/EPA agreement at Temple University directed by Dr. P. Donald Forbes. I am going to describe an outbred strain that is very common, the Skh-1 line, and an inbred derivative of that line, which I think will be very useful in photocarcinogenesis work in the future. I will talk about the photoresponses of these lines and of a number of other non-haired mouse lines. Then, I am going to talk about a contract at Emory University with Dr. Issac Willis, who is attempting to dissect the UV spectrum to show whether there are synergistic biological effects attributable to UVA and UVB light, again using the Skh-1 mouse. Lastly, I will talk about a postulated mechanism of UV carcinogenesis involving sterol derivatives.

Non-haired mice are not to be confused with nude (nu/nu) mice. I really want to emphasize this, because 90 percent of the people to whom I talked about hairless mice really think I am talking about the nude mice which are used commonly in immunological studies. The mice which I will be referring to are not nude (nu/nu) mice. They are not immunologically incompetent and they do not have the athymic condition that the nude mouse has. Furthermore, the term hairless is not always reserved for the genotype (hr/hr) which refers to a specific mutant, but is used interchangeably with the term non-haired.

Non-haired mice have been used in photocarcinogenesis studies as a convenience. In haired strains, tumors induced by UV irradiation are often confined to ears or non-haired extremities and are often of mesodermal rather than epidermal origin. Shaving or enzymatically removing hair from haired mice in photocarcinogenesis experiments is possible but is generally considered laborious. There are, however, some people who contend that the hairless strains are intrinsically better models for photocarcinogenesis than the hairless mice or non-haired varieties.

Several mutations lead to the non-haired phenotype. The recessive gene hr is encountered most frequently. Non-haired mice, although convenient sources of bare skin for irradiation, have some peculiar problems on their own. Since mouse pigmentation is closely associated with the hair follicle and is most noticeably expressed as coat color or hair color rather than skin color, hairless mice are only weakly pigmented due to the disruption of hair follicles. A hairless mouse skin type comparable to negroid skin does not exist, as far as I know. Non-albino hairless mice are capable of a tanning reaction, however.

Another common problem with non-haired mice is that the young will not always accept a non-haired mother. This necessitates a heterozygote breeding program, which cuts the efficiency of animal production in half.
It was also suspected, and to some extent shown experimentally, that non-haired mice suffer from a variety of immunodeficiencies, not as striking as the athymic condition in nude (nu/nu) mice but, nevertheless, important in considering photocarcinogenesis. Photobiologists have addressed these problems in a variety of ways. The result has been the establishment of numerous colonies of outbred and inbred non-haired mice with fundamentally different characteristics. In the 1960's and 1970's, it was becoming more and more obvious that different responses to UV irradiation or to chemical treatment coupled with UV irradiation could be attributed in part to differences in the strains of mice that were being used.

I would like to digress here for a minute. I first became interested in photobiology about four years ago when I was given responsibility for monitoring a contract at Temple University. I was coming from the field of immunology. It appeared to me when I first reviewed some projects in photobiology as if the field were in a situation similar to that of histocompatibility antigen research prior to the discovery of inbred strains. There were two major variables that had not been standardized in photocarcinogenesis research. I think these two variables accounted for most of the discrepancies in data and for most of the disagreements between photobiologists. One was the differences that were prevalent in the mice that were being used. Everyone had his own non-haired strain of mouse reared in the basement breeding colony. Everyone assumed that he had the same mouse that others were working with. This was not the case at all. The other variable that was extremely significant to the outcome of photocarcinogenesis experiments was the light source used to irradiate animals. Different light sources had widely different spectral characteristics. They were roughly classified as UV-B emitters or UV-A emitters and were employed with and without filters. The spectra of light they emitted were quite different. As we shall see, this has a major effect on the outcome of a radiation experiment.

In a number of respects UV induced skin cancer as it is being studied in animals and as it is observed clinically is better understood than any other type of cancer. The relationship between UV exposure (dose or irradiance) and tumor incidence is understood in general terms. Molecular mechanisms of initiation of carcinogenesis have been proposed and various hypotheses are amenable to experimentation. Skin cancers, because they are surface cancers, can be observed readily, can be biopsied, and for these reasons, progression or regression can be scored very simply in both animals and in man. There is good correlation between the information we have about humans and the information we are generating in animal experiments. Hence the subject area, skin cancer, could serve well as a proving ground for ideas about human risk assessment based on an interpretation of animal data. Furthermore, there has been considerable progress in analyzing the various genetic factors that are responsible for susceptibility or resistance to skin cancer.

Fig. 1 shows the spectrum from a solar simulator. It is pertinent to all of the papers on photocarcinogenesis. I am going to use it here to indicate what the atmosphere does to protect the surface of the earth from ultraviolet radiation.

The top line in Fig. 1 represents the actual solar spectrum as it passes through space before reaching the earth's atmosphere. UV light is divided arbitrarily into three categories, A, B and C. It is generally assumed that the UV-B component of ultraviolet light is the most active biologically and the most important type of light that reaches the surface of the earth. UV-C is also biologically important radiation, but it is effectively filtered by the ozone in the atmosphere. The biological role of
UVA is controversial. The bottom curve is the spectrum of the solar radiation (in this particular case experimentally simulated) as it reaches the earth.

In Figure 2, we can see that ozone does not act exclusively as a neutral density filter. When the effective thickness of the ozone layer changes, and this can be simulated in the laboratory by a series of Schott glass filters, there is a qualitative, as well as a quantitative shift in the UV spectrum. Filtration effectively eliminates most of the UVB that is biologically active. As the filtration decreases, either naturally (hypothetically) by cataclysmic loss of ozone from the atmosphere or, in the laboratory setting by decreasing the thickness of Schott glass filters, there is a disproportionate increase in incident irradiation in the biologically active region. For that reason, the potential changes in the atmosphere associated with ozone destruction are considered significant, because not only would they let in more UV light, but they would let in light at particularly active wavelengths.

Figure 2 gave an indication of the spectral quality of a solar simulator, which mimics very nicely what is called an effective ozone concentration. One of the very tedious jobs that was performed at the Temple University laboratory was to construct real ozone filters, and to measure filtration as a function of O₃ concentration. Dr. Forbes was able to show that the Schott glass filter system mimicked very nicely true ozone filtration with respect to the quality of the spectrum in the UV-B region.

The contract, which I am going to describe now, is one that was awarded to the Temple University, School of Medicine, Skin and Cancer Hospital in Philadelphia. The principal investigator is P. Donald Forbes. The contract was initiated to examine the extent of strain variation in response to irradiation with a solar simulator and to develop criteria for selecting one strain of hairless mouse for large scale production. The initial objective was not to look at the significance of strain variation, but to find the ideal hairless mouse to put into production for all photobiology work. That concept proved somewhat naive, although Dr. Forbes has obtained information that has resulted in a special interest in the inbred strain designated HRA/Skh. This strain has good breeding characteristics and high UV sensitivity.

Table I outlines the strains that are being examined by the Temple University group and gives designations of their genotypes, their UV sensitivity, that is, whether they are highly susceptible to UV-B induced tumors or solar simulator induced tumors, their acceptance of a non-haired mother, which is a parameter of importance in developing a colony because it gives the production efficiency, and the breeding schedule for these strains. I am using inbred here to imply brother/sister mating and I am using the word outbred loosely to indicate any schedule that does not involve specifically brother/sister mating.

Figure 3 illustrates the production problems that are encountered with the animals that will not accept a non-haired mother. The figure shows rhino mice. One parent mouse is a homozygous male. It is the male, which is mated with the heterozygous female. Since the rhino gene is recessive, the heterozygous female will be haired. The offspring are either haired (hr⁻/+) or non-haired (hr⁻/hr⁻). This gives you an example a forced heterozygosis breeding schedule.
### Table I

**Mouse Strain or Line Designations and Characteristics**

<table>
<thead>
<tr>
<th>Name</th>
<th>Genotype</th>
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<th>Breeding Schedule</th>
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H, M, L mean high, medium and low susceptibility to carcinogenesis induced by UV irradiation with a solar simulator.

Inbred implies a brother-sister mating schedule which may not have reached a 20th generation; outbred includes various schedules not specifying brother-sister mating.
Two of the offspring in Figure 3 are homozygous \((hr^{rh}/hr^{rh})\) rhinos that still have their juvenile hair coat. The mutation does not affect the juvenile hair coat; it only affects the adult hair coat. These mice are born haired and then they gradually lose their hair, starting from the face proceeding all the way down the back of the animal. Another one of the young in figure 3 is a heterozygote; it will be used in the subsequent breeding schedules if female. Most of them are discarded and this decreases the efficiency of the production of these mice.

Most of the mice in Table 1 which require a forced heterozygosis breeding schedule are bred homozygous male to heterozygous female. Fortunately, there are some strains that accept a non-haired mother. They can be bred directly by brother/sister mating. The most vigorous line is the \(HRA/Skh\), which is bred as a homozygous \((hr/hr)\) breeding pair on a brother/sister mating schedule. The pedigreed line is now approaching the F20 generation. It then will be designated an inbred strain. This strain has many useful characteristics, and has been designated for a large scale production and for use in the bioassay of psoralen derivatives. We suspect that this strain, the \(HRA/Skh\), will replace the frequently used outbred strain \(Skh\)-hairless-1 from which it was derived.

DR. CAMERON: Can you give us the reasons for picking an inbred over an outbred?

DR. ORME: We want eventually to examine questions related to the genetic control of susceptibility to UV carcinogenesis. To do that, we needed the inbred lines. I think that in conducting a wide variety of biochemical experiments in which you are specifically looking for genetic control over various phenomena, there is no choice but to go the inbred route. I also do not see any advantage in continuing to use an outbred line when the breeding characteristics of a comparable inbred line are good. The only legitimate reason for using outbred lines is production ease. I think we have in the \(HRA/Skh\) line, an inbred line that has good breeding characteristics. They are not quite as good as those of the outbred \(Skh\)-1 line, but Dr. Stanley Mann who directs the breeding operations at Temple is getting satisfactory litter sizes and satisfactory viability at weaning. My general preference is to design carcinogenesis and toxicological experiments exclusively with the inbred line, because it allows repetition of experiments and the use of genetic tools in analyzing the observed phenomena.

The idea that outbred mice are better models of a heterogeneous human population does not lead to any worthwhile experiments. If, for instance, only 5 of 50 outbred mice irradiated with \(UVR\) were to develop tumors, it would be impossible to prove that these 5 are unusually susceptible for genetic reasons and impossible to reproduce their genotypes. If, in fact, only those 5 were susceptible because of genotype, the statistically meaningful group size for experimental design, would have been reduced from 50 to 5, and 100% of the mice of that particular genotype receiving UV treatment would have had to respond with tumors before an effect could be detected. The topic of genotype specific responses is important as we shall see. But meaningful experiments cannot be conducted with outbred mice. Artificial heterogeneity is the only approach, and this artificial heterogeneity in a mouse population can be constructed by using defined numbers of inbred mice selected from a variety of strains. The conclusion is simple. If genetic tools are to be used to analyze in vivo carcinogenesis, use inbred lines. A corollary might be that in vivo experiments not amendable to genetic analysis are primitive in conception.
I would now like to describe the solar simulator. The solar simulator system and its relationship to other models for solar light and ozone filtration has been described by Dr. Forbes (Figure 4). This unit contains a xenon arc lamp. Each of the panels contains a system of filters. These can be either neutral density filters, cut-off filters or the Schott glass filters, which are used to simulate various effective thicknesses of ozone. With this set-up, Dr. Forbes can simultaneously irradiate different racks of mice with qualitatively different light spectra depending upon the system of filters put in at the various windows.

Figure 5 is another picture showing the xenon arc lamp in the middle of a bank of racks. Each one of these racks is getting a qualitatively different type of UV, but they can be irradiated for the same period of time and reirradiated simultaneously.

Figure 6 shows what is called the Mouse Sheraton in contrast to some simpler arrangements which are called the Mouse Holiday Inns. You can see what is actually happening in the cages. In one cage, a mouse is trying to hide. But mice cannot escape irradiation. They do preferentially turn while they are being irradiated, so that most of the radiation falls on the back. There are left-handed and right-handed mice, as pertains to the side they prefer to present to the irradiation apparatus.

The most common response during the irradiation period is for mice to curl up and to go to sleep. I imagine that is part of their simulated nocturnal/diurnal cycle.

DR. CAMERON: Tom, do you know if there is any degree of blindness or retinal degeneration?

DR. FORBES: These mice are albino. I do not know the answer to that, Tom. I do not know of any specific physical changes in the eye. Eye tumors, for instance, are not common. So, I would have to ask about any effects leading to blindness.

Figure 7 was made before we introduced good laboratory practices. It shows the position and the multiplicity of the lesions observed. As I said, the entire back of this animal is a susceptible target, rather than just the ears as might be the case with a haired mouse. Maps or diagrams of the actual position of the various tumors are maintained on a weekly basis, so that the progression, regression or coalescence of various tumors can be followed precisely. One of the things I mentioned earlier is the uniqueness of this system for measuring time to tumor and following the progression of lesions. That coupled with the fact that these tumors arise in 24 weeks makes it a very useful experimental model.

Figure 8 shows some advanced tumors.

I think it is important to present Figure 9 to explain one of the major experimental variables. One of the reasons why plots of tumor incidence versus time differ from laboratory to laboratory so significantly is that people have not standardized the tumor scoring procedure. Figure 9 pertains to the same group of mice, but scoring is dependent on different diameters of tumor. If in fact you start the scoring with 0.5 millimeter tumors, which are barely perceptible red dots, you record an early incidence. Dr. Forbes thinks the tumors that can be scored with some degree of certainty are tumors one millimeter in diameter or larger. The time course changes very significantly according to what you consider a tumor of scorable size. Since most of the publications in experimental photocarcinogenesis do not specify this parameter, you can understand why discrepancies in published data exist.
The first major experimental variable that Dr. Forbes wanted to investigate in these studies was immunocompetence. Since there was a prevalent notion that non-haired mice were immunologically deficient, he set up a screen of immunological parameters looking at both cell mediated immunity and antibody formation. The conclusion is described in a publication that has just been submitted by Drs. Sharon Smith and Don Forbes, and it is that all the lines, as far as major responses such as the ability to form antibody and react with specific antigens and such as the ability to mount a T cell mediated immune response, are immunocompetent. There are quantitative differences in the various strains, but there was no deficiency that could be called a major immunodeficiency which could be responsible for differences in strain susceptibility to UV carcinogenesis. That does not mean that there are not specific subsets of the various basic immunological tests that are completely lacking. For instance, Dr. Smith did not break down T and B cell mediated functions into subfunctions that could be tested independently.

Then comes the meat of the matter. I find this fascinating.

Figure 10 shows the response of the various non-haired lines at 24 weeks. The names differ somewhat from those shown in Table 1. For instance, the HRS/J is called JAX in Figure 10, and the HRS/Argonne is called AnJ. This is the response of the various lines to exactly the same irradiation conditions. The mice have been exposed five times a week to the solar simulator for a specified period of time. Those irradiation conditions are spelled out in the abstract.

Cryptothrix and absebia are the most resistant. They are mutations distinct from hairless (hr). They map at different sites and have quite a different physiology. The other mutations are all hairless (hr). Still, we can see a wide variation in the susceptibility with different backgrounds carrying the same mutation.

There is no correlation between susceptibility and the albino gene. For instance, Jax is an albino strain as is HRA/Skh. Their responses to the solar simulator differ significantly. There are major differences in the susceptibility even when the animals carry the albino gene. There is no association of susceptibility with the hairless (hr) gene.

The bar graph in the upper half of Figure 10 presents the percentage of the animals that have at least one tumor a millimeter in diameter at 24 weeks.

Not only is there a wide discrepancy in the number of mice or the percentage of mice affected, but the lower bar graph of Figure 10 shows the multiplicity of the tumors on the affected mice. Again, pronounced strain specific variation is indicated. The Skh-1 and the HRA showed multiplicities of tumors 40 and above within 24 weeks. What is being scored in Figure 10 are mainly papillomas, but a high percentage of them will progress to frank carcinomas.

That is really all I wanted to say about Dr. Forbes' work. Temple will exercise its option in a three-year incrementally funded contract to revise its work statement. Rather than screen more strains for susceptibility, they are going to start using the various differences that they have observed to analyze the mechanism of photocarcinogenesis.
A possible line of experimentation is to actually start making crosses between
the inbred strains that are available to find out whether the susceptibility relation­
ships are dominant or recessive. This could be done, for instance, with absebia, which
is on a Balb/c inbred background. It could be crossed very easily with the inbred HRA
line to look for different relationships there.

What I like about this series of experiments is that it is telling us something
that we have known for a long time about humans but which we continue to ignore.
Different people have different susceptibilities to irradiation. Probably the same
could be said about chemical carcinogens. I use this to illustrate what I think is a
great mistake in our approaches to human risk assessment.

We could have taken the data from any one of these lines, turned it over to a
statistician and said give me a dose response curve that we can use to extrapolate by
various fudge factors to the human population. Literally, we would have the full
range of possibilities. What we really want to know when we make a human risk
assessment is not the dose-response curve for or the probable susceptibility of the
whole population; what we really want to know is the size of the population at high
risk and why it is at high risk. I hope that this type of experiment is going to give us
some insights into how to start looking at the human risk assessment problem with
those considerations in mind.

I personally feel that this type of data shows the futility of applying mathe­
matical modeling to the data that are obtained from one species or one strain of
animal. It just may be totally atypical of the population at large. For this reason, I
think we have to start building into our experimental designs for risk assessment
some consideration of the variation in responses that we get.

DR. KELSEY: I just wanted to say that I am very excited about this area. I
have been following the hairless mice story from a different point of view, from
cholesterol metabolism. But showing the genetic susceptibility seems like an
interesting way, as you have just mentioned. I am wondering how widespread would
this type of analysis be in chemical carcinogenesis.

DR. ORMEE: Well, I have submitted a question for this afternoon's discussion
which treats that. My feeling is that if you were to do the same thing with a
chemical carcinogenesis screen, let's say test a carcinogen at the maximum tolerated
dose, which I think overrides genetic resistance in some cases, in ten different
strains, some might develop tumors and others not. I give you an example: C57 black
mice are known to be resistant to most of the chlorinated hydrocarbons. They are
not totally resistant, but you have to go a full two years before you start detecting
carcinogenesis in the C57 black. On the other hand, with the C3H line, similar
exposure conditions induce liver tumors rapidly.

With 2-AAF, the same pattern prevails: the C57 is resistant and will get tumors only
very late, while the C3H gets tumors right away. In fact, if you give 2-AAF in the
drinking water, a dose that kills C3H immediately is tolerated by C57 black for its
full lifetime. So you see that there are major metabolic differences in these lines.

The problem, and this is the matter that I want to bring up this afternoon, is
that we are not gearing our resources to multistrain testing. Current systems for
production of animals are designed to make large numbers of Fischer 344 rats and
B6C3F1 mice available and the use of a restricted number of strains is justified when
one considers animal health problems. The non-haired mice I have discussed are now in conventional facilities and they are susceptible to and are carrying a variety of undesirable viruses like Sendai and mouse hepatitis virus. We hope that those viruses are not influencing experimental results. But if we were to rederive and put into production all of these animals, the cost would be enormous. As I said, we have decided to do this with a single mouse line only so we are not getting much closer to multistrain testing.

DR. KELSEY: I guess my question would be particularly where you know have positive carcinogenic response from classic bioassays. Would it not then be a reasonable step to look at these effects in various other strains.

DR. ORME: Yes, I think that that ought to be done.

DR. KELSEY: How do you determine the metabolism? I realize that you may not be able to do a full blown bioassay.

DR. ORME: Yes, I think we get into a variety of problems by not doing more work in metabolism and pharmacokinetics. There are big loopholes in the basic theory of the bioassay. A good example pertains to the benzidine dyes that have been tested with the Fischer 344 rat. The benzidine dyes are very carcinogenic and metabolism studies have been done in the Fischer 344 rat showing that benzidine is excreted in the urine along with 4-amino-biphenyl. Unfortunately for the theory, 4-amino-biphenyl and benzidine have never been tested for carcinogenicity in the Fischer 344 rat but only in other strains of rats. So those people who are arguing that the measurement of urinary metabolites is presumptive evidence for the carcinogenicity of the parent benzidine dyes have one more experiment to do. And after this is done we will still wonder why the mouse is resistant to the benzidine dyes.
Figure 1

Solar Spectrum: Upper Line, in space; lower line; at sea level on Earth's Surface.
Figure 2

Figure 3

Heterozygous Breeding of Rhino Mice. (See text for explanation)
Figure 4
Solar Simulator
Figure 5

Solar Simulator in bank of racks.
Figure 6
Irradiation Rack
Figure 7
Early tumors
Figure 8

Advanced Tumors
Figure 9

Tumor Incidence vs. Minimal Size for Scoring.
Figure 10. Strain Variation in Tumor Incidence
Project: "Effects of Varying Doses of UV on Mammalian Skin: Simulation of Decreasing Stratospheric Ozone."

A. Introduction:

The malignancies and premalignancies unequivocally associated with sun-exposure include solar keratoses, basal cell epitheliomas, squamous cell carcinomas and keratoacanthomas. The evidence that such tumors might be associated with solar radiation includes their increased prevalence on parts of the body habitually exposed to sunlight, in lightly pigmented and, thereby, less protected individuals in areas of the world which have the greatest insolation, and in individuals who spend more time out of doors.

The problem of ultraviolet carcinogenesis is complex. Among the factors which must be considered are (a) relative effectiveness of different wavelengths in carcinogenesis, (b) dose-response relationships, (c) time-dose effects, (d) repair processes and (e) immunologic considerations. Closely related to the above are the environmental effects on UV carcinogenesis, which involve geographic, temporal, anatomical, and life-style-related considerations (e.g. modification of the stratospheric ozone layer by human activities). This work deals with factors a-c and the ozone depletion problem explained below.

Although it is generally agreed that the UVB portion of the solar spectrum (i.e. 290-320 nm) is most effective in causing skin cancer, knowledge of the relative effectiveness of these wavelengths is not well known (i.e., reliable action spectra do not exist). Moreover, the UVA region (320-400 nm), which was long thought to be unimportant in UV carcinogenesis is now known to augment UVB sunburn response and also appears to augment the UVB carcinogenesis. In preliminary experiments, we observed that the photo-augmentation effect for sunburn response is time-dependent; the time interval between exposure must not exceed six hours for the effect to be observed. The observation of UVA involvement is of great practical (as well as mechanistic) importance because of the predominance of UVA in the total UV solar spectrum.

Because of the complexities brought on by the above-mentioned photo-augmentation phenomenon, as well as by competing repair-, biochemical, and immunologic mechanisms, the dose-response or time-dose effects may not be straightforward. Indeed, time-dose reciprocity does not exist for UV-induced tumors (Urbach et al, 1979; our own data to be published). The detailed knowledge of time-dose-response characteristics are not known, but are required for explanation and prediction of observed carcinogenic effects.

In recent years, concerns have been raised that environmental pollution may affect skin cancer incidence by affecting the stratospheric ozone shield. The ozone shield removes more than 99% of ultraviolet radiation of wavelengths less than 320 nm from the incoming sun's rays. (Emmett, 1979). The ozone is formed by the interaction of oxygen and wavelengths shorter than 240 nm. The resultant ozone then screens out longer ultraviolet wavelengths up to about 320 nm. The ozone is naturally destroyed by various mechanisms.
including combination with OH ions from water and destruction catalyzed by

certain gases such as NO. The stratosphere differs from the lower atmosphere

trophiophere) in that it has a very low turnover rate so that any pollution
accumulates and is very slowly removed.

Man could, theoretically, reduce the stratospheric ozone concentration by
a variety of ways including thermonuclear explosions, exhaust gases from
SST's, and halomethanes used as spray propellants. Predictions of the degree
to which these activities will increase the actual frequency are difficult to
make because they require estimation of several relationships which are not
at present well known, namely the degree to which these man-made activities
will increase the amount of ambient UV radiation, and the degree to which these
increases will be effective in producing cancer. (We already have a glimpse of
the problems posed in elucidating the degree to which UVB increases will result
in skin cancer, for an appreciation of the formidable problems associated with
assessing the degree of ozone depletion, see J. L. Fox, Chem and Eng. News,
394-395) projects a 16% reduction in stratospheric ozone and consequent 44%
increase in UVB radiation reaching the earth's surface.

B. Objectives of this Work:

The overall objectives addressed by these studies are of a two-pronged
nature: they are concerned with (1) the effects of superimposing a constant
or varying band of UVA radiation on the action of UVB light ("photoaugmentation
effect") and (2) the effect of depleting the ozone layer. To accomplish this
goal, we irradiated albino hairless mice with "solar-simulating" ultraviolet
light and with "monochromatic" light at selected wavelengths in the UVA and
UVB regions. Solar-simulating UV was obtained from a 1600 watt ozone free
Xenon arc with its emission passed through a 45° dichroic mirror and a 2 mm
Corning #9863 filter. This combination limits the spectral output to the
range 290-400 nm. The #9863 filter does not significantly affect the short
wavelength cutoff, which is limited to 290 nm by the impure quartz used for
ozone-free emission. "Monochromatic" light (band width 10 nm) is isolated
from a 200 watt Xenon-mercury arc (Canrad Hanovia) by means of a Bausch and
Lomb high intensity grating monochromator. To simulate varying thicknesses
of stratospheric ozone, we vary the amount of UVB while keeping that of UVA
essentially constant. This is accomplished by filtering the solar simulating
(290-400 nm) radiation through a series of Schott WG-320 filters ranging
from 0.5 to 4.0 nm. These are sharp cut-off filters whose characteristics
are given in Table 1. UVA light is obtained by means of filtering 290-400 nm
solar simulating light through a Schott WG-345 filter, which cuts off essen­
tially all light below 324 nm (Table 1). Absorption data was obtained using
a GCA-McPherson Absorption Spectrophotometer.

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TABLE 1
Characteristics of Schott Filters used to Vary Amount of UVB Output from Solar Simulating Lamp.*

<table>
<thead>
<tr>
<th>Filter</th>
<th>Thickness (mm)</th>
<th>λ cut (nm)</th>
<th>λ 50% (nm)</th>
<th>λ 90% (nm)</th>
<th>λ 99% (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WG-320</td>
<td>0.5</td>
<td>310</td>
<td>305</td>
<td>296</td>
<td>292</td>
</tr>
<tr>
<td>&quot;</td>
<td>1.0</td>
<td>313</td>
<td>308</td>
<td>300</td>
<td>295</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.0</td>
<td>318</td>
<td>313</td>
<td>305</td>
<td>300</td>
</tr>
<tr>
<td>&quot;</td>
<td>3.0</td>
<td>321</td>
<td>316</td>
<td>307</td>
<td>303</td>
</tr>
<tr>
<td>&quot;</td>
<td>4.0</td>
<td>323</td>
<td>318</td>
<td>309</td>
<td>304</td>
</tr>
<tr>
<td>WG-345</td>
<td>2.0</td>
<td>346</td>
<td>341</td>
<td>330</td>
<td>324</td>
</tr>
</tbody>
</table>

*All wavelengths in nm. λ cut refers to approximate wavelength where filter starts to cut off. λ 50%, λ 90%, and λ 99% refer to wavelengths where 50, 90, and 99% of the incident light is absorbed.

C. Experimental:

I. Relative Carcinogenicity of "Monochromatic" Bands of UV Radiation

Experimental: Groups of five animals were exposed to "monochromatic" light (10 nm bandpass) at 280, 301, 307, 313, and 366 nm. For the first three wavelengths, the dose was 8.9 J·cm\(^{-2}\) for 5 days, and then decreased to 4.5 J·cm\(^{-2}\). For the 366 nm irradiation, the dose was 75 J·cm\(^{-2}\). The same test sites were exposed to the same light for five consecutive days per week. Observations were made on a daily basis, and responses were graded on a 6-point scale as follows:

- \(E_1\) - Mild to moderate macular erythema
- \(E_2\) - Intense macular erythema
- 1+ - Light scaling with or without accompanying erythema
- 2+ - Firm, scaling, palpable keratosis
- 3+ - Raised, palpable keratotic plaque, corresponding to early malignant changes as defined by Epstein et al (1969)
- 4+ - A papilloma or tumor corresponding to extensive malignant development.
In a companion experiment, irradiation at the above wavelengths was promptly followed by 6.5 J.cm\(^{-2}\) UVA light. For the 280 nm and 313 nm experiments, the fluence was 0.2 J.cm\(^{-2}\) day\(^{-1}\), for both augmentative and non-augmentative conditions. At 301 and at 307 nm, where the action is much greater, the fluence was 0.02 J.cm\(^{-2}\) day\(^{-1}\) and 0.01 J.cm\(^{-2}\) day\(^{-1}\) for 301 and 307 nm radiation respectively. In the latter cases, the output from the monochromator was attenuated by means of wire screens which had been calibrated with the thermopile. Precancerous responses were graded as described above. In cases where the mice burned the amount of energy required to produce each of these two types of "action" were recorded; their reciprocals were plotted as a function of excitation wavelength to yield preliminary "action spectra" for precancerous changes (minimal erythema) and burning (figures 1 and 2 respectively). In cases where burning resulted, no further irradiation was carried out. It is doubtful that either heat generated from the lamp or straight infrared radiation are the primary causes of burning since we observe a pronounced wavelength effect for the latter phenomenon (see below).

At selected intervals, mice were sacrificed and skin biopsies of the irradiated areas are taken. Histological preparations (see below) were monitored for morphologic and biochemical changes.

Results:

Precancerous lesions were induced at 280, 301, 307, and 313 nm. These same wavelengths also produced burning. No precancerous lesions were seen at 366 nm despite the much higher irradiation dosage. Both burning and E\(_2\) responses appear to peak at 307. The shape of the two action spectra appear to differ, especially on the short wavelength side where the effects seem to fall off more rapidly in the case of the burning. Similar effects have been noted by Urbach, 1969. These results also suggest that UVA can photoaugment the effect of UVB, even though it is a poor carcinogen by itself.

Preliminary investigations reveal the following: H and E preparations show that irradiation produces marked epidermal thickening and increase keratosis. These changes are wavelength-dependent and they seem to parallel the clinical responses. However, there were some ambiguities, which probably arose from difficulties in obtaining good biopsies due to the small size of the monochromator-induced tumor. This was especially in the case where UVA radiation from the solar-simulator was used in conjunction with the monochromator.

Comment: These data, though preliminary in nature and, therefore, subject to some modification, nevertheless illustrate some important points. First, they suggest that UV carcinogenesis may be preferentially produced by light near 307 nm. It is difficult to assess the chromophore(s) responsible for precancerous lesions and/or burning from this data, especially as the observed action spectrum may be considerably distorted from the true chromophore absorption, in the short wavelength region by internal scattering and absorption by non-active chromophores. By the same token, it is difficult at this juncture to ascertain whether the apparent differences between the action spectrum for skin cancer and the action spectrum for burning arise from differential action of endogenous chromophores or whether the changes arise from early chemical modifications which affect the subsequent absorption properties of the skin for action which occurs at a later stage (burning). Further mechanistic data is needed to help resolve these questions.
II. Solar-Simulator Experiments

Experimental: While the results of the preceding experiment indicate that shorter wavelengths do not necessarily increase carcinogenesis, we feel that data using monochromatic light is "artificial" and cannot be extrapolated to environmental conditions, since sunlight emission is a polychromatic continuum. We, therefore, conducted experiments with the 1600 watt solar-simulator as described above as well as with broad band "monochromatic" radiation of UVB centered at 300 nm (half value band width = 20 nm). In order to enable a meaningful comparison between the two sources and to gauge the magnitude of UVA in augmenting the UVB effects, an "effective" dose was calculated for both sources by convoluting the action spectrum determined above with the spectral distribution of the emitting source (Willis et al, in preparation). Experiments involving both sources were carried out under conditions of a) constant irradiation (~ 1 MED) and b) a regimen whereby the dose was increased at 20% increments (of the standing dose i.e. 1 MED) after every five days of irradiation. Additional experiments were carried out with broad band UVA (solar-simulating radiation filtered through a Schott WG 345 cut-off filter). Clinical and histological responses were graded as previously described. Twenty animals were used in each experiment.

Results:

a) UVB radiation. After 30 days (total dose 1.62 J/cm², effective dose 1.44 J/cm²) 75% of the animals had 1+ response, 20% had 2+, and 5% had 3+ responses. At the 3+ stage, histopathological changes were compatible with early squamous cell carcinoma, or carcinoma in situ. There were cells with large and bizarre nuclei, as well as "rounding off" of cells, with apparent loss of desmosomal attachments. In many (but not all regions, the basement membrane appeared to be intact. Since the epidermis becomes thickened on exposure to UV radiation, we felt that it should be possible to incrementally increase the dose without burning the skin. This turns out to be the case. For equivalent effective doses, the latter regimen results in increased clinical and histological severity of response. An example is given in Table II.

Table II - Comparison of Constant vs. Incrementally Increased Doses of UVB Radiation.

<table>
<thead>
<tr>
<th>Eff. Dose (J/cm²)</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant Dose</td>
<td>1.24</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Increased Dose</td>
<td>1.23</td>
<td>4</td>
<td>12</td>
</tr>
</tbody>
</table>

(See "Experimental" for detail of dosage, source, etc.)

b) UVA Radiation: Daily exposures of 62 J/cm² were given for 30 days at 5 days/week. By day 10, 65% of the animals exhibited 1+ responses, but this regressed until, by day 30, only E₁(minimal erythema) was present.
c) Effect of UVA + UVB: Twenty mice were irradiated with a constant daily
dose of 9.0 J/cm² (1 MED) effective dose = 0.113 J/cm². At the end of 30
days (effective 3.40 J/cm², 80% of the mice had developed 3+ reactions, as
compared to 5% for UVB alone. The effect of UVA in augmenting precancerous
and carcinogenic effects of UVB is further indicated in Table III.

Table III - Photoaugmentation of UVB by UVA Radiation at
Constant Dose

<table>
<thead>
<tr>
<th>Eff. Dose (J/cm²)</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVB</td>
<td>1.44</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>UVB + UVA</td>
<td>1.59</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Histopathologic changes paralleled those occurring for UVB radiation alone,
but were more pronounced.

As was the case for UVB radiation, when the doses were increased at 20% increments every fifth day, the response at a given dose was more severe than
for the constant regimen. After 18 days, three animals (15) had developed
4+ responses (advanced tumors) after 18 days, and the number of 4+ reactions
continued to increase up to day 30. At equivalent effective doses of UVB, no
animals had developed 4+ reactions.

Histopathologic changes in the 4+ mice showed atypical mitotic figures,
hyperplasia and hyperchromasia of cellular nuclei, disintegration of inter-
cellular bridges, and increasing variability in all sizes. Several specimens
obtained 4-6 weeks after irradiation revealed so-called "spindle-celled"
squamous cell carcinomas. This type of tumor closely resembled a fibrosarcoma
with spindle-shaped cells extending from the epidermis to deep into the dermis.
This unexpected histopathological finding is of extreme interest, since it is
the type reported to occur in areas of radiodermatitis in humans, and is
regarded as a relatively rare Grade-4 highly malignant metastatizing form of
squamous cell cancer.

Comment: These results indicate two major points: Firstly, time-dose
reciprocity does not exist, as evidenced by comparison of responses at equi-
valent effective doses of either UVB or UVA + UVB radiation (Table II).
Secondly, despite the relatively poor carcinogenic effectiveness of UVA ra-
diation, it can augment the carcinogenic effects of UVB, as evidenced by the
comparison in Table III. This demonstrates that UVA effects must be explicitly
considered when effects such as ozone depletion (see below) are considered.

III. Simulation of Ozone Depletion

Experimental: Schott WG-320 filters (Table I) were used in conjunction
with the solar-simulator, as described above, to produce conditions aimed at
mimicing the effect of varying ozone layer thickness. The spectral distribu-
tion of the solar-simulator under the various conditions was measured.
The wavelength at which the output is down to 1% of its value at
340 (plateau region) is given in Table IV. This wavelength is somewhat arbitrarily defined as a cutoff wavelength.

**TABLE IV**

<table>
<thead>
<tr>
<th>Filter (mm)</th>
<th>Cutoff Wavelength (nm)*</th>
<th>MED (J/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>301.0</td>
<td>7.05</td>
</tr>
<tr>
<td>0.5</td>
<td>303.5</td>
<td>13.4</td>
</tr>
<tr>
<td>1.0</td>
<td>305.5</td>
<td>17.3</td>
</tr>
<tr>
<td>2.0</td>
<td>307.5</td>
<td>22.4</td>
</tr>
<tr>
<td>3.0</td>
<td>308.5</td>
<td>25.7</td>
</tr>
</tbody>
</table>

*Wavelength where output is 1% of that at 340 nm.

Groups of 10 animals were exposed to the light from the solar-simulator which had been filtered with the various Schott filters (including no filter). Minimal erythemal doses (MED) were determined for each set of conditions. Following MED determination (Table IV) two sets of experiments were carried out, using the technique of incremental increases described above. Firstly, equal doses of radiation (0.9 MED for the "no filter" condition) were used for each filter combination. In the second set of experiments, doses equivalent to equal responses (i.e. 0.9 MED for each filter combination) were used. Responses were evaluated as previously described.

**Results:**

a) **Constant Dose:** Only the "no filter" and the 0.5 mm filter combinations produced lasting precancerous changes beyond the E₂ stage. These changes were along the lines of those described previously. The effects due to 0, 2.0 and 3.0 mm combinations eventually regressed back to the E₁ stage or they disappeared altogether.

b) **Dose Equivalent to Constant Biologic Effect:** Much different results were obtained for two groups (of 10 and 8 mice respectively per filter combination) which were irradiated with light equivalent to 0.9 MED at each filter combination. In this case, the "no filter" group clearly progressed to the 3+ (early cancer) stage after 30 irradiation days, whereas the remaining mice all exhibited effects which were difficult to distinguish from one another (mostly 2+ responses, some 3+ with or without moderate burning).
Comment: These experiments disclose several salient features. Firstly, they again attest to the superiority of the UVB component in causing precancerous and cancerous changes. The results again point out the non-reciprocal time-dose relationship. Indeed, it appears that in choosing our experimental conditions, we may have chosen two extreme cases—one (equal dose) in which little or no net response is observed and the other (equal response) in which the precancerous response in each system (obscured somewhat by burning) are relatively acute and difficult to tell apart. In order to obtain data which would allow a more quantitative distribution of these wavelength effects, it appears that some intermediate condition(s) for irradiation will have to be carried out. Conversely, these results also imply that observed increases in skin cancer may not be related to ozone depletion in a straightforward way: the effects of dose, angle, environmental and other factors previously mentioned will all influence the results inasmuch as they are determinants of solar dose and spectral distribution as functions of time.

General Prognosis: The above results indicate the complex nature of the problems of explaining and predicting the effect of broad band solar UV light in producing carcinogenesis, and of predicting the effects of ozone depletion on these processes. The combined effect of component wavelengths in polychromatic UVA + UVB light is different from the sum of its individual components, and the augmentation process must be better understood. Hence, reliable action spectra for UVB carcinogenesis in the presence and absence of UVA are needed, and such work is projected for the future. Since time-dose reciprocity does not hold, the provision of a much more detailed picture of the dose-response characteristics under different filtering conditions (i.e. "different ozone concentrations") is also projected. The overall aim is to eventually explain and predict the observed results on a more basic level. Therefore, future studies will involve the expansion of the scope of histological, biochemical experiments and to conduct mechanistic photochemical studies on the molecular level.
REFERENCES


EPA/NCI Grant # R806066010
"In vivo Analysis of UV-B Induced Photooxidations"
Norman I. Krinsky, Ph.D., Principal Investigator
Period: 8/28/78 – 4/28/80

The basic objective of this project is an attempt to elucidate the mechanism of UV-B induced photooxidations occurring in vivo, utilizing cholesterol synthesized in vivo as a substrate for photooxidative reactions. By analyzing the cholesterol oxidation products formed when mice are irradiated with UV-B, it is our intention to be able to describe the types of photooxidations initiated by UV-B in vivo.

During the first phase of this project (8/28/78 – 8/27/79) we concentrated on the development of a suitable HPLC system for the rapid and quantitative resolution of cholesterol and of some of the expected oxidation products. We have used an Altex 310/S0 HPLC with a variable wavelength detector coupled to a reverse-phase column for the separation and analysis. The following compounds have been prepared from cholesterol to serve as chromatographic standards:

- Retention time (min)
  - cholesterol: 11.5
  - cholesterol-5α, 6α-epoxide: 7.0
  - cholesterol-5β, 6β-epoxide: 6.0
  - 3β-hydroxycholest-6-ene-5α-hydroperoxide: 9.5
  - 3β, 5α, 6β-trihydroxycholesterol: 7-keto cholesterol 11.5

In addition, we have also prepared several of these compounds from 14C-cholesterol to serve as internal standards and, in particular, to locate some of these steroid derivatives that do not have an adequate absorption at 205 nm to permit detection in our variable wavelength detector. The 14C-steroids synthesized in our laboratory from 14C-cholesterol include: 14C-cholesterol-5α, 6α-epoxide, 14C-cholesterol-5β, 6β-epoxide and 3β, 5α, 6β-trihydroxycholesterol.

The retention times for several of these standards are listed above, using acetonitrile as the developing solvent.

For the purpose of providing a radioactive substrate for the UV-B photooxidations, we have utilized RS-(5-3H)-mevalonic and (*MVA) injected intraperitoneally into mice. Although there are no reports in mice, *MVA has been utilized in rats and found to give significant incorporation into skin steroids within 30 min (J. Lip. Res. 2:344, 1961). We have now been routinely injecting 0.25 μ curies of *MVA into each experimental animal in order to obtain an adequate quantity of *cholesterol in the skin steroids. Our present protocol consists of injecting *MVA and incubating in vivo for 60 min, either in darkness or exposed to 2 GTE Sylvania fluorescent lamps, F40T12/2021 at a distance of 15 cm.

The experimental animals are nude mice, Skh :hr-1, obtained from Temple University Skin and Cancer Animal Hospital, Philadelphia, PA. Following in vivo incubation, the skin is removed and the lipids are extracted into chloroform/methanol using a Brinkmann Polytron Homogenizer. The entire
process is carried out in a nitrogen-filled chamber under dim light to avoid spurious oxidation of the *cholesterol. After suitable preparation, the steroids are separated by preparative thin-layer chromatography to yield a "steroid" fraction. To remove polar lipids the TLC plate is irrigated 3 times. This material is then subjected to HPLC analysis, with the effluent collected directly in scintillation vials via a Gilson FL-100 fractionator.

Our results are still preliminary, but do indicate that the major radioactive skin steroid observed 1 hr after IP administration of *MVA is cholesterol. There are also smaller amounts of two other radioactive compounds, one more polar and the other less polar than cholesterol. The more polar compound has been tentatively identified as the 3β,5α,6β triol. As yet, we have not been able to identify the less polar compound, but we may be dealing with a precursor of cholesterol such as 7-dehydro cholesterol.

In the extracts of animals exposed to UV-B, we find increased amounts and types of more polar radioactive peaks. The precise characterization of these peaks is difficult, due to the very low levels of radioactivity observed and a problem in reproducibility. We are quite concerned that some of the changes we are observing may be due to the UV-B catalyzed formation of vitamin D₃, via pre-vitamin D₃, from 7-dehydrocholesterol, as recently described by Holick et al. (Biochem. 18, 1003-1979). We are in the process of obtaining some radioactive standards of the compounds described by Holick et al. to compare to our UV-B induced radioactive peaks.

Our remaining effort in this project period (8/28/79 - 8/27/80) will be to characterize these products of UV-B photooxidation of *cholesterol.
Dr. Cameron, NCI: I think this question has been raised before, but how much work has been done with skin painting of these various strains? I also have another naive question. What is a senecar mouse? Is that a hairless, too?

Dr. Orme, NCI: No. The senecar mouse is one that was developed by Tom Slaga at Oak Ridge. What they did over a number of generations was select mice that developed tumors after treatment with DMBA-TPA. Since the tumors were not lethal, they could breed tumorigen bearing animals, and, in that manner, developed a population with high susceptibility to DMBA-TPA. They also developed another line, which was supposedly the resistant counterpart.

I may not have that story completely straight because I have obtained it verbally and have never seen any publications on the senecar mouse, but Tom Slaga has described it as a very useful system, not only because of its high susceptibility for screening of skin carcinogens and looking at the promotion phenomenon, but also because he has explanted skin cells from the senecar mouse and has an in vitro transformation system which he says is remarkably similar to the in vivo system as far as its response to a spectrum of chemicals is concerned.

It has always been puzzling to me why hairless mice were not picked up for skin painting studies, but to my knowledge they have never been used extensively for that.

Dr. Cameron, NCI: Would you propose that that be an avenue for further research?

Dr. Orme, NCI: Yes. Dr. Fred Urbach at Temple has proposed to do some screening studies with hairless mice. He wants very badly to start screening for skin carcinogens using the HRA strain. That is a possible use.

Dr. Cameron, NCI: That would seem to me to have an occupational smattering, would it not? There would be different exposures for different occupations.

Dr. Gass, OSHA: I have one question - a kind of fun question. Do juvenile hair coats in susceptible strains offer any protection against the UV range?

Dr. Orme, NCI: I do not know if anybody has done that experiment.

Dr. Gass, OSHA: It would be a neat control.

Dr. Orme, NCI: It would be a hard one to do, because the juvenile hair coat is gone so fast.

Dr. Gass, OSHA: It lasts about six days?

Dr. Cameron, NCI: Their juvenile hair coat lasts about ten days, doesn't it?

Dr. Orme, NCI: I am not really sure.

Dr. Gass, OSHA: They do not get a hair coat; that is the problem. If hair does protect against UV, would the juvenile hair coat protect? That is the question.
Dr. Orme, NCI: I think the protection with the hair is just a neutral density protection. It is a neutral density filter protection.

Dr. Gass, OSHA: That is why I would use it for a control.

Dr. Orme, NCI: There is an interesting phenomenon which occurs in the C3H mouse, which is being used by Margaret Kripke in her immunology studies, where she has shown that there is a UV-B induced antigen on skin tumors which develop after UVB irradiation. These are all fibrosarcomas. I do not know, and I do not think anybody knows, why irradiation of some mice leads to fibrosarcomas and irradiation of others leads uniformly to squamous cell carcinomas. Hairless mice, for the most part, respond to UV irradiation with squamous cell carcinomas. Haired mice yield a variety of tumors.
There is a lot of public, political and scientific interest in evaluating the adverse health effects of low-level radiation exposures. This study is designed to quantify the risks of low-level radiation exposures in a population of cervical cancer patients that have received radium implants and external x-ray therapy.

The study has been misinterpreted as being clinically oriented in the sense of evaluating the efficacy of the radiation therapy, but in fact the study goal is to determine the radiation risks. The low-level exposure comes from the radium that is implanted in the uterus; the emitted gamma rays result in whole body exposures. So, although the doses to the pelvis are very large, the doses to the stomach, lung and breast are below or on the order of 100 rads of radiation.

This study is the largest human radiation study ever conducted. It is larger than the study of ankylosing spondylitics; it is larger than the atomic bomb survivor study. It is complex in design. What I am going to try to do this morning is give a broad sweep of the aspects involved in the study.

First, we are starting off with what I call the clinical sample. It involves 30 different clinics in nine countries in the world and approximately 30,000 women have currently been evaluated from 1960 to 1970. The clinical sample is being continued with an evaluation from 1970 to 1980. What is rather unique about these 30,000 women is that they have been monitored with blood studies every year for ten years. So they have been closely screened for hematopoietic effects, particularly leukemia. There are eight clinics in the United States and 22 clinics in Europe at this time; three in England, five in Germany, four in Denmark, four in France, two in Austria, one in Czechoslovakia, one in Greece, and two in Italy.

To enhance the population size in order to be able to evaluate effects of low-level radiation, we have included cohort follow-up studies in population-based cancer registries across the world. These include Connecticut in the United States and cancer registries in Denmark, Sweden, Finland, Norway, five in England, Yugoslavia and several Canadian registries. The addition of these cancer registries to the clinical sample results in a population size of several hundred thousand women that received radiation therapy for cervical cancer. It is important to note that cervical cancer is a disease with a good survival rate. The radium implants were used mainly for patients with low stage disease, who have very good survival, and thus the probability of detecting late radiation effects is enhanced.

To obtain more information on the characteristics of the patients, that is on medical history and other cancer risk factors, we have two approaches. One is by case-control studies, which have been initiated already in the United States. In the SEER cancer registries in the United States and in the cancer registries in Europe, and Canada, we are going to go back to the medical records and obtain information about medical radiation exposures and other cancer risk factors, such as reproductive histories, smoking histories and alcohol consumption. We are also planning to send mail questionnaires to those persons who are still alive today.
As mentioned, the clinical study was initiated in 1960 by the World Health Organization. We are continuing it and have completed a ten year analysis. In 1960, radiation was not as conclusive a human carcinogen as it is today, particularly with respect to leukemia. Only the atomic bomb survivors and the study of patients with ankylosing spondylitis in England had shown risks of leukemia that could be quantified; although radiologists were reported to be at increased risk in the 1940s, the radiation doses could not be determined.

The WHO study was initially set up to clearly show that radiation caused leukemia and to quantify the risks. These women received very large average doses to the bone marrow, 500 to 1,000 rads. It was such a large sample size that a leukemia excess should have been readily detectable.

The same advisory committee has remained with the study since its initiation in 1960. They include Dr. Brian MacMahon, Chairman of the Department of Epidemiology at Harvard, Dr. George Hutchinson, Professor of Epidemiology at Harvard, Dr. Herman Lisco, pathologist at Harvard Medical School and formerly Secretary of the U. N. Scientific Committee in 1960 when the study was designed, and Dr. Jim Nixon, a radiotherapist in Memphis. I am the epidemiologist at NCI coordinating the investigation.

I am going to just briefly present the results of the ten year follow-up. These were patients who were followed from 1960 to 1970, and no excess leukemia was observed. This is one of the most surprising things about the study. There were 30,000 women studied; the doses were enormous; there were actually fourteen leukemia cases observed and 15 were expected among these who received radiation. Essentially, the risk was zero with regard to radiation-induced leukemia. This was startling at the time, particularly since 40 to 80 excess cases would have been expected based on the studies of the A-bomb survivors and the spondylitics.

It is hard to imagine the types of biases that would have gone into this study, since there was essentially a 100% follow-up and each patient was clinically examined for up to ten years with blood studies. The average follow-up was about five years and the person-years at risk was about 148,000. Overall, there were 15 leukemias and about 16 expected. There did not seem to be any unusual variation by any of the countries reporting leukemia cases.

Once again, the dose from external x-ray or from radium to the total bone marrow was on the order of between 300 to 1,500 rads. This is a very large dose. There did not seem to be any variation by the type of treatment, whether they received radium alone, or external beam therapy, or radium and external, or any radiation.

As mentioned, what was extremely unusual was that based on the studies of the A-bomb survivors and the ankylosing spondylitics, there would have been expected to be an excess of between 40 and 80 leukemias and there was none observed.

The study is consistent, however, with other studies or cervical cancer patients. In several studies, women receiving large doses were not found to be at increased risk of leukemia.

I might add that the historical study dealt with leukemia and lymphoma, whereas the expanded study is to look at the risk of cancer in organs outside the pelvis. That is, to look for low-dose radiation effects.
There have been several possible explanations given as to why no radiation effect was observed. One involves radiation factors. It is possible that the doses were so large that the cells were killed and not transformed. In other words, cell sterilization occurred as opposed to cell transformation. There is some evidence from animal experiments that this may be the case. It is also possible that the women may not have been followed long enough, although from the studies of A-bomb survivors and the spondylitics the peak incidence of radiogenic leukemia is about four to six years. The minimal latent period for leukemia is about two years; the incidence peaks around six years and then returns to normal levels around 20 to 25 years. But it is possible that older women may have a different latent period or temporal pattern of excess risk than these other two studies which involved men predominantly. There is also a suggestion that females may be at slightly lower risk of radiation induced leukemia than males.

A recent animal study by Major and Mole at Harwell in England, shows a downturn in the incidence of myeloid leukemia in mice at high radiation doses. Dr. Upton has also done similar experiments in the 1960s and showed similar effects. There seems to be an effective dose to cause leukemia of around 200 to 300 rads. At the very high doses, there seems to be a decrease in incidence with increasing dose. This trend has been used by some to explain the findings in the cervical cancer studies to suggest that the dose may be so large that the cells were killed and transformation could thus not occur.

What is unusual about this analogy is that the cervical cancer patients received whole body exposures, and although it is true that the doses in the pelvis were quite large, on the order of 1,000 rads to the bone marrow, the bone marrow in the spine and the chest cavity and the femur and other parts of the body also received substantial radiation exposures that should have been leukemogenic. It is difficult to explain why these doses to the non-pelvic bone marrow did not produce any excess leukemia.

Data from the A-bomb survivor study, suggests that latent period is a function of age at exposure. If we follow the cervical cancer patients for another ten years or so, we may be able to find a slight excess if this pattern is a realistic representation of latent periods for older women. The data are particularly weak, but the A-bomb survivor studies do suggest that the temporal pattern of excess incidence seems to be related to age at the time of exposure. Cervical cancer patients have an average age of around 50 to 55 years. Perhaps if we follow them another ten years and have 20 years of follow up, we would be in the range where a leukemogenic effect might be detectable.

Before embarking on such a very large study to quantify the risks of low-level radiation, we felt the need for as much guidance as possible. We convened a study group consisting of scientists in the United States and in Europe. They met in October 1979 and people attending included scientists from the International Agency for Research on Cancer in Lyon, from England, the United States, EURATOM, which is the Atomic Energy Commission of the Common Market in Europe, epidemiologists from Italy, Germany, Finland and Norway. They evaluated the study protocol and design and concluded that it was scientifically valuable to conduct such a study. Although we do not need any more studies to show that radiation causes cancer, there is a need to be able to quantify the risks at various low levels and also to determine, if possible, why various organs vary in sensitivity and why age may be an important factor in determining subsequent risk.
The study group did raise certain concerns though. Can we measure the radiation doses in these patients? Can we follow patients in European countries? Are the sample sizes large enough to detect low-level effects? Can we obtain information on other cancer risk factors, such as smoking or breast cancer risk factors, in order to control them in the analysis?

The study group recommended three things. One is that we conduct feasibility studies, getting information from hospital records regarding risk factors. Two, that we perform dosimetry studies. Three, that we expand the study to include the cancer registries. For the last six to eight months, we have been embarking on these feasibility studies in the United States and in Europe.

Regarding radiation dosimetry, we have a subcommittee consisting of physicists at the M. D. Anderson Hospital in Houston, the Harvard Joint Center for Radiation Therapy, and the Bureau of Radiological Health. Other European physicists have also been contacted.

The dosimetry is complex. It involves external beam therapy and internal radium. We have made measurements now on patients who are today being treated with radium and external beam therapy. We have made dosimetry measurements on phantoms. We are trying to characterize neutron exposures, which come from high energy betatron machines and very high energy accelerators. We are also working on computer simulations and questionnaire designs to contact the physicists at all the clinics and the cancer registries to characterize all the radiation exposures.

Our preliminary measurements indicate that we can accurately characterize the organ doses that specific patients received. The work was done mainly at M. D. Anderson. For typical treatment modalities, the range of doses is as described. The ovaries are in the pelvic area and they would receive very large doses, 1,400 to 1,700 rads. The stomach is much lower, 90 to 300; the pancreas a little lower; the lung still lower, and the breast, 15 to 40 rads. This is in the dose range of current interest, under 100 rads for the organs outside the pelvic range. The thyroid received 6 to 20 rads, which is also a low dose; the brain also receives a very low dose.

To refine these estimates will probably take us another few years, but we do feel confident that we can accurately characterize the doses.

Of the eight clinics in the United States, we conducted a study of a ten percent sample patients to learn what information is available in the medical records. This included a sample of about 600 records. Most of the important information is in fact available in the records. The birthdates are usually available. Even occupations for these women is usually available. Marital status is always available. The number of children is there. Age at first birth is not recorded usually. Family history of breast cancer was given 70 percent of the time. The smoking habits and drinking habits were recorded in the medical records 66 to 56 percent of the time. Regarding information based on medical histories, the medical records had places for whether second cancers occurred, which was entered about 60 percent of the time. This indicates that we will have to do additional follow-up to characterize the population, and this is planned. The additional information was reasonable for many of the risk factors relating to breast cancer. The radium dose was available 99 percent of the time and external radiation therapy was available 98 percent of the time. Forty-two percent of the sample had already died.
The other aspect of the study concerns our conducting cohort studies in tumor registries across the world. Next week the study group is meeting again and the tumor registries from the nine or ten different countries will be combining their analyses of the risk of second tumors among cervical cancer patients that were treated with radiation.

We have also completed analyses using data from the Connecticut Tumor Registry. This is a particularly unique registry because it is the earliest in the world, starting in 1935. It has the potential for long term follow-up, at least for about 40 years. This is the analysis of about 6,000 patients in Connecticut and shows observed and expected incidence of various cancer sites. By itself, I do not think that any of the numbers are exceptionally meaningful. There is a lot of variation in them. A lot of factors are not controlled. However, when they are combined with the other 15 registries, certain patterns and clues about radiation effects might be indicated. We also plan to go back into the records in Connecticut and the tumor registries across the world to get detailed information about radiation exposures and other risk factors. But there was a 40 percent risk overall of a second cancer among all the patients who received radiation therapy. There were 339 second tumors observed in about 5,000 patients and 244 would have been expected based on the Connecticut age and calendar year incidence rates.

Rectal cancer was in excess. This might have been expected since it receives very large doses. The bladder was in excess, and the kidney. The bladder and the kidney are particularly interesting. This excess occurred 15 years after exposure, and therefore is more aligned with the expected latent period for solid tumors. There was a very slight excess of stomach cancer, not very much. This would be at the lower dose. Ovaries had a slight excess. There was also an excess in the body of the uterus, the corpus.

Consistent with all the other studies, there is no excess of leukemia, or only a very slight one, eight observed versus six expected. There was an excess of lymphoma, actually non-Hodgkin's lymphomas. Lung cancer was substantially in excess. This was actually one of the most startling findings, 48 lung cancers were observed and nine expected. It is questionable whether this is a radiation effect since the great bulk of the excess occurred within the first five or ten years and this is not consistent with what is known from most other radiation experiences. In other studies excess risk due to lung cancers occurs 10 to 15 years after exposure. Cervical cancer patients, perhaps, can be characterized by a low socio-economic status, perhaps they smoke more than the general population, and these factors may be related in part to some of the excess.

Pancreatic cancer was not in excess. Interestingly, breast cancer was in significant deficit. This is consistent with studies of women who have been treated for benign menstrual diseases with castrating doses of radiation. It seems that in human studies, if the ovaries are ablated, the subsequent risk of breast cancer is reduced because of the cessation of ovarian activity. This finding is consistent with this hypothesis.

There were two bone cancers. They were osteosarcomas and both occurred in the pelvic area. There was no excess of brain cancer.

Thus, I have presented a quick overview of the entire study. There will be another three years of data collection and another five years before the final results are completed.
Dr. Gass, OSHA: Do you have any evidence of leukopenia or thrombocytopenia from the blood studies that would indicate that you might have an overdose of radiation to the hematopoietic system? We know for example that we can detect acute dose delivery at 25 rad and at 100 rads. Surely, with these doses you might see it. A related question to that is what was the average dose delivery internally and then externally? How did you relate that to your data?

Dr. Boice, NCI: Details on the blood studies are not available. The clinicians in the thirty clinics took the blood samples and when something particularly unusual was found, the slide was sent to the hematologist, Dr. Moloney, at the Peter Brent Brigham Hospital in Boston. So, the data were not systematically collected regarding the blood studies, except that they were referred to us when there were severe abnormalities.

Dr. Gass, OSHA: There is no biochemical dosimetry either, such as uric acid or purine or pyrimidine metabolites?

Dr. Boice, NCI: No.

The second question was about the radiation doses. Since there was no excess leukemia, we did not go into exquisite detail regarding dosimetry. We did a first approximation and the range in doses of milligram hours of radium was perhaps 4,000 to 8,000 milligram hours. The external doses were 2,000 to 10,000 rads to the pelvic area.

The exposures varied by stage. This was the other complexity. If you had low stage disease, you received radium only. If you had higher stage disease, you received mainly external with some radium. Currently we are looking into the dosimetry problems in great detail. But from the former study, there was not the need because there was nothing to quantify. There was no excess risk.

Dr. Cameron, NCI: I have a political question. Why did the Japanese and Russians not participate? Where they asked? Especially with Japan, because we had the U.S.-Japanese cancer collaborative effort.

Dr. Boice, NCI: Dr. MacMahon, who is on our steering committee, is also chairman of an NCI/Russian Scientific Committee. He is going to Russia shortly and plans to quiz them on whether they might be interested in collaborating with us. I am not too keen on the idea, however, because one of the scientific requirements for this study is that good follow-up on patients is available as well as good medical records. This is not generally the case, but if large hospitals with large numbers and good follow-up can be found, we would welcome them into the study.

The Japanese have not been approached with regard to this study.

The original population had been selected from the "Annual Report on the Results of Treatment in Carcinoma of the Uterus," Vol. 13, 1963. All hospitals treating large numbers of cervical cancer patients were invited to participate.
Dr. Orme, NCI: I have a couple of naive questions to ask. I presume that none of these women are in a childbearing state. Were they sterilized for the treatment?

Dr. Boice, NCI: They are definitely sterilized after treatment because of the high radiation dose ablating the ovaries. Some women had their uterus removed prior to radiation. The average age was about 55 for cervical cancer patients in this study. There was, however, a small percentage of young women with cervical cancer. I think the youngest age was about 25. There was a small percentage of women 25 to 40.

Dr. Gass, OSHA: Did they use any thermoluminescent dosimetry? Did they use any lithium or glass dosimetry on these people?

Dr. Boice, NCI: We are doing that now. In the M. D. Anderson dosimetry study thermal luminescent dosimeters and lithium fluoride were used. These TLDs were placed on patient's breasts and thyroid to estimate doses from radium implants and from external beam therapy from a betatron and from cobalt-60. For units that are not used anymore, like orthovoltage and other low energy x-ray machines, we conducted phantom studies. We are also conducting computer simulation studies regarding patient treatment.

Dr. Orme, NCI: I have one more question. In the studies that you showed where the dosimetry seems to have a maximum at the lower end and then it goes off at the end, what is the survival of the animals that get the high dose radiation? Is that sufficient time for them to develop leukemia?

Dr. Boice, NCI: Actually that is a very important question. It is the same question that I asked Dr. Mole during a recent seminar. Adjustment for competing causes of risk was made in their percentages. Although in several strains of mice 600 rads is a lethal dose, in the particular strain used, 850 rads was the critical lethal dose. Thus the decrease in leukemia risk in mice that received over 600 rads does not appear to be related to mice killing but rather to cell killing.

Dr. Orme, NCI: Isn't it true that over 1,000 rads will kill the mice within two weeks?

Dr. Cameron, NCI: I remember years back when I was at Roswell Park I think they used 600 rads to wipe out the spleen. They had to replant from donor mice. I think the dose they used there was 600.

Dr. Boice, NCI: Six hundred rads is a lethal dose for most mice strains although 850 rads appears to be the lethal dose in the Major-Mole experiments.

Dr. Orme, NCI: I do not even see how they can correct for that, if it is what I think it is. Those mice will not live more than two weeks.

Dr. Boice, NCI: I think there is some survival. I think they call it the LD$_{50}$, which is probably around 400 or 500 in most strains.

Dr. Cameron, NCI: This gentleman said the LD$_{50/30}$ is about 480 to 600 depending on the strain.

Dr. Gass, OSHA: That is right. I say it is about 480 to 600 depending on the strain.
Dr. Boice, NCI: The $LD_{50/30}$ means that half would live longer than 30 days, and then it would be the percent of those survivors that would develop leukemia.

Dr. Orme: I am very suspicious about that. Even if they survive more than 30 days, they may all be dead at 50 or 90 days. We are still outside of the latent period for leukemia.

Dr. Cameron, NCI: I would agree with you. The upper limit seems to be amazing.

Dr. Boice, NCI: I agree, but the authors appear to have a strain of special mice. It is also hard for me to believe, however, that "cell-killing" totally explains our lack of an effect in the cervical cancer study. We think further follow-up just in terms of leukemia alone will be very interesting and informative in terms of radiation biology and carcinogenesis in general.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Thursday Morning, May 8

CONCURRENT SESSION II:
DATA BASES/MONITORING

SESSION CHAIRPERSON

Dr. George Simon
Environmental Protection Agency
CHEMICALS FOUND IN HUMAN BIOLOGICAL MEDIA, A DATA BASE

Cindy Stroup, EPA Project Officer
Office of Pesticides and Toxic Substances
Survey and Analysis Division
Design and Development Branch

For those not familiar with this program, the establishment of a data base on chemicals that have been measured in human tissues and body fluids, a little history may be in order.

In 1975, it was suggested by members of the Interagency Collaborative Group on Environmental Carcinogenesis (ICGEC) and staff at the EPA, that a more relevant exposure assessment to toxic agents and environmental carcinogens could be achieved by the acquisition of data on human body burden. Residue levels of inorganic and organic compounds are reflections of exposures to food, air, and water contaminants and pharmaceuticals. These data are needed:

- in the identification of industrial chemicals of concern;
- to place chemicals in a priority order for agency attention;
- to complement data already on hand such as environmental, human exposure, and materials balance information;
- in the setting of appropriate regulatory limitations;
- in the National Cancer Institute nomination and selection process for carcinogenesis bioassay;

and for a number of other purposes.

In 1976, the Task Group on Chemicals in Human Tissues was established under the aegis of the ICGEC to investigate potential activities in the area of human body burden. Members of the Task Group represent a broad spectrum of federal agencies:
One of the first objectives identified by the Task Group was the establishment of a body-burden data base. To that end, the Oak Ridge National Laboratory's Information Center Complex, under contract to the National Library of Medicine, was identified to perform a feasibility study for computerized searching of appropriate on-line data files for a selected list of chemicals. The retrieval of a large number of inappropriate articles as well as the omission of many pertinent ones revealed that available descriptors apparently were not adequate for the efficient automated retrieval of body-burden information from existing computer files.

The consensus of the Task Group was that a manual literature search might yield better results than a computer search and so a second feasibility study for manual searching was undertaken, this time by Tracor-Jitco, Inc., under contract to the Office of Toxic Substances. The successful completion of the twenty-one month (January 1977 through September 1978) search of forty-two selected journals confirmed the Task Group's hypothesis and the manual approach was adopted.

During this same time period, the NCI/EPA Cooperative Program was evolving. The establishment of a body-burden data base was ultimately identified as an activity to be a part of that interagency effort. The level of funding for the first four years of this six-year program is as follows:

FY 79: $181,272 expended
FY 80: $164,946 expended of planned $263,000;
FY 81: $303,000 planned; and
FY 82: $303,000 planned.

This program is being accomplished for us by Oak Ridge National Laboratory through an interagency agreement involving the EPA and the Department of Energy. Oak Ridge is under contract to DOE. The work began in September 1978 at the Oak
Ridge National Laboratory's Information Center Complex Health and Environmental Studies Program.

In the first year and a half of this program, our efforts have focused on:

the identification of a core list of journals for routine manual searching retrospective to 1974;

identification of alternate sources of body-burden information, such as US federal data bases and international contacts;

efficient procedures for the identification of source documents, i.e., pertinent body-burden articles;

data extraction techniques including appropriate keywords;

efficient data storage and retrieval systems;

a format for the tabular display of data in an annual publication; and

preparation of the first annual publication of the data base.

As appropriate data sources are identified through manual searches or otherwise, a wide variety of pertinent information is extracted and entered into the data base. The major fields used for data extraction are:

type of data source (report, journal, data base);

language (other than English);

chemical name, as used in the data source;

CAS preferred name and registry number;

chemical formula;

major use(s);

immediate source or possible source of chemical;

number of cases in the research;

levels measured in biological media, mean and range;

chemical analytical technique employed;

route of exposure;
half-life;
pertinent demographic information;
toxicity and health effects;
pathology and morphology information;
pertinent explanatory comments or caveats; and
keywords.

Data in these fields were entered into the data base only if available in the source document itself.

At this time, we have completed the retrospective search of our core list of approximately 84 journals. Journals continue to be added or deleted as appropriate. So far, body-burden data has been extracted from over 760 of the 2,000-plus documents produced by the manual search. As of May 1st, the data base contained approximately 2,730 records of information on 432 chemicals that have been reported in human tissues and body fluids.

In the published version of the data base, body-burden information is presented in tabular format arranged alphabetically by chemical, using CAS preferred names. Generally, the CAS names are from the 9th Collective Index, and in some cases, names from both the 8th and 9th Collective Indices are given.

Physical and chemical data are generally not given in the source documents but are obtained from a number of standard references and supplied in the published version of the data base for the convenience of the user.

Within an individual chemical, records are arranged in alphabetical order by tissue. A GENERAL INFORMATION column contains a variety of pertinent information such as experimental design, demography, health effects, pathology, morphology, toxicity, source, half-life, and use. Obviously, it is impossible to include all details of the research in the table. In general, supporting information deemed important for understanding the data presented will appear in this column. KEYWORDS also appear in this column.

The first annual publication of the data base is now available. It has been our goal to make this unique reference tool as easy to use as we possibly can and toward that end have regular review and comments from members of the Task Group on Chemicals in Human Tissues have been obtained.
A number of indices allow the user to access the data base in a variety of ways in addition to by chemical name. The table can be searched by author, corporate author, tissue and keywords. Cross-referenced chemical lists provide assistance in finding CAS preferred names from common names, or vice versa, as needed.

Future editions of the data base will contain data collected subsequent to this report. However, the indices will be cumulative and so will refer to the entire series of tables published to date.

Preparation of the first publication was started last October. Emphasis on inputting recent literature and significant research documents has resulted in a chronological mix of articles from 1974 to the present. The 1100-plus page report contains 1,580 data records from approximately 440 source documents which reflects 244 chemicals measured in human biological media.

Just a word about the distribution of this document. Since, to our knowledge, this is the only comprehensive body-burden data collection of its kind, and due to the keen interest that has been expressed in human body burden, we are providing wide distribution of this first annual report. Copies have been sent to:

all US medical schools;

all US schools of public health;

DHEW Committee to Coordinate Environmental and Related Programs;

NCI Interagency Collaborative Group on Environmental Carcinogenesis; and

ICGEC Task Group on Chemicals in Human Tissues.

In addition, a number of government and private investigators, both domestic and foreign, were provided copies. All recipients have been invited to, and I hope will, respond with constructive suggestions that can serve to make future publications of the data base a more useful and valuable resource.

We have been overwhelmed with the unexpected amount of human body-burden data and the mammoth job of retrieval, extraction, input and publication. This is the first such effort, and good start, but there is certainly room for improvement.
We are looking to the future with some ideas or goals in mind. With routine procedures for data acquisition now fairly well established, the data base has been expanded to include pertinent supporting information on food contaminants and on feral populations.

Such supporting information is available from a number of sources including existing data bases at various federal agencies such as the US Department of Agriculture, the Food and Drug Administration, and the Fish and Wildlife Service. The pertinence of such information as indicators of environmental contamination and subsequent potential human body burden is indisputable and the appropriate mechanisms with which to identify, collect, and incorporate supporting data into the human data base are being actively examined.

The investigation of existing on-line data systems for placement of the data base has been underway for several months. In order to achieve maximum accessibility for potential users, it is desirable to place the data base in as many on-line systems as possible. An offer by the National Library of Medicine has been received. It is also anticipated that the data base will ultimately become part of the Chemical Substances Information Network currently under development. It is expected that later this year the data base will have evolved to a point to allow it to be placed on-line at several locations.

A potential problem area is the requests for computerized searches of the data base. If the demand to date for this document, and it has just been released, is any indication of the level of interest in a centralized source of body-burden data, we are going to be swamped with these requests.

The current mechanism for obtaining computerized searches has been to contact the EPA project officer. Requests for computerized searches of the data base have been received from Congressional and state government offices, and federal scientists who usually need the information immediately.

Ideally, the development of appropriate mechanisms to allow computerized scanning of the literature for efficient and cost effective identification of body-burden data will eventually eliminate the need for manual searching. As those in the business of publishing body-burden data (e.g., journal editors and abstractors) become sensitized to the need to facilitate the identification of such information, appropriate techniques will be developed and implemented.

Finally, concern has been expressed about the fact that the data base contains unvalidated data. The intention of this program has never been to provide a validated data base nor to
obviate the need for users to ultimately refer to the original source documents and to exercise good scientific judgment in the use of others' research. The need that was recognized by the Task Group was for a mechanism to readily identify a collection of body-burden data, to easily find out what sort of information exists about a particular chemical or tissue.

A suggestion has been offered that a mechanism be identified for the review and evaluation of data in the database. Preliminary suggestions include the formation of a scientific panel to rank the research data against a number of criteria that may include quality control/quality assurance measures, precision and accuracy of techniques, and statistical design and evaluation of resultant data.

Names and addresses of all persons interested in receiving copies of the report may be submitted to the EPA project officer. Everyone using the document or interested in this program is encouraged to provide constructive criticisms, suggestions for improvements, or any appropriate comments.
ICGEC

Task Group on Chemicals in Human Tissues

Armed Forces Institute of Pathology
Center for Disease Control
Department of Agriculture
Environmental Protection Agency
Food and Drug Administration
National Bureau of Standards
National Cancer Institute
National Center for Toxicological Research
National Institute for Environmental Health Sciences
National Institute for Occupational Safety and Health
National Library of Medicine
BACKGROUND AND FINANCIAL DATA

0  Feasibility study for computerized approach
   Oak Ridge National Laboratory Information Center Complex
   1976

0  Feasibility study for manual approach
   Tracor-Jitco, Inc., 1977-78

0  National Cancer Institute/Environmental Protection Agency
   Interagency Agreement for the Collaborative Program, 1978

0  Environmental Protection Agency/Department of Energy
   Interagency Agreement, September 1978
   Oak Ridge National Laboratory
   Information Center Complex
   Health and Environmental Studies Program

FY 79:   $181,272 expended
FY 80:   $263,000 planned
FY 81:   $303,000 planned
FY 82:   $303,000 planned
ACTIVITIES

0  CORE LIST OF JOURNALS FOR MANUAL SEARCHING

0  ALTERNATE SOURCES OF BODY-BURDEN INFORMATION

0  IDENTIFICATION OF SOURCE DOCUMENTS

0  DATA EXTRACTION

0  DATA STORAGE AND RETRIEVAL SYSTEMS

0  TABULAR DISPLAY OF DATA

0  PUBLICATION OF FIRST ANNUAL REPORT

695
DATA EXTRACTION FIELDS

- TYPE OF DATA SOURCE (REPORT, JOURNAL, LETTER)
- LANGUAGE (OTHER THAN ENGLISH)
- CHEMICAL NAME, AS USED IN THE DATA SOURCE
- CAS PREFERRED NAME AND REGISTRY NUMBER
- CHEMICAL FORMULA
- MAJOR USE(S)
- SOURCE
- NUMBER OF CASES
- LEVELS MEASURED IN BIOLOGICAL MEDIA, MEAN, RANGE
- CHEMICAL ANALYTICAL TECHNIQUE EMPLOYED
- ROUTE OF EXPOSURE
- HALF-LIFE
- PERTINENT DEMOGRAPHIC INFORMATION
- TOXICITY, HEALTH EFFECTS
- PATHOLOGY, MORPHOLOGY INFORMATION
- PERTINENT EXPLANATORY COMMENTS OR CAVEATS
- KEYWORDS
CURRENT STATUS OF DATABASE

- RETROSPECTIVE SEARCH TO 1974 COMPLETE
- 84 JOURNALS
- COLLECTED OVER 2,000 DOCUMENTS
- EXTRACTED DATA FROM OVER 760 DOCUMENTS
- OVER 2,730 DATA RECORDS IN DATABASE
- 432 CHEMICALS REPRESENTED
ARSENIC
7440-38-2
As
AtW 74.9216, MP 817 °C at 28 atm, BP 613 °C (sublimes), VP 1 mm Hg at 380 °C, 10 mm Hg at 440 °C

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<th>Range</th>
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CONCENTRATIONS EXPRESSED AS ELEMENTAL ARSENIC (III).
COPPER SMELTER WORKERS AGES FROM 26-65, MEAN OF 46 YRS.
ARSENIC: METHYL RADICALS:
URINE: COPPER: SMELTERS: OCCUPATIONAL HAZARDS:
PARTICULATES
FIRST ANNUAL REPORT
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References for Physical and Chemical Data

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Cross-Referenced Chemical Lists
Tissues and Body Fluids in Data Base

INDICES:

Author
Corporate Author
Tissue
Keyword
Directory of Chemicals

DATABASE (Table)

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CONTENTS OF FIRST ANNUAL REPORT

- Data input through October 1979
- Data extracted from 440 documents
- 1,580 data records input
- 244 chemicals represented
ANNUAL REPORT DISTRIBUTION

0 US MEDICAL SCHOOLS

0 US SCHOOLS OF PUBLIC HEALTH

0 DHEW COMMITTEE TO COORDINATE ENVIRONMENTAL AND RELATED PROGRAMS

0 NCI INTERAGENCY COLLABORATIVE GROUP ON ENVIRONMENTAL CARCINOGENESIS

0 ICGEC TASK GROUP ON CHEMICALS IN HUMAN TISSUES

0 PRIVATE INVESTIGATORS, DOMESTIC AND FOREIGN
IDEAS AND PLANS

0 Expansion to include pertinent supporting data

0 Placing the data base on-line in 1980

0 Responding to increased requests for computerized searches

0 Development of improved keywords for computerized scanning of the open literature

0 Establishment of an evaluation system for the data base
CINDY STROUP

DESIGN AND DEVELOPMENT BRANCH
SURVEY AND ANALYSIS DIVISION (TS-793)
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
ENVIRONMENTAL PROTECTION AGENCY
401 M STREET SW
WASHINGTON, DC 20460

755-8294
Dr. Weisburger (NCI): On what basis was the decision made to include certain chemicals within the list; for example, I find a whole page of ascorbic acids and citric acids and compounds of that sort which are, one would think, usually occurring in the well-nourished human body. I just wonder on what basis it was decided that a certain chemical should be selected for this list.

Ms. Stroup (EPA): At this stage of the game, we made the decision not to exclude any substance. There is interest in the compounds you mentioned by different factions of the scientific community. For instance, the American Institute on Nutrition has exhibited a lot of interest in the data base, and in particular, in the dietary and nutritional elements that we at EPA are not traditionally interested in.

This is the first go-round, and if we get many requests to exclude certain substances, then perhaps we will. At this point, yours is the first such comment.

Dr. Weisburger (NCI): The original question was on what basis the compounds were selected for inclusion in this data base because there is a whole page of listings on ascorbic acid and citric acid, as mentioned, and some other compounds that are found ordinarily in humans.

My next comment is that there are really thousands of chemicals running around in the human body, and if you include all of these, you know, is that going to be something considered physiological, or is it supposed to be a listing of abnormal chemicals found in the human body?

Ms. Stroup (EPA): The objective of this program is to identify all chemicals that have been measured in human biological media. It is conceivable that the scope may be narrowed in the future, but at this time, there doesn't appear to be the need to do so.

Dr. Simon (EPA): Are there any other questions? Dr. Kraybill?

Dr. Kraybill (NCI): Do you suppose that the development of this data base could lead, ultimately, to some projects -- I do not say that you necessarily support, or anyone will -- on a tissue bank, where actual measurements will be made on autopsy tissue or surgical biopsy tissue? I know this was brought up a couple of years ago. What are your thoughts on it?

Ms. Stroup (EPA): I believe such an effort is ongoing out of George Goldstein's shop at EPA and also involves the National Bureau of Standards. I do not know the present state of that project, but there certainly is a lot of interest in this area.
Mr. Feinstein: Are you also collecting data on what subpopulations the samples came from, like, for example, if it might have been an agricultural worker?

Ms. Stroup (EPA): It usually is. Our data extractors have been instructed to pull out all information deemed pertinent to interpreting the analytic results. This is, of course, dependent on the information provided in the source document.

Mr. Feinstein: Thank you.

Dr. Kraybill (NCI): I am sorry, again. I think you alluded to the other data base, and maybe the rest of you in here are not familiar with it. The Department of Agriculture, by regulation and by law, is required to annually make a surveillance of all the meat animals, and you should be aware that they record data on bovine, sheep, swine, et cetera. This would include pesticides, drugs, environmental chemicals, what have you.

The beauty of this is that these animals are just like people; they are a monitor. Years ago, at the Perrine, Florida lab in the pesticide program down there, they were going out and getting wildlife, and I guess even the armadillos, and recording what the body burden was for these chemicals.

Another historical event in this whole thing was about 5 years ago. They said, well, we are looking at chemicals in air and water, food and diet, and this was brought up in the NCI testing program that we might as well be aware of what was in people; that is, at the cell level, like we all know about DDT and DDE and PCB and PBB's and things of that sort. That is, a direct insult right there at the cellular level.

So these monitors, I think, are very important because it should tie in with the multi-media exposure, and if we can see what is in animals and man, that will be most useful to us.

Dr. Simon (EPA): Okay, one more question.

Mr. Cooper: I was curious as to what the indexing criteria was for a particular chemical. Is it the name which was used by the author or is it the correct name? I noticed that you have benzene hexachloride, and you use as a synonym the 6-chlorinated cyclohexane. They are clearly different chemicals, and yet they are listed as though they were a single chemical.

Ms. Stroup (EPA): I am sorry, I am not sure I heard all of your question. Are you asking how we decided what to name a given chemical?

Dr. Cooper: That is correct.

Ms. Stroup: We use the compound name provided in the source document. Do you find a conflict?
Dr. Cooper (NCI): Well, yes. Our experience in PHS-149 is that very frequently the person who writes the articles uses the wrong name.

Ms. Stroup (EPA): Yes, I know.

Dr. Cooper (NCI): Do you attempt to correct this?

Ms. Stroup (EPA): We do not change what is represented in the source document. We do have a chemist involved in data extraction and she may translate when she feels certain that what the author is calling a given chemical is incorrect. Then she will translate into the CAS preferred name. Otherwise, there are no changes from the source document.

Dr. Kraybill (NCI): I think Dr. Cooper raised a very pertinent question because there are many synonyms -- what is the other word, acronyms, synonyms?

Speaker: Antonyms.

Dr. Kraybill (NCI): Antonyms? No, synonyms. But if you look up a lot of these chemicals, they may have eight or ten. One safe way to do it is to list it by chemical abstract name, right? That would be ideal.

Ms. Stroup (EPA): That is what we do.

Dr. Kraybill (NCI): Well, the other day I looked up a drug, Flagyl, and I could not find it. If I had known that Flagyl was a metronidazole, I would have found it very quickly, so I had to trace back and that is how I came to get it. So you need to sometimes list several names.

If these people that are abstracting the literature could go back and list a couple of other names, that would be helpful. I think that is what you are getting at, aren't you John, having several recordings.

Dr. Simon (EPA): This is going to have to be the last comment because we have a couple of other papers to get to.

Ms. Stroup (EPA): Apparently there is disagreement over the use of the 6-chlorinated cyclohexane as a synonym for benzene hexachloride. If that usage is incorrect, then it is also incorrect in the National Library of Medicine's CHEMLINE which is the source of all synonyms in the data base.

Thank you.
In reporting the vital statistics for 1972, the National Center for Health Statistics (NCHS) included information from a 50% sample of certificates. While this sample is argued to be representative of the total United States, it is not representative of individual states or counties of the U.S. Due to the historic interest of the NCI in investigating cancer mortality at the county-level, I became involved in attempting to convince the NCHS to code the missing 50% for 1972.

The NCHS adopted this coding procedure for 1972 in an attempt to catch up, and, thus, be able to present summary vital statistics data in a more timely manner. Due to the fact that the sample was not stratified by cause of death, age, sex, and race, one has no way of knowing what percent of the total number of events one has for a specific cause of death at the local level.

With support from the Office of the Secretary, DHEW, we at NCI were charged with supporting the coding of the missing 50%. This translates to 983,108 death certificates.

At the present time, 73% of that 50% have all of the demographic data coded. Included in this category are sex, race, age, county of death, state of birth, residence, both state and county, and information with regard to whether or not an autopsy was performed.

For the 983,108 certificates which require the assignment of an underlying cause of death, 38% are now completed. The project will be completed in another year and a half. At that time these data will be made publicly available.
Unidentified Speaker: Has occupation been included in this?

Dr. Mason, NCI: No.

Unidentified Speaker: Why not?

Dr. Mason, NCI: They do not code occupation, and perhaps that is a blessing in disguise. Several states are trying it now with the National Center for Health Statistics' data. North Carolina is one. I believe Michigan and Minnesota are others that are attempting it.

I believe more thought needs to be given to this aspect. We are lobbying for several additional states where we believe it would be desirable to look at it. It has been our experience, in taking the information from the death certificate and attempting to code occupation from it, in densely populated urban places, it is bad to be retired and it is bad to be a housewife, and beyond that you do not learn much.

We are going to see if we cannot do something about that, and I think the way to proceed is through additional education of the persons who put the information on the certificate. Once it is there, it is there, and there is not much you can do about it. I think that it is only going to be through some of these pilot studies and through some of this that we will be able to see it.

Yes we think it is important, but if you just take it the way it is right at the moment, I do not believe you are going to get much from it. In some senses, in some places, in areas with smaller populations, where it is more likely that the individual who signs off the certificate actually knew the individual, you have a better opportunity. I mean, we are fortunate to have, still, a number of places in the United States that are relatively rural, that are one-industry places, where you can indeed get some information from the certificate with regard to occupation and pursue it. In many instances, however, you cannot just simply take it from it. It breaks down; you do not get any good information from it.
INTRODUCTION

The subject of this talk is national mortality databases which are being used for studies in environmental epidemiology. Before these databases are discussed, UPGRADE will be described. UPGRADE is the system with which the mortality databases are analyzed. A brief overview of the data available in the UPGRADE system will be presented with the mortality data being described in more detail. The paper will conclude with a glimpse at the data and some specific questions and some general analyses suggested by the data.
UPGRADE is an analysis system developed for the President's Council on Environmental Quality (CEQ). The UPGRADE system has been under development for five years and has been funded by a number of federal agencies including the National Cancer Institute (NCI) and the Environmental Protection Agency (EPA). An UPGRADE User's Manual became available in January, facilitating increased use of the system.

UPGRADE is interactive and English-language prompted. The analyst sits at a computer terminal and works with the data by responding to straight-forward prompts. Many prompts can be answered by "yes" or "no". Others require the analyst to select an option. If the proper response is not obvious, available choices can be displayed by typing "help". The system is designed to be used directly by the analyst and is essentially self-teaching.

A wide variety of procedures are available in UPGRADE. First are the necessary, but not so glamorous, data manipulation procedures such as filtering, partitioning, listing, sorting, and transforming. Then there are graphics procedures such as bar charting, scatter plotting, polygon plotting and regression plotting. A wide variety of plot options are available. These allow the user to tailor a graph and produce a version suitable for presentation. Another UPGRADE procedure is mapping. Five different types of national and regional maps can be drawn. Finally, UPGRADE contains statistical procedures such as a basic statistics report and regression. Other statistical procedures are available through UPGRADE's interface with SAS, a well-known and sophisticated statistical package developed by SAS Institute, Raleigh, North Carolina. The SAS job is set up interactively in UPGRADE and runs independently from but concurrently with UPGRADE. A SAS job is usually ready in a matter of seconds.

DATA IN THE UPGRADE SYSTEM

Along with the development of the analysis system has been the development of databases. Because UPGRADE was developed for CEQ and because CEQ uses UPGRADE for analyses which go into the CEQ Annual Report on Environmental Quality, a great deal of environmental data has been accumulated for use with the system. There are site-specific, time-series water quality data from EPA's STORET system and USGS's NASQAN database along with air quality data from EPA's SAROAD system.

The data most often used in epidemiological studies are contained in the UPGRADE Integrated Database (IDB). These data are geographically defined, e.g., on the county- or state-level. Other geographic units proposed for inclusion are SMSA's and census tracts. The IDB has been designed so that data from a variety of sources using a common access code, such as the FIPS state-county code, can be put into one dataset and analyzed using UPGRADE. In this way morbidity or mortality data from the National Center for Health Statistics, demographic and socio-economic data from the Bureau of the Census, and environmental data from...
EPA can be put together and analyzed. For example, mortality could be graphed versus population, education, or an environmental factor. Or a regression could be run with the mortality rate as the dependent variable and the other factors as independent variables.

**NATIONAL MORTALITY DATA IN UPGRADE**

These are two types of mortality data available for analysis in UPGRADE. The first consists of mortality rates for grouped causes of death; the other consists of the number of deaths by individual four-digit ICDA codes in a number of age, race, and sex categories.

The mortality rates for grouped causes were tabulated for NCHS by Herbert Sauer of the University of Missouri at Columbia. For each county there are mortality rates for 70 causes of death. Of these, 25 are rates for cancer such as stomach cancer, breast cancer, or respiratory cancer. Eight additional rates are broader categories of cancer such as cancer of the genital organs or non-respiratory cancer. These broader categories are sums of the more specific rates.

The mortality rates are for two time periods. There is one set of rates calculated on deaths occurring from 1968 to 1972. An earlier set of rates is based on deaths occurring from 1959 to 1961. Because deaths for these two time periods were classified according to different versions of the ICDA, comparability ratios calculated by NCHS are available so that these two sets of rates can be compared. A later set of rates for the years 1973 to 1976 may be obtained for the system.

These rates have been calculated separately for four race-sex groups -- white female, white male, black female, and black male. The rates are age-adjusted to both the 1940 and the 1970 United States population. Hence, the set of mortality rates currently in UPGRADE is age-adjusted and race-sex specific. Also on hand, but not yet included in the IDB, are age-specific rates for eleven age groups (under 1, 1-4, 5-14, 15-24, 25-34, 35-44, 45-54, 55-64, 65-74, 75-84, and 85 plus).

The second type of mortality data available for use by UPGRADE has been tabulated from NCHS detail mortality tapes. These data consist of counts of deaths rather than mortality rates. For each county, 1970 population and 1970 deaths by individual four-digit ICDA code are classified according to the following factors:

- **Sex** - female, male
- **Race** - white, black, other
- **Age** - five-year age groups
  (under 1, 1-4, 5-9, 10-14, ... 80-84, 85+)

These mortality data are being expanded and in another year (by June, 1981) should include data for individual years from 1965 to 1976 (with the exception of 1972), two sex groups, nine race classifications, 21 five-year age groups, and two autopsy categories.
EXAMPLE OF ANALYSIS

In order to demonstrate a few UPGRADE capabilities and to illustrate the mortality databases, two analyses will be suggested. The first dealing with cancer of the liver, gall bladder, etc. and cirrhosis of the liver in white males illustrates use of the mortality rates. A look at two causes of death for which the number of deaths are few -- malignant neoplasms of the eye and the thyroid gland -- illustrate the second type of mortality data, the raw death counts.

Using the age-adjusted, race-sex specific mortality rates described previously, the geographic distribution of cancer of the liver, gall bladder, etc. and cirrhosis of the liver in white males will be compared using UPGRADE's mapping capability. Figure 1 shows the distribution of mortality rates for cancer of the liver in white males. Included in this mortality category are all sections under ICDA codes 155 and 156. These include cancer of the gall bladder and bile ducts as well as cancer of the liver. Rates are per 1,000,000 and are calculated on deaths occurring from 1968 to 1972. For this map the range of mortality rates for all U.S. counties was divided into four equal parts. Because the distribution of these rates is skewed with more rates falling on the lower end of the range, there are not an equal number of counties in each interval. More counties fall in the lower shading intervals and fewer fall in the higher intervals. Figure 1 shows that the high rates for this cause are concentrated in the Rockies and plains states.

Figure 2 presents the distribution of white male rates for cirrhosis of the liver. This cause includes all sections of ICDA code 571. Again these are 1968-72 rates per million population. For this map the range of the cirrhosis rates has been divided so that one-third of the counties fall into each shading interval. From the map concentrations of high rates occur in the west coast, the southwest and the northeast. In Figure 3, this same cause is mapped with a different selection of shading intervals. In this figure only ten percent of counties are shaded in the darkest pattern and the concentration of counties with the highest rates is in California.

The maps suggest a number of questions each of which might lead to further analyses. Figure 1 suggests that cancer of the liver should be mapped separately from liver of the gall bladder and bile ducts to see if these causes have the same or different geographic distributions. It might be expected that as the treatment for cirrhosis improves, the mortality from cirrhosis would decrease and the mortality from liver cancer might then increase because cancer would have the latent period to develop. This could be investigated by looking at mortality rates for these causes from different time periods. Perhaps since these two maps show different pictures, there are two different risk factors for these diseases which are geographically distributed. An investigator would want to consider other factors such as demographic and socioeconomic variables and alcohol consumption. This type of analysis is possible using UPGRADE's Integrated Database which allows the user to access variables from a variety of sources.

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Using the second type of mortality data -- the number of deaths per county by individual four-digit ICDA code -- deaths from all cancer causes were tabulated by age, sex, and race, and two rare causes of cancer were selected for investigation. There were 352 deaths due to malignant neoplasms of the eye in the United States in 1970. There were 1067 deaths from malignant neoplasms of the thyroid gland in this year. Sex and race distributions of these deaths are shown in Table 1. Looking at a list of counties in which deaths from these two cancers occurred, the deaths appeared to be concentrated in urban counties. For 20 of the largest SMSA's there existed air quality data in the form of the Pollution Standard Index (PSI) developed by EPA. An approximate annual average was calculated for 1973 for each SMAS. These annual averages were plotted versus the number of deaths in each SMSA. These plots are shown in Figures 4 and 5. In both cases the correlation coefficient between the number of deaths and the PSI was 0.57.

As in the case of the previous maps, these graphs suggest a number of interesting questions. Other urban factors than air quality should be considered. It would be nice to map deaths from rare causes using spot maps rather than shaded maps. In spot maps a symbol is plotted which is proportional in size to the variable being plotted. This capability is being added to the UPGRADE system. As is always the case in descriptive epidemiology, analysis of this type are best suited to generating hypotheses for further research and cannot by themselves provide evidence of increased risk.

CONCLUSION

This purpose of this presentation was not to present scientific studies on cancer, but to illustrate the UPGRADE system and the associated national mortality databases. UPGRADE has been developed as an exploratory analysis tool. The Integrated Database brings together information from a variety of sources. It is hoped that the analyses presented here show how the system can be used to investigate the relationships between environmental factors and health indices.
Table 1. Sex and race distribution of 1970 deaths from cancer of the eye and from cancer of the thyroid gland.

**Cancer of the Eye**

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**Cancer of the Thyroid Gland**

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<td><strong>706</strong></td>
<td><strong>361</strong></td>
<td><strong>1,067</strong></td>
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Figure 1. Geographic distribution of age-adjusted mortality rates for white males from cancer of the liver, gall bladder, and bile ducts (ICDA codes 155 and 156). Rates are per 1,000,000 population and are calculated on deaths occurring 1968 to 1972.

Figure 2. Geographic distribution of age-adjusted mortality rates for white males from cirrhosis of the liver (ICDA code 571). Rates are per 1,000,000 population and are calculated on deaths occurring from 1968 - 1972.

Figure 3. Geographic distribution of cirrhosis of the liver with the ten percent of all counties having the highest rates shaded the darkest.
AGE-ADJUSTED MORTALITY FROM CANCER OF THE LIVER,
(ALL BLADDER AND BOWELS) 1968-1972 PER 1,000,000
POPULATION AT RISK, WHITE MALES, 1968-1972

NATIONAL CENTER FOR HEALTH STATISTICS
U.S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE

COUNCIL ON ENVIRONMENTAL QUALITY

Figure 1
AGE-ADJUSTED MORTALITY FROM CIRRHOSIS OF THE LIVER
ICDA 571 PER 1,000,000 POPULATION AT RISK
WHITE MALES, 1968-1972

NATIONAL CENTER FOR HEALTH STATISTICS
U.S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
COUNCIL ON ENVIRONMENTAL QUALITY
Figure 4. Regression plot of deaths from cancer of the eye (1970) versus the average annual PSI (1973) for 20 large SMSA's.
Figure 5. Regression plot of deaths from cancer of the thyroid gland (1970) versus the average annual PSI for 1973.
Dr. Mason (NCI): I think it would be helpful for those who are not as familiar with the system as several of us are, if you would share with the audience where it is resident, how one goes about getting access to it, what charges are incurred, does one have to be a sponsor, et cetera. If you would do that, then I would like to interact with you about a few of these things.

Dr. Graves: That is a good question. It lets me do a little marketing. The system was developed on NIH computers and has recently been moved to the Boeing Computer Services computer facility in McLean, Virginia. Boeing has a computer network so that access to the system is a local call from most places around the country. The system continues to be available to government agencies who have sponsored its development. In addition, it is now more easily available to government agencies whether or not they are interested in sponsoring the system. Now an agency can just pay for off-the-shelf use. Because of the public access to Boeing, the system will also be available to other government agencies, i.e., to state agencies, educational institutions, consulting firms, et cetera. In summary, there are two ways to access the system. Federal agencies for the most part will access the system through agreements with CEQ. Non-federal access will be through Sigma Data Computing Corporation.

The current cost is the Boeing computer charge plus a percentage for the maintenance and administration of the accounts.

Dr. Mason (NCI): Okay, so that if someone from NIOSH or from EPA or Industrial Health or wherever were interested in getting access to UPGRADE -- and by this I mean really creating a subfile of data, say mortality rates for a particular disease that they are interested in -- they would do the following. They would contact CEQ and say, "These are the things that I am interested in. Can you provide them?" All they would have to do is reimburse CEQ for the creation of that file.

Dr. Graves: Yes. charges for creating a file would include both computer and personnel costs. After the file is created the only charge would be for that person's or agency's computer usage.

Dr. Mason (NCI): Okay, and one last point. I would encourage you, when you give this presentation and show these particular maps, not to make too much out of tiny numbers in any one year, and rather lean on the strength of the system. The system is interactive. The system gives the research-oriented person an opportunity to sit down at a CRT and look at the data to see how rich they are, to look at how many measurements
are there, to look at the range, look at characteristics of the under­
lying distribution, look at factors as they relate to climatic conditions,
and things such as that. And I think the strength of the system is that
an analyst has an opportunity to sit down in a cost-effective manner and
look at, in whatever way is deemed appropriate, these particular data.
The analyst can then branch over to appropriate analysis procedures.

I would encourage you to perhaps make this point a little stronger
rather than stressing a regression equation fitted to tiny numbers in
one year; rather argue that you have the full complement as far as staff
and facilities to do whatever analysis is deemed appropriate.

Dr. Graves: Yes, I agree with you. These slides were put together, as
I said, more to illustrate the system than to illustrate the science.

However there are several points I'd like to make. The rates which were
mapped were not calculated from deaths in a single year but for the
years 1968 to 1972. Because several years are included, the rates are
based on a larger number of deaths.

The second point I want to make is that in looking at these rare causes
of cancer, we are interested in their geographic distribution. These
maps are not the most suitable for this purpose. For this reason a spot
mapping capability is being added to UPGRADE. In spot mapping a symbol
appears wherever a death or other event has occurred rather than shading
an entire geographic area such as a county. That is another tool and it
should be very useful.

Dr. Kraybill (NCI): Looking at that map and not being an epidemiologist,
I am going to ask a simple question. The map of liver cancer rates
shows high rates in the Midwest. What is your explanation, or does any
epidemiologist here have any explanation for that? Is it real?

Dr. Mason (NCI): No, and that is why it looks that way. What has been
done in order to get numbers is fine. Here cancers of liver and gall-
bladder and bile ducts have been combined, when in fact, the determinants
of liver cancer and the determinants of gallbladder cancer are totally
distinct. If you look at liver cancer by itself, you see aggregations
in Texas and other such places. This distribution of cases has prompted
a number of follow-up studies in which we are looking at everything from
the hepatitis B carrier state to the potential for dietary aflatoxin to
the potential for specific classes of compounds which are known to be
carcinogenic to the liver.

If you look at gallbladder cancer by itself, you see a midwestern
concentration of cases. This is more consistent with the dietary
practices of the ethnic groups who have settled there. The ethnic diet
leads to the development of gallstones which is the primary risk factor,
the first and most important risk factor, for gallbladder cancer. So
the maps do not show liver cancer alone. They show liver and gallbladder
combined.
It is interesting, if you pick up on the liver-cirrhosis type of thing, you do indeed see disparate distributions. Some of our earlier work never got into press because we were not convinced that it was not an artifact -- that perhaps what was being called cancer in certain places was really advanced cirrhosis and vice versa. So we have gone into the field to attempt to sort this thing out in a much more analytic way.

Dr. Graves: The fact that people are interested in just liver cancer or just gallbladder cancer and not a combination is one reason we are so enthusiastic about going to the detailed mortality data that I mentioned. With the detailed data, we can break it down, if you want, to the fourth digit ICDA code. But this first set of rates gives people some ideas, and as you have done, they can go on from there.
MAPPING CHEMICAL EXPOSURES

BY

Kenneth D. Kreitel
The Hazard Section of the Surveillance Branch is actively pursuing several new techniques for focusing on potential worker exposures to chemicals in use in industry. This paper reports on a technique to display the geographic concentration of potential worker exposures through the linking of large computer files and data bases.

The computer files from NIOSH's National Occupational Hazard Survey are searched for specific instances where a chemical material of interest was identified during the site visit phase of the survey. Those companies in which the material was observed are classified by their four-digit Standard Industrial Classification (SIC) code. The names and addresses of similar companies are then extracted from the Dun & Bradstreet computer files. The resultant computer file is then analyzed statistically and a cartography system at The National Oceanic and Atmospheric Administration (NOAA) produces a map of the continental United States with each county shaded appropriately.

For the purpose of providing immediate visual impact, areas of the United States which contain large numbers of companies similar to those which were observed using the materials in question are shaded very dark. Areas less likely to contain significant quantities of such chemicals are shaded in lighter half-tones.
The system is capable of producing maps for each of the 8,000 potentially hazardous materials encountered during the National Occupational Hazard Survey, either individually, or in combination.
I. INTRODUCTION

The purpose of this paper is to give a preliminary report on a new technique that the Hazard Section of NIOSH is actively pursuing.

The Hazard Surveillance Section of NIOSH is charged with the responsibility to develop, compile, and analyze information on the number and distribution of workers exposed to potential occupational hazards to enhance the preventive aspects of occupational health.

It is natural, therefore, for the Hazard Section to be interested about the geographical dispersion of various industrial materials throughout the United States. Our interest in this stems from the often expressed need for greater awareness of potential occupational exposures to hazardous materials.

This awareness is very difficult to achieve because of two built-in confounding factors. Chief among these factors is the practice of tradenaming products. That factor only slightly overshadows the other; which is inadequate labelling requirements. Taken together, these two constitute a rather large impediment to the kind of awareness we feel it is necessary to build.

The Hazard Section does, however, have access to a unique resource which is useful in penetrating the mystique surrounding

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the question of who is potentially exposed to what in the workplace. This report is an attempt to briefly describe that unique resource, and to introduce an intriguing new use of its data.

II. GOAL

The goal of the mapping project is to develop maps of the continental United States showing suspected locations and concentrations (if possible) of potentially hazardous exposure agents. Furthermore, insofar as practical, we would like to compare these maps and the underlying data with other data sources such as NCI's "Cancer Mortality by County: 1950-1959" (1) and their "Atlas of Cancer Mortality for U. S. Counties: 1950-1969." (2)

III. RESOURCES

The principal resource used for the mapping project was NIOSH's National Occupational Hazard Survey Data Base. The National Occupational Hazard Survey (NOHS) was conducted during the period 1972 through 1974. It was a nationwide effort to gather information on potential workplace exposures to hazardous material through the use of 20 field surveyors who actually visited over 4,500 different plant sites throughout the United States.
The surveyor's job, to simplify it greatly, was to first interview the plant management about current practices within the plant, and then to conduct a detailed walk-through survey of the plant, noting occupational exposures to potential chemical, physical, and biological hazards. The surveyors also noted the conditions under which the exposures were occurring, and the control measures that were being applied.

The plants that were surveyed represented a national probability sample of selected industries. The result of that effort is a computerized data base which contains almost five million records, and which is useful for describing potential occupational exposures by industry, by occupation, and by exposure agent. (3)

NIOSH's experience in compiling this data base indicated that most of the workers' exposures were to products that were trade-named as opposed to being in pure chemical form with the chemical adequately labelled. Some 70% of all exposures noted during the survey were, in fact, to tradenamed products. NIOSH then began a program of follow-up by writing to the manufacturers of the tradenamed products to obtain the ingredients and the formulation of the product. This auxiliary effort, dubbed "TNIC" or Trade Name Ingredient Clarification yielded information which has proven to be invaluable for the mapping project. Now integrated into the main NOHS data base, the TNIC data provides
very valuable insights into the potential for occupational exposures
to hazardous materials that were formerly obscured or disguised
due to the twin problems of tradenaming and inadequate labelling.
Of the approximately 80,000 different tradenamed products encountered
in the course of the NOHS survey, about 64,000 or 80% have been
resolved into components through the cooperation of the manufacturers.

The secondary resource that the mapping project draws upon is the
Dun & Bradstreet file. This computerized file contains information
on 4.3 million companies throughout the United States. Each
company record includes the company name and address, its size
in terms of the number of people employed there, and its Standard
Industrial Classification (SIC) code.

IV. METHODOLOGY

As a first step, a single important industrial material suspected
of being in widespread usage throughout the United States was
chosen as an appropriate vehicle for developing the methodology.
This material was chosen because it was suspected of being
incorporated into a wide range of products which enjoyed a
wide variety of uses within industry.

This material, asbestos, was used as the basis for a computerized
search of the entire NOHS data base. The result was a compilation
of all the plants in which NOHS surveyors had noted at least one
worker potentially exposed to the material by virtue of his or
her job, during the period 1972 through 1974. Any worker who
indicated he or she used this material (or a tradenamed product which, upon resolution was found to contain this material) for periods totaling more than one-half hour per week in the aggregate served to nominate an entire industry for further consideration.

The list of industries nominated through this process by the NOHS data base was lengthy. It included one-hundred and forty-seven distinctly different industries, as delineated by the four-digit Standard Industrial Classification (SIC) code.

The length of the list was due, in part, to the ability of the integrated NOHS and tradenames data bases to penetrate the tradename barriers and detect obscure or unrecognizable exposures to the material. This is expected to become a regular occurrence in future attempts at mapping.

The list of nominated industries was then analyzed a number of ways, to determine the extent to which the NOHS study accurately reflected each of the industries in question. A set of decision rules was then developed which was capable of separating the list into two smaller lists of industries; one qualified for further consideration, and one unqualified. The decision rules were carefully applied to each of the industries on the candidate list.
The first rule specified that NIOSH surveyors must have observed the material in question at least twice in an industry during 1972-1974. The second rule specified that in addition to Rule #1, the NIOSH surveyors must have noted exposures to the material in question in at least 25% of the plants of that industry type that were visited. The two tests were designed to eliminate from further consideration those industries in which the NOHS data was too limited to provide a good case for continuing.

The fully qualified list of industries (see Table 1) then formed the basis for extracting the records of similar business establishments throughout the United States from the Dun & Bradstreet file. The entire Dun & Bradstreet file was searched for companies whose industry codes matched the twenty-five (25) on the "fully qualified industries" list. Records of approximately 60,000 business establishments were extracted using the matching procedure.

These records were then organized by county and analyzed with the aid of a widely-available computerized statistical analysis system. Results were tabulated and displayed on a county-by-county basis as a means of providing the researcher with some preliminary insight prior to readying the data for the cartography system.

The cartography system was county-based. It contained X and Y coordinates of all the county lines, and required only the proper county code and a code to indicate the desired shade of darkness for each of the counties. The proper country identification code was not
the one extracted from the Dun & Bradstreet file, however. Therefore, a code conversion table was built and software developed which was capable of automatically converting the Dun & Bradstreet code to the preferred code.

The shade code was assigned to each county on the basis of the number of qualified industrial facilities within the county. To provide clarity and to achieve the greatest visual impact, the counties with the highest number of qualified facilities were shaded the darkest. Nine different shading protocols were investigated as a means of becoming familiar with the variation in subsequent outcomes that the different protocols afforded. As the maps show, it is possible to prepare shading protocol that becomes more selective until only the very, very high interest counties remain shaded on the map.

V. RESULTS

The maps appear to corroborate the conventional wisdom that asbestos and asbestos-containing tradenamed products conceivably were used in industrial settings across most of the face of America.

The industries that were rated "fully qualified" tend to be found in conjunction with large population centers. There are, however, some notable exceptions. Fargo, North Dakota, and Sioux Falls, South Dakota, for example, with populations less than 100,000 persons, cannot be considered major population centers, yet each contains several "fully qualified" industries. Two large population centers in particular, Cook County, Illinois, and Los Angeles
County, California, contain very large numbers of businesses that fit the "fully qualified" description.

These maps are not "rate" maps. That is, they are independent of population considerations and thus they serve only to locate geographical areas with large numbers of fully qualified industries. They do not attempt to depict nor to predict high incidence rates of asbestos-related illnesses.

VI. LIMITATIONS

There are two principal limitations inherent in this technique which must be understood for correct interpretation of the results. First, the NOHS survey was not designed to be statistically representative of industries at the four-digit SIC code level. Some industries represented by four-digit codes, in fact were not visited during the survey. Those industries are not represented anywhere in the data. In addition, more four-digit industries in general were not sampled with enough frequency to assume that the sample that was drawn was representative. The decision rules detailed in the methodology section above formed the sole basis for qualifying industries to the list of highly interesting industries.

The second principal limitation upon the interpretation of the results is more mechanical than statistical. The NOHS data was described in terms of 1967 SIC codes. The Dun & Bradstreet
file uses the 1972 version of the same publication. (4) There were some industry classification changes between the two versions of the publication. The changes were minor in nature. The major problem involved in the methodology above is the great "leap of faith" that was made in assuming that an industry as delineated by a four-digit SIC code in 1972 is essentially the same as the industry typified by the same SIC code in 1979. No attempt was made to account for possible changes in technology, methods of production, changes in regulations, or geographic shifts in industry in the years between 1972 and 1979.

The maps simply represent the distribution of industries that qualified as being of "high interest" through the methodology above at a single point in time.

Asbestos was chosen only as a first attempt to map chemical exposures. The Hazard Section is continuing to develop this technique, and will map other industrial materials in response to the Institute's Surveillance needs.
TABLE 1

STANDARD INDUSTRIAL CLASSIFICATION CODES OF INDUSTRIES CONSIDERED "FULLY QUALIFIED"

<table>
<thead>
<tr>
<th>1967 SIC CODE</th>
<th>INDUSTRY DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1742</td>
<td>Plastering, Drywall, and Insulation</td>
</tr>
<tr>
<td>1752</td>
<td>Floor Laying &amp; Floor Work N.E.C.</td>
</tr>
<tr>
<td>1761</td>
<td>Roofing and Sheetmetal Work</td>
</tr>
<tr>
<td>2011</td>
<td>Meat Packing Plants</td>
</tr>
<tr>
<td>2821</td>
<td>Plastics Materials and Resins</td>
</tr>
<tr>
<td>2851</td>
<td>Paints and Allied Products</td>
</tr>
<tr>
<td>2911</td>
<td>Petroleum Refining</td>
</tr>
<tr>
<td>2952</td>
<td>Asphalt Felts and Coatings</td>
</tr>
<tr>
<td>3241</td>
<td>Cement, Hydraulic</td>
</tr>
<tr>
<td>3291</td>
<td>Abrasive Products</td>
</tr>
<tr>
<td>3292</td>
<td>Asbestos Products</td>
</tr>
<tr>
<td>3312</td>
<td>Blast Furnaces and Steel Mills</td>
</tr>
<tr>
<td>3352</td>
<td>Aluminum Rolling &amp; Drawing</td>
</tr>
<tr>
<td>3433</td>
<td>Heating Equipment, Except Electric</td>
</tr>
<tr>
<td>3443</td>
<td>Fabricated Platework (Boiler Shops)</td>
</tr>
<tr>
<td>3519</td>
<td>Internal Combustion Engines, N.E.C.</td>
</tr>
<tr>
<td>3661</td>
<td>Telephone and Telegraph Apparatus</td>
</tr>
<tr>
<td>3711</td>
<td>Motor Vehicles and Car Bodies</td>
</tr>
<tr>
<td>3713</td>
<td>Truck and Bus Bodies</td>
</tr>
<tr>
<td>3721</td>
<td>Aircraft</td>
</tr>
<tr>
<td>3731</td>
<td>Ship Building and Repairing</td>
</tr>
<tr>
<td>3742</td>
<td>Railroad Equipment</td>
</tr>
<tr>
<td>3791</td>
<td>Trailer Coaches</td>
</tr>
<tr>
<td>3843</td>
<td>Dental Equipment and Supplies</td>
</tr>
<tr>
<td>3996</td>
<td>Hard Surface Floor Coverings</td>
</tr>
</tbody>
</table>
BIBLIOGRAPHY


Dr. Jenkins (EPA): How are you planning to utilize the data you are now collecting from both the labor unions and industry on specific chemicals and specific plants, and how is this going to interface in the future with updating this mapping system?

Mr. Kreitel (NIOSH): It is actually a little too soon to tell that. The information that we are collecting from the unions that relates specific chemicals to specific plants is not coming in on anything that resembles a nationwide basis at all, so it really would have to have much more complete coverage before we could do anything like this with it, so it probably will not interface for a large number of years.

Dr. Bellin (EPA): I am just curious, as an ancillary question, what is your experience with trade name products? How often does their composition change? Can we assume that something that had a certain composition in 1972 has the same composition now?

Mr. Kreitel (NIOSH): As a matter of fact, that is probably a very dangerous assumption to make. We believe that there is a product life cycle out there and that products in certain industries turn over much faster than products in other industries and other uses.

There are a few more presentations that will be made today on our plans to update that data base and on a brief description of that data base itself.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Thursday Morning, May 8

CONCURRENT SESSION II
DATA BASES/MONITORING (CONTINUED)

SESSION CHAIRPERSON

Dr. Elizabeth Weisburger
National Cancer Institute
In order to make intelligent decisions concerning priorities for action, NIOSH requires a current and continuing supply of information on nationwide patterns of occupational exposure to health hazards. The National Occupational Hazard Survey of 1972-1974 (NOHS I) which developed such information among a probability sample of nearly 5,000 plants, has had important applications in a wide range of NIOSH research activities. It will become increasingly necessary to update the results of that survey in order to reflect current conditions.

The foundation of experience which was developed during NOHS I will be used to support and guide the planning and implementation of a second national survey. Although methodologies used in the new survey will closely resemble those of NOHS I, the previous survey forms, data processing systems, programs, and procedures will be critically examined to identify areas where improvements can be made.

A sample of approximately 5,000 facilities will be selected to cover a wide range of industry types, facility sizes and geographical locations, and will be constructed so as to permit the development of national exposure estimates. This sample will be selected in the fourth quarter of fiscal year 1980. Twenty-one industrial hygienists will be recruited and trained intensively in observational survey techniques during the fourth quarter. The surveyors will then be subdivided into teams and deployed into the selected facilities beginning in fiscal year 1981 in order to gather data on the nature and extent of potential occupational exposures to chemical and physical agents, including trade name products. The field phase of the survey will last approximately two years, and preliminary data should be available during the year immediately following the survey.
JUSTIFICATION AND BACKGROUND

An intelligent and informed sense of priorities for action requires a current and continuing supply of information on nationwide patterns of occupational exposures to health hazards. It is important to know what hazards are associated with certain industries and occupations, and to have some idea of the relative importance of those hazards, both in terms of total number of workers at risk, and the toxic properties of the substances. An effective program of hazard surveillance requires a diverse array of data collection activities. It must identify and seek out networks which supply intelligence on occupational hazards, and must be sensitive enough to detect early indications of potentially hazardous situations in time for effective intervention on behalf of those affected. Hazard surveillance aims to provide the information necessary to prevent occupational disease, so that the in vivo experiment need not be carried to its obvious conclusion. It is a goal which challenges us for a number of reasons. Chief among these is the fact that new chemicals are introduced into the workplace in numbers which largely overwhelm the capacity of toxicological testing facilities. A second factor involves the difficulty in extrapolating animal data to humans. Another confounding factor is the extensive practice of masking chemicals with trade name designations, thus frustrating attempts to even identify the chemicals to which workers are exposed.

Recognizing these factors, NIOSH has constituted a program of hazard surveillance which: (1) attempts to monitor a broad range of current information on chemical toxicology, (2) conducts periodic special surveys to develop intelligence on the distribution patterns of health hazards at the workplace, and (3) persistently seeks to gather information on the chemical ingredients of industrial trade name products.
NIOSH's first nationwide special survey of occupational hazards was conducted from 1972-1974 in a probability sample of nearly 5,000 facilities. A team of twenty surveyors conducted wall-to-wall observational surveys in a broad sample of workplaces, inventorying chemical and physical agents, including trade name products. A total of more than 9,000 different potential hazards were discovered, and more than 85,000 trade name products were listed. After an extensive and time-consuming period during which over 10,500 manufacturers were contacted and asked to supply ingredient information on their products, the data base was available for producing estimates of the number of people exposed to selected hazards.

The foundation of experience which was developed during the original National Occupational Hazard Survey is being used to support and guide the planning and implementation of a second national survey.

OBJECTIVES

The broad objectives of NOHS II can be simply stated:

(a) To develop a data base capable of producing reliable estimates of the total number of workers in the target population exposed to the health hazards which are observed during the survey.

(b) To develop data which is capable of reliably describing each industry type comprising the target population in terms of the nature and extent of exposures to health hazards, and the degree to which facilities have implemented programs to reduce occupational health problems.

(c) To compile the data in such a way that analyses of industrial hazard exposure trends can be made by comparison with NOHS I information.

There are a number of important parallel activities which must be conducted to initiating the field phase of the survey. The ultimate utility of the data and the speed with which it can be made available for dissemination and use depend on the successful timing and execution of these tasks.

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FORMS DESIGN

One task which is a necessary prerequisite to many others is the drafting of forms for data collection and guidelines to enable the surveyors to use the forms. NOHS I used a 3-part form, and the same basic structure will be employed in the second survey. Part I of the form is filled out during an initial interview with plant management. It consists of a series of approximately 50 questions which elicit information about the health and safety program at the facility, and the degree to which the program has been effective in reducing illnesses and injuries. We have encouraged widespread input of candidate questions for inclusion in Part I. Part 2 of the form is filled out during the walk-through survey, and captures data on the types of workers (sex and occupational title) observed, and their exposures. Additional data on the duration of the exposures, the physical form of the substance, and the control measures being used are also collected at this time. Part 3 contains information on the amount of surveyor time spent travelling to a plant, walking through the facility, and writing up the results.

SAMPLE FRAME

A second major task which must be completed prior to initiating the field phase of the survey is the construction of a sample frame or comprehensive listing of facilities from which a probability sample can be drawn. Since there are more than 4,000,000 facilities in the United States, a certain portion of which enter or leave the scene each year, the assembly of a current and accurate frame requires some effort. A NOHS II sample frame will be built from existing data sources, and modified where necessary. The construction industry, which includes general contractors and special trade contractors, will create special problems due to the fact that the majority of exposures probably occur at the job site rather than at the location of the business headquarters. Separate listings of construction projects in progress will likely be required to adequately describe this important activity.
The target population for NOHS II will be slightly different than that chosen for NOHS I. Extremely small facilities (7 employees or less) will still be excluded from the frame, but an attempt will be made to achieve a larger, more complete sampling of the manufacturing industries. This will mean that fewer facilities in industries like wholesale and retail trade, financial institutions, and educational services will be surveyed.

After the target population has been described, a sample strategy will be developed which maximizes the use of the surveyor resources and meets the objectives of the survey. It is anticipated that some form of stratification by Standard Industrial Classification (SIC) and size of facility will be used, and that geographical clustering will be required. Part of the sampling strategy will involve developing exigency procedures to deal with situations where a facility is unsurveyable. While NIOSH has been granted statutory authority to enter workplaces during the conduct of its research activities, there may be occasions where this authority will be challenged. In such cases a decision will be made on whether to undertake the legal action required to effect entry, or to draw another facility as a replacement.

The final stage in design of a sampling strategy is to produce a list of facilities which constitutes the sample. It will probably be necessary to validate the list of facilities by verifying the critical data through phone contact with management personnel at each candidate facility.

A specific task which is closely related to the development of a sampling strategy is the design of analytical methods which will accept the survey data as input and produce national estimates of numbers of workers exposed to selected hazards. This projection algorithm must be computationally efficient and capable of identifying those estimates for which measures of variance can be computed.

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RECRUITING AND TRAINING SURVEYORS

It will be extremely important to select and train the surveyors properly. All surveyors will be required to have a bachelor's degree in a scientific discipline such as chemistry, chemical engineering, or health sciences. A minimum level of training in organic chemistry will be necessary. Six to eight weeks of classroom training in general principles of industrial hygiene with special emphasis on recognition of hazards is planned. A two week module tailored to the specific goals and methods of the survey will follow, after which a period of two to three weeks will be spent in in-plant instruction in the use of the forms.

CLARIFYING TRADE NAME INGREDIENTS

The entire process of acquiring ingredient information on trade name products will be critically examined to identify areas where improvements can be made. It will be important to check the product names from NOHS II against those identified in NOHS I to avoid requesting the same information from a manufacturer a second time. It may also be possible to access other sources of ingredient information which provides a satisfactory level of detail. The strategy of offering the option of comprehensive reporting on an entire product line to manufacturers is being considered as one means of minimizing the need for repeated contact. Current procedures for safeguarding confidential data will be evaluated for possible improvements.

DATA PROCESSING

It is hoped that the extensive collection of systems and software developed during and following NOHS I can be used as the basis for the development of the data automating system which will be required for NOHS II. If the survey data is automated and edited as soon as it is collected, it will be possible to spot errors in time to communicate this information to the surveyors for correction while they are still in the field, and before it becomes a widespread problem.
FIELD PHASE

The operational aspects of the field phase of the survey will be modified somewhat to improve the quality and quantity of data collected. The survey force will be divided into several smaller teams, and a team leader for each group will be identified. It will be the responsibility of this individual to coordinate the activities of the group, schedule and assign facility surveys within a region, desk check and organize the data forms for submission to headquarters, and serve as a conduit for information between the surveyors and headquarters staff. The surveyors will spend more time disseminating general information at the plant site, including information about NIOSH services such as the Health Hazard Evaluation program. They will also be on the alert for any special problems that arise, including previously undiscovered health problems associated with new chemicals or new uses of common chemicals. They will thus function as a continuing source of current intelligence on emerging problems. All such information will be relayed to headquarters for appropriate action.

DISSEMINATION PLANS

The ultimate worth of such a large-scale survey effort is judged on the basis of how the data is disseminated and used. Many of the changes in survey design from NOHS I to NOHS II are designed to reduce the interval between termination of the field phase of the survey and availability of survey data. Rather than publishing a single volume of summary statistics, a series of interim, special topic reports is contemplated. These special topic reports could take the form of profiles of selected industries or occupations, or could be designed to deal with classes of chemicals. Efforts will be devoted to loading the data under a data base management system to facilitate specialized retrievals soon after automation is completed.

In a survey of this magnitude and complexity there will always be unanticipated developments which necessitate modifications to the original plan. Realization of this fact increases the ability of the survey managers to accommodate the unexpected. NOHS I provided many essential lessons which will not be lost on the architects of NOHS II. It is with considerable enthusiastic anticipation that we look forward to this challenge.
Dr. Green (NIOSH): I have two questions. You mentioned that when you get some trade name information, you will go back and check and see if it overlaps with requests to manufacturers from NOHS I, and there was a point brought up that the ingredients may have changed in the interim, so perhaps you might consider that, and a second contact might be worthwhile.

Mr. Sundin (NIOSH): Yes. I should clarify my statements here. We are undertaking at the current moment a research effort which analyzes our existing trade name file. One component of that research effort is to gain some sense of the rate of change in production formula.

A similar study was conducted on CPSC's file of trade name ingredients, and we are looking with some interest at the results of that study.

In any case, I think it will be important not to simply go out and re-request information on a product for which we have even antiquated data. Rather, an intelligent strategy would seem to be to at least give the manufacturer the opportunity to indicate whether the product formula as we have it has changed or is the same. I think that the people that are looking at respondent burden would want us to operate in such a fashion. We are sensitive to the fact that we cannot just use old information that we have on file, but we will at least establish whether or not we have any information on that particular product and then attempt to validate with the manufacturer whether it is current information.

Dr. Green (NIOSH): The other thing I wanted to bring up, in the first survey there was a certain percentage of the exposed population whose estimate was based on what was called the generic exposure. Can you discuss what that means and what are the criteria for selecting people as falling into that group?

Mr. Sundin (NIOSH): A "generic procedure" was used to derive data on exposures for those people that were exposed to trade name products for which we did not have ingredient information from the manufacturers.

The whole process of clarifying trade name products has been an extremely difficult one for a number of different reasons, which is why we are directing a lot of our attention during the current planning stages to improve that effort.

Any kind of response you could possibly imagine occurring, did in fact occur at some time during the course of contacting these 10,000 manufacturers. Our file of ingredient information grew rather slowly, and the pressures to examine survey data began almost immediately after the field phase was completed, as you might expect.
Everytime a trade name product was noted in the field, an accompanying record was input by the surveyor concerning what that product was doing, what type of product it was. Was it a surfactant, a cleaning compound, and so forth? Under contract, we then researched the contemporary chemical literature and assembled a list of probable ingredients for product formulas that we have not yet received from manufacturers. At such time as we receive actual ingredient information from manufacturers, we then replace the generic resolution with the actual data.

With a view toward NOHS II and some of the problems that we have noticed with using generic resolution, we are attempting to compare the set of ingredient information which we actually got in specific product classes from manufacturers with that generic list and see how good the generic list actually was and modify it based on actual data received. We realize we will still be faced with that kind of a situation, since to get trade name ingredient information requires some time, but we hope our generic resolution process will be a little more accurate the second time.

Dr. Weisburger (NCI): There have been some comments made that some times the NOHS overestimates the number of workers that are exposed to a particular chemical. Could you comment on that, please.

Mr. Sundin (NIOSH): In this business, nobody is ever satisfied with your estimates. Either they are too high or they are too low. But in most instances where we have researched what seemed to us to be a serious challenge to the estimates, what we find is that there are so many non-standard, secondary, widely dispersed uses of certain products which the manufacturers or the processors or the distributors of the chemical are totally unaware of, that we indeed find that the NOHS estimates derived, as they are, from a plant level sort of survey procedure, tend to represent potential exposures fairly well.

Realizing that we are not talking about exposures that were defined by taking environmental measurements, but rather about a compound that is in the workplace of a worker, being used at something more than 30 minutes per week, the other process that we sometimes use to answer these sorts of questions is to look at only full time; i.e., more than 4 hours a day, or part time, and an amazing number of exposures occur in very short bursts. The numbers drop drastically when you talk about only full time exposures, and that tends to be closer to the classic definition of exposure in many people's minds. We realize however that even part time exposures for certain chemicals are important to consider.

So in most cases when we examine apparent discrepancies, it turns out that there is some validity to our estimates.
Dr. Bellin (EPA): Down to what level do manufacturers identify their products, and is there any attempt to gain information on hazardous contaminants that we have some idea might exist?

Mr. Sundin (NIOSH): That is something we will probably be examining closely, with an eye to potentially modifying during the second survey. We asked the manufacturers to report anything that was there at greater than one percent by weight or volume, except in the cases of carcinogens which should be reported regardless of the percentage -- but the definition of carcinogen was not presented to manufacturers, and I am sure they interpreted it fairly liberally.

We have seen cases where a pesticide, for example, will come back as 100 percent petroleum distillate; the active ingredient being in the product at less than one percent, and therefore the manufacturer is choosing to ignore it. We are therefore contemplating a change in the wording of our request form to include a request for total reporting of any active ingredients and impurities where the manufacturers have such knowledge.

We permitted also a plus or minus 5 percent margin on the percentage of composition reporting. That does not affect the way in which we use the data back in NOHS. I think it is probably an adequate level of reporting and gives us fewer problems with certain manufacturers if they are permitted to use a plus or minus 5 percent tolerance on individual percentages of the components.
"Extent of Industrial Exposure to Epichlorohydrin, Vinyl Fluoride, Vinyl Bromide and Ethylene Dibromide"

Talk Presented at
First NCI/EPA/NIOSH Collaborative Workshop
Rockville, Maryland
May 8, 1980

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Cincinnati, Ohio

750
ABSTRACT

Industrial hygiene studies were conducted in several industries to evaluate present levels of worker exposure to hazardous chemicals and to determine the feasibility of worker mortality studies.

The greatest exposure to epichlorohydrin in the chemical and resin manufacturing processes occurs with chemical operators. In the chemical manufacturing processes, operator exposures were found to be less than 2.1 ppm for 20 TWA personal samples and in the resin manufacturing processes, operator exposures were found to be less than 0.8 ppm for 39 TWA personal samples.

Chemical operator exposure levels for vinyl fluoride in the manufacture and polymer processes were generally found to be less than 5 ppm for 18 TWA personal and general area samples. One sample was found to be 21 ppm during the initial start-up shift. Operator exposure to vinyl bromide in the manufacturing process was found to be less than 0.4 ppm for 4 TWA personal samples. Tank car loading crewmen were found to have the greatest exposure, and 1.2 and 6.3 ppm VBr, respectively for two TWA personal samples taken.

Ethylene dibromide median exposures by similar job types in the manufacturing processes ranged 10 to 500 ppb for 35 TWA personal samples; while, median exposures by similar job types in antiknock blending operations ranged 0.2 - 54 ppb for 39 TWA personal samples. EDB ceiling levels for quality control sampling and for loading or unloading tank cars ranged 0.04 to 23 ppm for 7 samples and 0.09 to 2.4 ppm for 4 samples, respectively.

The reported levels are generally within permissible exposure limits. Many of the processes are closed chemical processing which minimize worker exposure. However, continued attention needs to be given to the open system processes and equipment maintenance to prevent worker exposure.
I. INTRODUCTION

The purpose of this paper is to present and summarize the pertinent findings of industrial hygiene studies of epichlorohydrin, vinyl halides (vinyl fluoride and vinyl bromide) and ethylene dibromide. Study of these substance agents have been conducted by NIOSH under contract with Tracor Jitco, Inc. and SRI, International. More complete information concerning the findings of these studies is contained in the published NIOSH technical reports: (1) Epichlorohydrin Manufacture and Use - Industrial Hygiene Survey, (2) Vinyl Fluoride and Vinyl Bromide Industrial Hygiene Survey Report, and (3) An Industrywide Industrial Hygiene Study of Ethylene Dibromide.

II. STUDY PROCEDURE

These chemical agents were selected by NIOSH for industrial hygiene study because they are suspect cancer agents and limited information exists on exposure levels in industry.

Walk-through surveys were conducted for each facility to obtain preliminary information about the process, production activity, potential exposures and worker exposure. Based on the walk-through survey, in-depth survey requirements are defined for each plant. Essentially, the in-depth survey procedures are directed toward determining 8-hour TWA personal exposure and characterizing work practices and controls. Sampling is conducted for all shifts (usually three) and potentially exposed workers. Job descriptions are characterized for potentially exposed workers. Appropriate survey sampling and analytical methods are determined. The NIOSH recommended sampling and analytical methods formed the basis for methodologies employed in the study. Recommended vinyl fluoride sampling methods were not available at the time of the study. Personal sampling for vinyl fluoride was conducted using a 7.7 liter Teflon bag attached to the sampling pump operating at flow rates of 14 to 100 cc/min. The samples were analyzed by gas chromatography within two days after sampling. Laboratory decay tests indica-
ted 10% loss in four days and 50% loss in two weeks for the Teflon bag used.

All other samples were collected on the standard 150 mg charcoal tube and analyzed by gas chromatographic techniques.

III. Epichlorohydrin

Studies were conducted at two epichlorohydrin manufacturing and user facilities and at three additional resin manufacturing facilities in 1976.

A. HEALTH CONCERN

The current Federal Standard for epichlorohydrin is 5 ppm (19 mg/m³) as an 8-hour time-weighted average permissible exposure level. This is based on the known acute (short term) health effects to humans from over exposure, i.e., respiratory tract irritation and systemic poisoning. Skin contact can result in dermatosis and systemic effects. Exposures occur principally by inhalation and direct skin contact and to a less extent by ingestion. After a comprehensive literature review, NIOSH concluded that exposure risks may include carcinogenesis, mutagenesis, and sterility in humans and recommended a time-weighted average occupational exposure limit of 2 mg/m³ of air (0.5 ppm) and a ceiling limit of 19 mg/m³ (5 ppm) as reported in the Criteria Document (Sept. 1976). More recent information on human exposure data has prompted NIOSH to issue a Current Intelligence Bulletin recommending exposures be reduced to the extent feasible. (Oct. 1978) The 1978 TLV for epichlorohydrin has been revised downward to 2 ppm for the 8-hour TWA and a short term exposure level of 5 ppm.

B. SURVEY RESULTS

1. Epichlorohydrin Manufacturing Process
Manufacturing plants for epichlorohydrin are of conventional open structure chemical process design. Both facilities manufacture epichlorohydrin for shipment and for use in their own manufacture of glycerine and epoxy resins.

Epichlorohydrin is made by the chlorination of allyl chloride yielding a mixture of dichlorohydrin. These products are washed with a cold dilute alkali solution to remove hydrochloric acid and yield impure epichlorohydrin. The epichlorohydrin is then further refined by distillation processes.

Survey Data

Plant A

The results of 8-hour TWA personal sampling at the first facility ranged from not detected (less than 0.05 ppm) to 0.4 ppm for 8 chemical operator samples (median of 0.3 ppm). Lower exposures were re-reported for the shift foreman, drumming operator and maintenance personnel. An exposure level of 0.3 ppm was reported for the tank car loader.

Plant B

Similar survey data was collected at the second manufacturing facility. Personal samples ranged from not detected (less than 0.05 ppm) to 2.1 ppm for 12 chemical operator samples (median of 0.1 ppm). However, two of these samples were considered significant levels, i.e., 1.9 ppm and 2.1 ppm. Lower exposures were reported for the shift and maintenance foreman. An exposure of 0.3 ppm was reported for the tank car loader. No drumming operations were performed during the survey of Plant B.

Controls

The production of epichlorohydrin operations are located out of doors.
with automated operations monitored from a control room. For normal operation, operators are seldom in the process areas. They are in the production areas for routine inspection of equipment, product sampling and occasional on-stream maintenance. Maintenance work is supervised by the shift foreman with work practices in effect. Pumps and pipe flange seals are maintained to prevent leakage.

Tank cars and trucks are cleaned and inspected before loading by outside contractors. Loading involves connecting supply lines and vent lines, loading tank car in which the operator is located at a control site some distance away and the disconnecting once the tanks are filled. The most significant exposure to the loader is during the disconnect procedure. The loading operation takes about $2\frac{1}{2}$ hours with about $\frac{1}{2}$ hour for connect and disconnect time. Drumming operations employ local exhaust ventilation systems.

2. Epoxy Resin Manufacture

Five epoxy resin facilities were surveyed for epichlorohydrin exposure. The first two are also manufacturers of epichlorohydrin.

Process

The basic process involves reacting epichlorohydrin with bisphenol A under alkaline conditions. Depending on the particular resin being produced, reactants and/or solvents may be introduced to modify the resin properties and the viscosity of the liquified resin products. The reactants, depending on resin specifications may include bisphenol A, tetrabromo bisphenol A, o-cresol, paraformaldehyde, caustic soda, oxalic acid, and p-tertiary butyl phenol. The solvents may include methyl ethyl ketone, methyl isobutyl ketone, acetone, toluene, and xylene. Epoxy resins may be produced as thermoset (cured) resins, containing no free or unreacted epichlorohydrin.

Survey Data

Plant A & B

Exposure to epichlorohydrin were found to be less for the epoxy resin
production operations. Resin process chemical operator exposures were found to be less than 0.8 ppm for 22 TWA personal samples taken in the two epichlorohydrin manufacturing facilities. Lower exposures are reported for foremen and maintenance.

Plants C, D and E

At three additional epoxy manufacturing facilities, chemical operator exposures were less than 0.2 for 17 TWA samples. The highest level reported (1.5 ppm) is that for a general area sample taken in the isolated pump room at Plant C. This demonstrates the potential for exposure from equipment leakage.

Control

Epoxy resin processes are located both indoors and outside, with a variety of natural, general and local types of ventilation control. Reaction kettles are equipped with vent lines. Epichlorohydrin was fully reacted in processes studied so that packaging and shipping of finished products did not present epichlorohydrin exposure to workers.

IV. Vinyl Halides

Studies were conducted for vinyl fluoride exposure at a manufacturing facility and at a polymerization facility. Vinyl bromide was studied at a manufacturing facility.

Health Concern

The vinyl halides including vinyl fluoride and vinyl bromide have gained prominent health concern as a result of the recently reported cancer associated with vinyl chloride workers. In the absence of definitive Federal Standards for vinyl fluoride and vinyl bromide, NIOSH recommends that worker exposures be minimized and that levels be maintained below the established Federal Standard for vinyl
chloride. The Federal Standard for vinyl chloride is one ppm as an 8-hour TWA exposure limit. The 1978 ACGIH Threshold Limit Value for vinyl bromide has been revised downward for the 8-hour TWA exposure limit.

B. Survey Results

1. Vinyl Fluoride Manufacturer Process

Plant A is a manufacturer of vinyl fluoride. The basic process involves a pressurized reaction of hydrofluoric acid and acetylene. Difluoroethane is formed as an intermediate product that is cracked to yield ethylene fluoride and hydrogen fluoride. The reaction products are refined by distillation and the off-products are recycled to the process stream. Liquid vinyl fluoride is piped to insulated storage tanks and from there to insulated tank cars for shipment.

Survey Results and Control

Sampling results at Plant A were generally not detected due to interference from difluoroethane. Difluoroethane was measured to be 5 ppm and less. The vinyl fluoride was estimated to be less than 2 ppm for 7 personal and general area samples. One TWA personal operator sample was 21 ppm for the start-up process on the first shift. This demonstrates that during a work shift with normal or unusual work operations, the potential for greater exposure exists. With continued caution on the part of the operators and the use of respiratory protection, protective clothing and face shields during these operations, the actual worker exposure is minimized.

2. Vinyl Fluoride Polymerization Process

Plant B is a manufacturer of polyvinyl fluoride. Vinyl fluoride is received, transferred to storage tanks and piped to the vinyl fluoride polymerization building. The monomer is continuously pumped to a
supply tank at the process building and from there injected into water and pumped to the reactor to which an aqueous solution of the reaction initiator is simultaneously added. The reactor is barricaded from the remainder of the processing area since the reaction is conducted under high pressure carefully controlled. The reacted vinyl fluoride, a finely divided precipitate, is separated from the reactor aqueous liquor. Unreacted vinyl fluoride is recycled to the supply tank. The polymer is stored as a 5% aqueous slurry and fed to a rotary filter. The process is a closed system until the slurry (completely polymerized) is fed to the rotary filter.

The resulting filter cake (white odorless product) is further dried, collected in bag filters, classified and stored for further processing.

Survey Results

Sampling results for the vinyl fluoride polymer plant, Plant B, indicate TWA exposure levels below 5 ppm vinyl fluoride. Samples ranged from 1 to 4 ppm with a median of 2 ppm for 7 TWA personal samples of polymer operators. One general area sample in the pump room was 5 ppm vinyl fluoride.

3. Vinyl Bromide Manufacturing Process

Plant C is a manufacturer of vinyl bromide. Ethylene dibromide is continuously fed to a reactor where caustic reacts with the dibromide and yields vinyl bromide. The unreacted ethylene dibromide is removed by distillation and recycled. The vinyl bromide is pumped to storage tanks and transferred to railroad cars for shipment. The process is enclosed and the plant structure is open and outside. The process control instrumentation is located in a separate building, where operators monitor the process.

Survey Data
Operator exposures to vinyl bromide in Plant C range from 0.1 to 0.4 ppm with a median of 0.3 for 4 samples. Higher levels were associated with the laboratory analysis and loading operations. A level of 6.3 ppm for a one hour sample during loading vinyl bromide tank cars was reported. A level of 1.2 ppm was reported for the 8-hour TWA sample.

Control

The primary control in these processes involve enclosed processes and process equipment located out of doors. The integrity of process systems are maintained to present minimal exposure hazards to plant workers.

Certain potential exposure situations such as sample collection, coupling or decoupling tank lines and maintenance of process equipment require specialized controls and work practices.

V. Ethylene Dibromide

Two manufacturing and two blending operations were studied for exposure to ethylene dibromide.

A. Health Concern

The current Federal Standard for ethylene dibromide is 20 ppm determined as an 8-hour time-weighted average occupational exposure level. This is based on the acute health effects to the respiratory tract and systemic poisoning. Skin contact may result in skin irritation and systemic effects. Exposures occur principally by inhalation and skin contact and to a less extent by ingestion. A comprehensive review of the literature by NIOSH of animal studies indicate reproductive effects, carcinogenicity, mutagenicity and teratogenicity. Based on these adverse effects possibly associated with human exposure,
NIOSH recommended an employee exposure ceiling of 0.13 ppm (1.0 mg/m³) in the Criteria Document of August, 1977. Based on the preliminary results of animal studies of a toxic interaction between disulfiram and ethylene dibromide, NIOSH published a Current Intelligence Bulletin-23, April 11, 1978. NIOSH recommended that workers should not be exposed to ethylene dibromide during the course of disulfiram therapy, used for treatment of alcoholics in industry. The TLV committee recognizes the carcinogenic potential of EDB as reported in animal studies and is considering a reduced recommended exposure limit.

B. Survey Results

1. EDB Manufacturing Process

Plants A and B are manufacturers of ethylene dibromide in continuous flow, closed system operations. Ethylene dibromide is produced by the exothermic reaction of bromine and ethylene in a countercurrent flow reactor. With bromine entering the top and gaseous ethylene entering the bottom, the reaction occurs in the upper portion of a column packed with ceramic chips. The crude liquid product is further refined, stored and loaded into tank cars for shipment.

Survey Data

The results of 8-hour TWA personal sampling at two manufacturing operations range from not detected (less than 0.02 PPB) to 1600 PPB. The higher levels were reported for the surveillance technician and lab technician at Plant A and the crew leader and product leader at Plant B. For normal plant operations these higher levels of exposure to ethylene dibromide result from open system operations.

Control

These processes are operated and monitored from remote control rooms.
Potential exposures to operating personnel occur for quality control sampling and analysis, loading and maintenance operations.

2. EDB Blending Process

Plants C and D produce antiknock blends using ethylene dibromide. Antiknock blends consist of homogeneous mixtures of ethylene dibromide, ethylene dichloride, tetraalkyl lead and may contain toluene and a dye. Raw materials are generally received by tank car, stored and pumped to the process area. Blending is performed in outdoor blending tanks. The batch-type process is activated and monitored from a control room. Manual operations involving potential worker exposure include loading and unloading tank cars, quality control sampling and analysis and drum loading.

Survey Data

The results of sampling for various operations for antiknock blending operations indicate lower overall exposures than occur in manufacturing. The results of 8-hour TWA personal sampling for two plants ranged from 0.1 to 8.2 ppb for 39 samples. Again, the higher exposures result from open system operations such as loading and unloading, drum cleaning, and sample collection. Drum loading is conducted using enclosed local exhaust hoods. The drums are reusable and undergo cleaning prior to reuse. Drum cleaning is conducted in an enclosed exhaust hood and the operators wearing respiratory protection.

3. Ceiling Levels

The final slide presents short term exposure data for quality control sampling, loading and unloading of tank cars at the various plants. These levels are expressed in ppm rather than ppb for the two prior slides.

The sample collection process varies from 5 to 30 minutes depending.
on the number and location of sampling. Process stream sampling is conducted generally at the beginning of each shift. Tank car loading requires about one to two hours and may be sporadic depending on shipment schedule.

Most of these levels are above the NIOSH Criteria Document recommended ceiling level of 0.13 ppm for a 15 minute period.

VI. Conclusion

The reported exposure levels are considered typical of the industries represented. The primary control for each operation is the closed processing of chemicals. This is typical of the chemical processing industry in general. Particular attention needs to be given to those operation and processes which can not be conducted as closed processes and routine maintenance to detect and correct leaks or accidental chemical releases. The potential for health effects associated with the materials studied is significant to warrant application of ventilation systems, personal protection and safe work practices.

No discussion followed this paper.
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<tr>
<th></th>
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<td>0.5 PPM</td>
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N.D. - NOT DETECTED BASED ON THE SAMPLING AND ANALYTICAL METHOD.
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N.D. - NOT DETECTED BASED ON THE SAMPLING AND ANALYTICAL METHOD.
TABLE 2.1
EPICHLOROHYDRIN SAMPLING DATA SUMMARY
RESIN MANUFACTURERS
8-HR. TWA PERSONAL SAMPLES

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<thead>
<tr>
<th>PLANT / JOB</th>
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<td>PLANT D:</td>
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N.D. = NOT DETECTED BASED ON THE SAMPLING AND ANALYTICAL METHOD.
VINYL HALIDES

VINYL FLUORIDE:

FEDERAL STANDARD  -
NIOSH CRITERIA DOC.  1 PPM
1978 T.L.V.  -

VINYL BROMIDE:

FEDERAL STANDARD  -
NIOSH CRITERIA DOC.  1 PPM
1978 T.L.V.  5 PPM

VINYL CHLORIDE:

FEDERAL STANDARD  1 PPM
NIOSH CRITERIA DOC.  1 PPM
1978 T.L.V.  5 PPM
TABLE 3.1  
VINYL HALIDE SAMPLING DATA SUMMARY  
VINYL FLUORIDE  
8 HR. TWA SAMPLES  

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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POLYMER OPERATOR</td>
<td>7</td>
<td>1-4</td>
<td>2</td>
</tr>
<tr>
<td>G.A. IN SUPERVISOR'S OFFICE</td>
<td>3</td>
<td>1-2</td>
<td>2</td>
</tr>
<tr>
<td>G.A. IN PUMP ROOM</td>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

G.A. = GENERAL AREA SAMPLES.  
N.D. = NOT DETECTED BASED ON SAMPLING AND ANALYTICAL METHOD.
### Table 3.2

**Vinyl Halide Sampling Data Summary**

**Vinyl Bromide**

<table>
<thead>
<tr>
<th>Plant/Job/Location</th>
<th>Sample Size</th>
<th>Range (PPM)</th>
<th>Median (PPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant C:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant Operator</td>
<td>4</td>
<td>0.1 - 0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Lab. Technician</td>
<td>2</td>
<td>0.3 - 0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Loading Crewman</td>
<td>1</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Loading Crewman</td>
<td>1</td>
<td>6.3</td>
<td>6.3</td>
</tr>
</tbody>
</table>

*Results are 8-HR. samples unless indicated.*
ETHYLENE DIBROMIDE

<table>
<thead>
<tr>
<th></th>
<th>IWA</th>
<th>CEILING</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEDERAL STANDARD</td>
<td>20 PPM</td>
<td>—</td>
</tr>
<tr>
<td>NIOSH CRITERIA DOC.</td>
<td>—</td>
<td>0.13 PPM</td>
</tr>
<tr>
<td>1978 T.L.V.</td>
<td>—</td>
<td>0.13 PPM</td>
</tr>
</tbody>
</table>

* SUSPECT CARCINOGEN AWAITING REASSIGNMENT
### Table 4.1

**Ethylene Dibromide Sampling Data Summary**

8 HR. TWA PERSONAL SAMPLE RESULTS
EDB MANUFACTURING

<table>
<thead>
<tr>
<th>Plant / Job</th>
<th>Sample Size</th>
<th>Range (PPB)</th>
<th>Median (PPB)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plant A:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Room Operator</td>
<td>4</td>
<td>20 - 140</td>
<td>80</td>
</tr>
<tr>
<td>Surveillance Technician</td>
<td>8</td>
<td>N.D. - 1600</td>
<td>370</td>
</tr>
<tr>
<td>Lab. Technician</td>
<td>4</td>
<td>N.D. - 570</td>
<td>140</td>
</tr>
<tr>
<td>Brine Field Technician</td>
<td>4</td>
<td>N.D. - 30</td>
<td>10</td>
</tr>
<tr>
<td><strong>Plant B:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Room Operator</td>
<td>7</td>
<td>3 - 160</td>
<td>40</td>
</tr>
<tr>
<td>Crew Leader</td>
<td>2</td>
<td>40 - 950</td>
<td>495</td>
</tr>
<tr>
<td>Product Loader</td>
<td>4</td>
<td>50 - 620</td>
<td>360</td>
</tr>
<tr>
<td>Laboratory Technician</td>
<td>2</td>
<td>10 - 80</td>
<td>45</td>
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N.D. - NOT DETECTED BASED ON SAMPLING AND ANALYTICAL METHOD.
<table>
<thead>
<tr>
<th>PLANT / JOB</th>
<th>SAMPLE SIZE</th>
<th>RANGE (PPB)</th>
<th>MEDIAN (PPB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLANT C:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLEND OPERATOR</td>
<td>5</td>
<td>4 - 58</td>
<td>22</td>
</tr>
<tr>
<td>LAB. TECHNICIAN</td>
<td>6</td>
<td>0.2 - 12</td>
<td>4</td>
</tr>
<tr>
<td>SHIFT SUPERINTENDANT</td>
<td>3</td>
<td>0.1 - 0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>PLANT D:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLEND OPERATOR</td>
<td>3</td>
<td>1 - 9</td>
<td>6</td>
</tr>
<tr>
<td>RELIEF OPERATOR</td>
<td>2</td>
<td>0.5 - 7</td>
<td>4</td>
</tr>
<tr>
<td>REACTOR OPERATOR</td>
<td>2</td>
<td>1 - 3</td>
<td>2</td>
</tr>
<tr>
<td>DRUM LOADER</td>
<td>4</td>
<td>8 - 18</td>
<td>14</td>
</tr>
<tr>
<td>DRUM PROCESSING</td>
<td>3</td>
<td>12 - 36</td>
<td>16</td>
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<tr>
<td>RAW MATERIAL HANDLER</td>
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<td>0.27 - 82</td>
<td>54</td>
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<tr>
<td>LAB. TECHNICIAN</td>
<td>4</td>
<td>0.1 - 0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>COMPOUND BULK OPERATOR</td>
<td>2</td>
<td>1 - 8</td>
<td>4</td>
</tr>
</tbody>
</table>

N.D. - NOT DETECTED BASED ON SAMPLING AND ANALYTICAL METHOD.
<table>
<thead>
<tr>
<th>TASK/PLANT</th>
<th>SAMPLE SIZE</th>
<th>RANGE (PPM)</th>
<th>MEDIUM (PPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUALITY CONTROL SAMPLING:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLANT A</td>
<td>3</td>
<td>5.3 - 23.4</td>
<td>12.0</td>
</tr>
<tr>
<td>PLANT B</td>
<td>2</td>
<td>0.3 - 0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>PLANT C</td>
<td>1</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>PLANT D</td>
<td>2</td>
<td>0.04 - 0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>LOADING TANK CAR:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLANT D</td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>UNLOADING TANK CAR:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLANT D</td>
<td>1</td>
<td>1.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>
ENVIRONMENTAL LEVELS AND URINE CONTENT OF WORKERS EXPOSED TO AZO DYES

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ABSTRACT

Benzidine has been agreed to by both industry and government as being a proven human bladder carcinogen. Henceforth, the use of benzidine and the handling of it has either been curtailed or its exposure to workers greatly minimized. However, the azo dye products of benzidine have received little serious concern and have for at least 75 years been considered as being innocuous. Chemically and biologically, however, the azo bond is quite labile to reductive cleavage.

Because of recent suggestive evidence that these dyes may be broken down to their component amines in the body, NIOSH initiated field studies into the dye manufacturing and dye consuming industries where potential exposure to benzidine derived dyes were suspected. Both environmental and biological urine sampling was performed in order to evaluate actual exposure and the excretion of benzidine and its metabolites, hypothesizing that the dyes are metabolized to benzidine in vivo. The findings of this study are presented for each of the six field surveys performed and the results are discussed briefly.

Based on this and other evidence, NIOSH has recently recommended that benzidine derived azo dye be treated as carcinogens and their manufacture and use be discontinued.
INTRODUCTION

During the relatively brief history of the synthetic dye industry, benzidine has undoubtedly played a major role. Much of what we now know about dye chemistry was laid down between 1870 to 1910, a period which is sometimes called the classical period of dye chemistry. In 1884, P. Boettiger discovered Congo Red. This was the first direct cotton dye derived from benzidine (1). Since then more than 200 benzidine dyes have been listed in the Colour Index (2). In 1948, for instance, some four million pounds of benzidine and 31 million pounds of benzidine derived dyes were produced (3). This accounted for 21% of all dyes reported to be manufactured and almost all of the direct class dyes on the market in that year. While there is still a good demand for these dyes by industries, most manufacturing plants have stopped producing dyes made from benzidine, primarily because of environmental and health concerns. Today, there is only one domestic producer of these dyes; however, imports have risen appreciably. Benzidine derived dyes now used in the U.S. represent less than 1% of the at least 1200 different dyes made in the United States, and the additional 800 dyes imported (4,5). However, benzidine based Direct Black 38 has remained the single largest dye produced among all dyes.

During 1973 the proportional use of benzidine derived dyes, by industry, was estimated to be: 40% used to color paper, 25% to color textiles, 15% for leather, and 20% for diverse applications (6). They may be used
by crafts, artists, and the general public (7).

The object of the present study was to characterize the industrial environment in terms of worker exposure to benzidine derived dyes and to monitor their urinary excretion of benzidine and its metabolites.

A thorough literature review of the pertinent information on benzidine derived dyes has been recently published by NIOSH as a Special Hazard Review (8). In addition, the composite report for this study is to be published shortly (7).

STUDY DESIGN

Procedures had been developed for determining the concentrations of airborne dye exposure and for quantitating the urinary excretion of benzidine and its metabolites. It was desirable to locate industrial facilities where the probable exposure was relatively pure, e.g. where the primary chemical exposures during the work day were to benzidine dyes. With the cooperation of the American Textile Manufacturers Association and the Tanners Council of America, as well as other various sources of information, prospective facilities using benzidine dyes were queried and selected. In all, two textile dyeing and finishing facilities, a leather tanning facility and a specialty paper company were surveyed. The two benzidine dye manufacturing facilities operating at that time were also surveyed.
Employees in each facility were selected with the aid of the managements. Each employee who participated was monitored for personal airborne exposure and urinary excretion of benzidine.

ENVIRONMENTAL SAMPLING AND ANALYSIS

Personal airborne dyestuffs exposure was determined by sampling a known volume of air through a pre-weighed closed face 37-mm glass fiber filter in a three piece cassette. The sampled air was drawn through the filter by a calibrated personal sampling pump at approximately 1.8 liters per minute.

Bulk samples of the benzidine dyes used by the workers during the surveys were obtained for determination of residual-free benzidine present as both the base and salt.

Air filter samples were analyzed gravimetrically, which is a routine procedure designed to measure total gross particulates in the air. As a supplementary procedure for a quasi-specific method for identifying the proportional quantity of a benzidine dye on the same filter sample, NIOSH method P&CAM 234 was used (9). The principle of this method is that a sample filter is extracted with an appropriate solvent, and a spectrographic scan of the solution is performed in the 400-700 nm range. The absorbance maxima are compared to the absorbance maxima of standard
solutions prepared from the bulk samples of the azo dyes. This procedure was of use in the present study since only one to four different color dyes were used during most of the surveys over any one day.

Bulk samples of the dyes were analyzed for residual free benzidine and its salts using a liquid chromatographic procedure using a 280 nm UV detector. Studies have shown that this method yielded a recovery of approximately 100% for benzidine and its salts. The detection limit for benzidine is 1 ppm (w/w) from a one gram sample (7).

BIOLOGICAL SAMPLING AND ANALYSIS

Urine samples were collected in 180 ml polyethylene screw top bottles. The period of collection usually began during the beginning of the first shift that was monitored and continued (excluding the non-work period) into the middle of the next day. However, monitoring periods were extended to include up to a five day period, depending on the dye usage period. The time was recorded on each sample submitted, which was frozen immediately on dry ice and remained frozen until analyzed. Many samples were split so that they could be analyzed by both NIOSH at the Clinical and Biochemical Support Section, Division of Behavioral and Biomedical Sciences, and the Chemistry Division, National Center for Toxicological Research (NCTR).
Urines were analyzed by a screening colorimetric method adapted for use on human urines. The procedure was based on the pH5 extraction of aromatic amines from urine with chloroform, back extraction into HCL and reaction with 2,4,6-trinitrobenzene sulfonic acid (TNBS) to produce a yellow chromophore absorbing at 400 nm. The sensitivity of the method was 1 ppb based on a 100 ml urine sample. The presence of benzidine could be confirmed by thin layer chromatography if concentrations of benzidine exceeded 3 ppb. Complete details of the method have been published in Volume 5 of the NIOSH Manual of Analytical Methods (10).

Selected specimens were also analyzed by the electron-capture gas chromatograph method described earlier at this workshop in a presentation by Lowry and reported by Nony et. al. (11,12). This method was used to confirm the presence of specific metabolites and benzidine. The lower limit of detection for benzidine (Bzd) was 1.4 ppb and monoacetylbenzidine (AcBzd) 5.8 ppb. The method was also used to detect 3,3-dimethylbenzidine (DiMeBzd) and 3,3-dimethoxybenzidine (DiMxBzd) at a lower detection limit of 3 and 3.6 ppb, respectively. The identity of metabolites was confirmed by chemical synthesis of metabolites and derivatives followed by gas chromatography-mass spectrometry (GC-MS) analysis. Confirmation was also obtained to establish that these amines were in fact metabolites and not a result of chemical reduction of the intact dye by the analytical procedures.
RESULTS

Air filter samples were analyzed and reported as milligrams of total airborne particulate per cubic meter of air sampled. Detailed results of the environmental and spectrophotometric analysis on the samples are presented elsewhere (7).

Urine samples from 23 NIOSH office workers were submitted for analysis with the colormetric/thin layer chromatography procedure used to screen workers urine. Table I indicates that fewer than 35% excreted one or more ppb aromatic amine. No benzidine was detected. These results from this non-exposed group were useful for comparison purposes.

In the first dyestuff plant that was visited only spot urine samples were collected during a walk-through survey. Eight of thirty four dye-stuff workers who were potentially exposed to the finished dye submitted urine samples. Using the screening method, less than 1 ppb aromatic amine was found in eight of ten workers. No benzidine was detected; however, 3 and 7 ppb monoacetylbenzidine was reported in two workers as shown in Table II. A return survey was not possible due to the discontinuance of benzidine dye manufacture at that facility.

The low level of aromatic amine excretion among these workers might be expected since cartridge filter respirators and local ventilation were commonly employed in this process.
Table II
Urinary Excretion in Dye Manufacturer I

<table>
<thead>
<tr>
<th>Urine Specimen</th>
<th>Benzidine or Monoacetylbenzidine (MAB)</th>
<th>Aromatic Amines (ng/100 mL)</th>
<th>Thin-Layer Chromatography</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 ppb MAB</td>
<td>120</td>
<td>N.D.</td>
</tr>
<tr>
<td>2</td>
<td>N.D.</td>
<td>80</td>
<td>N.D.</td>
</tr>
<tr>
<td>3</td>
<td>N.D.</td>
<td>100</td>
<td>N.D.</td>
</tr>
<tr>
<td>4</td>
<td>N.D.</td>
<td>80</td>
<td>N.D.</td>
</tr>
<tr>
<td>5</td>
<td>N.D.</td>
<td>90</td>
<td>N.D.</td>
</tr>
<tr>
<td>6</td>
<td>N.D.</td>
<td>80</td>
<td>N.D.</td>
</tr>
<tr>
<td>7</td>
<td>7 ppb MAB</td>
<td>90</td>
<td>N.D.</td>
</tr>
<tr>
<td>8</td>
<td>N.D.</td>
<td>60</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D.--not detected

The second survey to the other dye manufacturing facility discovered the highest worker exposures of the entire study. A small company with minimal engineering control of the process, no formal respirator program, and no industrial hygiene or medical programs were implemented. Of about 55 production workers, about 34 were likely to be potentially exposed. While environmental monitoring data was collected in all dye finishing departments, employee cooperation was poor and only four provided intermittent urine samples. All urine samples contained benzidine and/or monoacetylbenzidine. Two workers also excreted 3,3'-dimethylbenzidine (o-tolidine) which was also used to make dyes. Environmental and urine monitoring results are tabulated for the four workers in Table III. Bulk samples of eleven dyes known or suspected to be benzidine derived were collected for residual benzidine analysis. All contained less than 20 ppm benzidine as the amine or salt.

The following results are from two dye finishing areas at two textile manufacturing facilities.
In the first facility surveyed, personal exposure concentrations were determined while the urine samples were analyzed by both the screening procedure and the EC-GC method. Benzidine and/or monoacetylbenzidine were found in three of seven workers who were monitored. Urinary concentration of non-specific aromatic amines were all above 1 ppb and generally considerably above the NIOSH comparison data. Table IV summarizes these results. Two bulk samples of the dyes used were found to contain 1 and 4 ppm residual total benzidine.

In the second textile facility visited, ten workers were monitored. None of the urine samples analyzed contain benzidine; however, one contained 4 ppb monoacetylbenzidine. The screening method indicated that 40% of the workers excreted one ppb or more aromatic amine in their urine. Environmental samples indicate that daily worker inhalation exposure in the dye room and otherwheres was less than 1.5 mg/m$^3$. Bulk samples of the dye contained up to 20 ppm (w/w) total benzidine. Table V summarizes the results of the monitoring data.

Employees at a leather facility were also monitored. Only Direct Brown 95 was used at the time of the survey. Three employees with the highest likely exposure were monitored as in the other facilities. None of the urine samples collected from the dye weigher or the two dye drum operators contained any detectable benzidine or monoacetylbenzidine. It is probable that appropriate work practices including wearing of respirators, eating in clean lunch facilities, using shower and wash facilities
after work, and changing out of work clothes after work probably contributed to these results. Sampling results are summarized in Table VI.

The last facility surveyed produced colored specialty paper. Black paper using 2500 pounds of Direct Black 38 was produced over a three day period. Urine samples were collected for up to five days from employees over all three work shifts each day. In all, 47 urine samples were submitted. Seven production workers participated from this facility. Environmental and urine monitoring data is summarized in Table VII. Workers I, III, and IV are dyestuff weighers, while the other four workers are operators of the pulp beaters. Like other dye using industries, these seven workers are probably the only ones on a regular basis to be directly exposed to dyes.

In addition to the analysis of benzidine and its metabolites in the urine of these workers, a recently discovered contaminant of Direct Black 38, diaminoazobenzene, was detected. Diaminoazobenzene (DAAB), also known as Basic Orange 2 or Chrysoidine, is an animal carcinogen. The dye used in the paper dyeing facility was not analyzed for DAAB. However, DAAB was found in the urine of four of the seven workers. Benzidine and/or monoacetylbenzidine was also found in four of seven of the workers, though concentrations were generally near the lower limits of detection. In addition, 57% of the urine samples submitted contained 1 ppb or greater non-specific aromatic amines. This is a higher percentage than was found in the NIOSH comparison group.
It should be noted that the above workers who weighed dyes did so while wearing a half face NIOSH approved respirator. Exhaust ventilation near the dye weighing scales was also utilized to lower exposures. Airborne concentrations were generally below 5 mg/m$^3$, while actual exposure would have been less when using a respirator.

CONCLUSIONS

In total, urine samples were collected over varying lengths of time from 38 industrial employees who were regarded as potentially exposed to benzidine derived dyes. Of that number, benzidine or monoacetylbenzidine in quantities ranging from one part per billion to 112 ppb benzidine and 590 ppb MAB were found in 12 of the 38 workers monitored. Environmental exposures and work practices were recorded in an attempt to associate these factors with biological excretion.

Evidently, benzidine derived dyes can be used in the work place without detecting benzidine or its metabolites in the urine. By comparing the results from the six facilities surveyed it appears that total airborne particulate concentrations above 3-5 mg/m$^3$ frequently resulted in detecting benzidine, its metabolites, or elevated, non-specific aromatic amines in the workers urine. Worker exposure to airborne particulates less than 3 mg/m$^3$ are less often associated with finding aromatic amines in the urine. However, full shift airborne exposures as low as 1.1 mg/m$^3$ resulted in considerable benzidine in some workers urine, thus suggesting the difficulty in providing sufficient controls.
Piotrowski (13) has provided evidence that urinary benzidine concentrations of 100 ppb or greater are associated with an elevated risk of bladder cancer in man. In addition, the National Cancer Institutes recent testing of three benzidine derived dyes Direct Black 38, Direct Brown 95 and Direct Blue 6 indicate that the benzidine derived dyes may be more carcinogenic in the rat than benzidine alone (14). In view of this and other recently published information on these dyes, it is difficult to see how they can be used in a sufficiently controlled and safe manner. Therefore, NIOSH has recommended that benzidine derived dyes be treated as carcinogens and that steps be taken to substitute or minimize employee exposure as much as possible.
REFERENCES


Table I. NIOSH control urine results.

<table>
<thead>
<tr>
<th>Individual</th>
<th>Aromatic Amines* (ng/100ml)</th>
<th>Approximate ppb</th>
<th>TLC** Confirmation</th>
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<tbody>
<tr>
<td>1++</td>
<td>205</td>
<td>2</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>ND</td>
<td>&lt; 1</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>&lt; 1</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>&lt; 1</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>1</td>
<td>ND</td>
</tr>
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<td>6++</td>
<td>ND</td>
<td>&lt; 1</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
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<tr>
<td>8</td>
<td>120</td>
<td>1.2</td>
<td>ND</td>
</tr>
<tr>
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<td>300</td>
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<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>ND</td>
<td>&lt; 1</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>ND</td>
<td>&lt; 1</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>ND</td>
<td>&lt; 1</td>
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<tr>
<td>17</td>
<td>ND</td>
<td>&lt; 1</td>
<td>ND</td>
</tr>
<tr>
<td>18</td>
<td>145</td>
<td>1.4</td>
<td>ND</td>
</tr>
<tr>
<td>19</td>
<td>100</td>
<td>1.0</td>
<td>ND</td>
</tr>
<tr>
<td>20</td>
<td>100</td>
<td>1.0</td>
<td>ND</td>
</tr>
<tr>
<td>21</td>
<td>200</td>
<td>2.0</td>
<td>ND</td>
</tr>
<tr>
<td>22</td>
<td>ND</td>
<td>&lt; 1</td>
<td>ND</td>
</tr>
<tr>
<td>23</td>
<td>ND</td>
<td>&lt; 1</td>
<td>ND</td>
</tr>
<tr>
<td>Standard 1 (Bzd)</td>
<td>100</td>
<td>1.0</td>
<td>Positive</td>
</tr>
<tr>
<td>Standard 2 (Bzd)</td>
<td>250</td>
<td>2.5</td>
<td>Positive</td>
</tr>
<tr>
<td>Standard 3 (Bzd)</td>
<td>350</td>
<td>3.5</td>
<td>Positive</td>
</tr>
</tbody>
</table>

*Expressed as benzidine.

**Thin-layer chromatography

+Allergy medication taken

++Pipe smokers

Note: Lower limit of detection is 100 ng/100 mL of urine.
### Table III. Environmental and biological sampling data from Dye Manufacturer--Facility B.

<table>
<thead>
<tr>
<th>Job Description</th>
<th>Daily Personal Exposure</th>
<th>Urinary Excretion*</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulverizer 1</td>
<td>Day 1) 12 mg/m³ total 2) 5.9 mg/m³ total, 2.5 mg/m³ respirable</td>
<td>52 ppb Bzd; 248 MAB</td>
<td>Wore cartridge respirator. Occasional exposure to very high levels during adjustments.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18 ppb Bzd</td>
<td></td>
</tr>
<tr>
<td>Spray dry</td>
<td>1) 17.4 mg/m³ - area near chute</td>
<td>112 ppb Bzd; 590 ppb MAB: 50 ppb DiMeBzd</td>
<td>Wore no respirator - most of day spent outside building. The presence of DiMeBzd would indicate previous exposure as no DiMeBzd dyes were being used on day of sampling.</td>
</tr>
<tr>
<td>Pulverizer 2</td>
<td>1) 6.2 mg/m³ total 2) 14.1 mg/m³ total</td>
<td>10 ppb aromatic amines expressed as Bzd; Bzd confirmed on TLC 5 ppb aromatic amine as benzidine</td>
<td>Wore cotton gauze respirator; became very dirty from dyestuffs worked mostly with a non benzidine dye.</td>
</tr>
<tr>
<td>Tray Oven</td>
<td>1) 7.0 mg/m³ total 2) 6.5 mg/m³ total</td>
<td>11 ppb Bzd; 22 ppb MAB; 15 ppb, DiMeBzd</td>
<td>Wore cartridge dust respirator; emptied dried oven trays into drums.</td>
</tr>
</tbody>
</table>

* Abbreviations:  
  - Bzd - benzidine  
  - MAB - monoacetylbenzidine  
  - DiMeBzd - 3,3' - dimethylbenzidine  

* Limit of Detection (ppb)  
  - Bzd - 1.4  
  - MAB - 5.8  
  - DiMeBzd - 3.0
Table IV. Environmental and urinary excretion in Textile Facility C

<table>
<thead>
<tr>
<th>Workers Personal Exposure</th>
<th>Urinary Excretion*</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aromatic Amines</td>
<td>Benzidine</td>
</tr>
<tr>
<td>1) 1.39 mg/m³</td>
<td>3.2 ppb</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>4.4 ppb</td>
<td></td>
</tr>
<tr>
<td>2) 1.06 mg/m³</td>
<td>8 ppb benzidine</td>
<td>5 ppb</td>
</tr>
<tr>
<td></td>
<td>confirmed by TLC</td>
<td></td>
</tr>
<tr>
<td>3) 1.68 mg/m³</td>
<td>5.8 ppb</td>
<td>39 ppb</td>
</tr>
<tr>
<td>4) 2.06 mg/m³</td>
<td>3 ppb</td>
<td>ND</td>
</tr>
<tr>
<td>5) 1.07 mg/m³</td>
<td>4.5 ppb</td>
<td>ND</td>
</tr>
<tr>
<td>6) 1.12 mg/m³</td>
<td>9 ppb benzidine</td>
<td>16 ppb</td>
</tr>
<tr>
<td></td>
<td>confirmed by TLC</td>
<td></td>
</tr>
<tr>
<td>7) 1.98 mg/m³</td>
<td>3.4 ppb</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>3.0 ppb</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0 ppb</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>3.2 ppb</td>
<td></td>
</tr>
</tbody>
</table>

*Notes: 
- Dye weigher wore no respirator. General ventilation only.
- Dye weigher wore no respirator. General ventilation only. Boiled up dye by hand.
- Dye weigher wore no respirator. Beginning of shift dustiest.
- Dye weigher wore no respirator. Beginning of shift dustiest.
- Pad dye operator carried dye solution to dye baths and diluted to desired conc. Spent 80% of time in non-exposure area loading gray goods into Pad Dyeing Machine. Wore no respirator. No contact with dry dye.
- Jigg Dyer wore no respirator. Spent much time near steamy jigg baths making adjustments on cloth rolls. No contact with dry dyes.
Table V. Results of urine and environmental monitoring at Facility D—a textile dyer.

<table>
<thead>
<tr>
<th>Personal Worker Exposure*</th>
<th>Urinary Concentrations</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aromatic Amines</td>
<td>Benzidine</td>
</tr>
<tr>
<td>1) 1.54 mg/m³ 1.31 mg/m³ 1.45 mg/m³ TWA</td>
<td>4 ppb</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>4.8 ppb</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>3.6 ppb</td>
<td>ND</td>
</tr>
<tr>
<td>2) 1.15 mg/m³ 1.11 mg/m³ 1.13 mg/m³ TWA</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Area sample over scales 0.55 mg/m³</td>
<td>1.3 ppb</td>
<td>ND</td>
</tr>
<tr>
<td>3) 5.31 mg/m³ (void)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4) 0.90 mg/m³</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5) 1.58 mg/m³</td>
<td>3.2 ppb</td>
<td>ND</td>
</tr>
<tr>
<td>6) 0.67 mg/m³</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7) 0.63 mg/m³</td>
<td>3.2 ppb</td>
<td>ND</td>
</tr>
<tr>
<td>8) 0.20 mg/m³</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9) 0.60 mg/m³</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10) 0.48 mg/m³</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Environmental concentrations expressed as total airborne particulates per cubic meter of samples air.
Table VI. Dye Exposure among three workers at a Leather Tannery--Facility E.

<table>
<thead>
<tr>
<th>Personal Worker Exposure</th>
<th>Urinary Excretion</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 12.05 mg/m³</td>
<td>Day 1</td>
<td>Dyestuff Weigher</td>
</tr>
<tr>
<td>2) 12.95 mg/m³</td>
<td>ND+</td>
<td>Only benzidine-derived</td>
</tr>
<tr>
<td>3) 14.72 mg/m³</td>
<td>Day 2</td>
<td>C.I. Directo Black 38</td>
</tr>
<tr>
<td>4) 1.27 mg/m³</td>
<td>ND</td>
<td>and C.I. Direct Brown</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95 were used. Dye</td>
</tr>
<tr>
<td></td>
<td></td>
<td>weigher spent 80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>of time weighing these</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dyes into paper bags.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Half-face cartridge</td>
</tr>
<tr>
<td></td>
<td></td>
<td>respirator was worn</td>
</tr>
<tr>
<td></td>
<td></td>
<td>during weighing.</td>
</tr>
<tr>
<td>10.65 mg/m³ TWA*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5) 1.42 mg/m³</td>
<td>Day 1</td>
<td>Dye Drum Operator 1</td>
</tr>
<tr>
<td>6) 0.44 mg/m³</td>
<td>ND</td>
<td>Operator picks up</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bagged dyes from</td>
</tr>
<tr>
<td></td>
<td></td>
<td>weigher and empties bag</td>
</tr>
<tr>
<td></td>
<td></td>
<td>into solvating tub.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Potential exposure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>would only occur during</td>
</tr>
<tr>
<td></td>
<td></td>
<td>emptying. Both operators</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wore half-face car-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tridge respirators during</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dye handling.</td>
</tr>
<tr>
<td>0.69 mg/m³ TWA</td>
<td></td>
<td>Other responsibilities</td>
</tr>
<tr>
<td></td>
<td></td>
<td>include loading and un-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>loading dyed hides from</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dye bin.</td>
</tr>
<tr>
<td>8) 1.12 mg/m³</td>
<td>Day 1</td>
<td>Same as above</td>
</tr>
<tr>
<td>9) 1.65 mg/m³</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>10) 16.79 mg/m³</td>
<td>Day 2</td>
<td></td>
</tr>
<tr>
<td>5.79 mg/m³ TWA</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

*TWA = Time Weighted Average
+Benzidine not detectable
### Table VII. Results of environmental and urine monitoring at Paper Facility F

<table>
<thead>
<tr>
<th></th>
<th>Env. Conc. (mg/m³)</th>
<th>Urine (ppb)</th>
<th>Env. Conc. (mg/m³)</th>
<th>Urine (ppb)</th>
<th>Env. Conc. (mg/m³)</th>
<th>Urine (ppb)</th>
<th>Env. Conc. (mg/m³)</th>
<th>Urine (ppb)</th>
<th>Env. Conc. (mg/m³)</th>
<th>Urine (ppb)</th>
<th>Env. Conc. (mg/m³)</th>
<th>Urine (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worker II</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Worker III</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Worker IV</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Worker V</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Worker VI</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Worker VII</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**FOOTNOTES:**

* Environmental concentrations expressed as milligrams total airborne particulates per cubic meter air.

+ Concentration of specific aromatic amines in ppb with the following detection limits: benzidine 1.0ppb; MAB, 1.8ppb; DAAB, 0.8ppb; diacetylbenzidine, 0.2ppb

++ Concentration of non-specific colorimetric procedure with the limit of detection at 1ppb.

**NOTE:** Area within heavy black line signifies period of Direct Black 38 usage.
Discussion

Dr. Weisburger (NCI): We have time for one or two questions. Yes?

Dr. Landrigan (CDC): Did you analyze bulk samples of the dye or samples of the airborne particulates that you captured on your filters to see whether or not there was benzidine in that material, or are you convinced that the benzidine which was detectable in the urine arose entirely by metabolism within the bodies of workers?

Mr. Boeniger (NIOSH): This was an important step in the research protocol. In every case where benzidine-derived dyes were used in the workplace, the dyes were collected and analyzed for residual free benzidine content.

We have summarized considerable data on domestically produced benzidine dyes and imported derived dyes for their content of residual benzidine.

At the levels of residual free benzidine in these dyes, we tried to calculate from historical information what the likely excretion from simply the residual quantity of benzidine was, not accounting for possible metabolism of the dyes themselves, and in all cases, even with leniency, the levels of excretion would have been below the minimal level of detection of our procedures.

So while we have not been able to prove the metabolism of the Direct Black 38 or any of the other benzidine-derived dyes, it is highly suggestive that this work is in agreement with the animal experiments which have demonstrated metabolism of the benzidine dyes.

Dr. Landrigan (CDC): Have OSHA or the Department of Commerce picked up on the NIOSH recommendation to limit or ban the use of this material, and are they working on regulations either to restrict industrial use or to forbid the importation?

Mr. Boeniger (NIOSH): At this time, my understanding is that they have not taken the position of eliminating them or banning the benzidine-derived dyes, but simply to minimize the exposure to lowest feasible levels.

Dr. Saffotti (NCI): I wanted to ask you something about the presence of several aromatic amines concurrently. You have indicated, and I am sorry I missed the paper given by Dr. Lowry on the previous session, that you find benzidine and 4-aminobiphenyl and some other compounds as a breakdown product of the dyes.

We have been doing some work in in vitro systems on combined effect of various carcinogens, and one of the series we have recently studied is a series of aromatic amines, for which we have gotten a number of data in the Ames test, which again I would like to eventually compare with those that were reported by Dr. Lowry.
In studying the combinations of various aromatic amines together, we have found a number of them inhibiting each other in the Ames test and on occasion some synergism. One of the combinations that seems to be repeatedly positive as synergistic is benzidine and 4-aminobiphenyl.

In comparing the mutagenic activity of the Direct Black 38 with that of benzidine or 4-aminobiphenyl alone, one certainly finds a much higher level of activity of Direct Black 38 than the individual compounds, although I would like to have eventually some discussion about the solvent systems used and other things like that.

In relation to the monitoring in the human, do you have evidence of 4-aminobiphenyl or other compounds being detectable in the urine and possibly their concurrent presence with benzidine?

Mr. Boeniger (NIOSH): Initially, our methodologies were rather simple, but as we progressed into the last survey, and only in the last survey were we actually looking for other metabolites or other aromatic amines other than benzidine or its metabolites -- for instance, 4-aminobiphenyl -- and in the last facility we did not find 4-aminobiphenyl in any of the workers' urines there. So that was the only indication where they were actually looked for.

We did find it using Direct Black 38 in animal experiments and just do not know how to correlate that right now.

Dr. Jenkins (EPA): What other facilities have you now monitored over several days trying to establish some of the kinetics of the excretion patterns of benzidine besides that one paper facility?

Mr. Boeniger (NIOSH): Well, that was the last one in our study. We had at that time run out of both time and funds anticipated for the completion of this study.

There were some surveys previous to that where we looked at workers during the day of exposure and the following day of exposure, so there was a 2-day follow-up period there.

Dr. Jenkins (EPA): But no other long exposures like that paper?

Mr. Boeniger (NIOSH): No, I am afraid not.
INTRODUCTION

Coal liquefaction represents a technology that may decrease our dependence on imported petroleum. Research in this area has been conducted within the United States since 1934. However, in spite of the time and effort expanded on these processes many questions still remain to be answered before liquefaction becomes an established technology. One of the questionable areas now under investigation is that of occupational health. Work in this area was initiated several years ago by the National Institute for Occupational Safety and Health (NIOSH) through a series of studies designed to identify potential health hazards in the coal liquefaction environment.

This paper is a review of the results of one of these NIOSH studies. This study entitled "A Study of Coal Liquefaction Processes" is designed to characterize the occupational environment of different liquefaction processes in two stages:

1. The identification of contaminant classes, and
2. The determination of exposure levels for selected contaminants.

The results reported here are based on completed surveys in three of the five facilities under study. Although the physical agents and particulates were investigated, the findings for the organic contaminants are emphasized in this paper.
PROCESS

The facilities involved in this study utilized the direct liquefaction process in which coal is converted directly into a liquid product as opposed to the indirect process where coal is first gasified and then catalytically converted to a liquid fuel. Briefly, the direct process involves the formation of a slurry of fine-meshed coal in a coal-derived liquid. This slurry is then subjected to elevated temperatures and pressures to dissolve, depolymerize, and hydrogenate the carbonaceous material in coal to form the liquid product. Gases and solids are by-products of the process.

The study was divided into two stages. The first stage is qualitative analysis; the second is quantitative analysis of those compounds identified in stage 1.

Slide 1 gives the operating size and operating parameters for the dissolver/reactor stage of the process for the three facilities discussed in this paper. Facility II differs from the other facilities by having a catalytic hydrogenation step. As can be seen from the coal processing rate, all the plants studied were pilot plant facilities.

Protocol and Results/Stage 1

The contaminant classes present within the coal liquefaction working environment of the two facilities were identified through an analysis of 8-hour area air samples. Samples were collected on
### SLIDE 1

**OPERATING PARAMETERS AT THREE FACILITIES**

<table>
<thead>
<tr>
<th>FACILITY</th>
<th>SIZE</th>
<th>PRESSURE</th>
<th>TEMPERATURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>50 (150 BBL/d)</td>
<td>1500-2000</td>
<td>800-875</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>150-400</td>
<td>500-750</td>
</tr>
<tr>
<td>III</td>
<td>6 (18 BBL/d)</td>
<td>1400-2500</td>
<td>800-875</td>
</tr>
</tbody>
</table>
Slide 2. HIGH-VOLUME SAMPLING DEVICE FOR PNA
600mg charcoal tubes and 875mg silica gel tubes for the identification of hydrocarbons and aromatic compounds, e.g. amines, phenols. These samples were collected at a flow rate of 100 ml/min.

A special sampling cassette consisting of a silver membrane filter and Chromosorb 102 was designed for collecting polynuclear aromatics (PNAs). This cassette shown in Slide 2 includes:

- A silver membrane filter
- Stainless-steel screen
- Chromosorb 102 - 3 grams
- Support pad

A glass filter was not used in this sampling device because particulate levels at the surveyed facilities were not expected to clog the silver membrane filter. In operation, this assumption proved correct. The Chromosorb adsorbent was added to the system to capture vapor phase PNAs and species evaporating from the filter collected particulates. Area samples were collected at a flow rate of 9.2 Lpm.

A general scan of charcoal and silica gel tube area samples from various processing units was conducted for more than 25,000 compounds using a gas chromatograph/mass spectrometer (GC/MS) with qualitative identification by a computer data base. Charcoal and silica gel samples were also analyzed by gas chromatography for benzene, toluene, and xyylene, and the aromatic amines and aromatic
The PNA sample cassettes were also quantitatively analyzed for the 30 PNAs listed in Slide 4 for which analytical standards were available. Analyses followed the GC/MS and high performance liquid chromatography methodology developed by the University of Iowa Hygienic Laboratory, Iowa City, Iowa. In this method the silver membrane filter is ultrasonically extracted with cyclohexane; the Chromosorb 102 is extracted in a soxhlet extractor using a 50:50 mixture of methylene chloride-methanol. Sensitivity of the method for each of 30 PNAs is in the low nanogram range per sample.

Stage 1 Results and Discussion

A total of 86 compounds were identified in the 23 samples taken at Facility I, and 68 were identified in 21 samples taken at Facility II (Slide 5). Facility III was not involved in the Stage 1 survey. From the qualitative analysis, more different kinds of aromatic than aliphatic were found among the compounds which could be detected.
# Slide 3

**List of Selected Compounds**

<table>
<thead>
<tr>
<th>Aromatic Amines</th>
<th>Aromatic Alcohols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline</td>
<td>Phenol</td>
</tr>
<tr>
<td>N,N-Dimethylaniline</td>
<td>P-Phenol</td>
</tr>
<tr>
<td>2, 4-Dimethylaniline</td>
<td>0-Ethylphenol</td>
</tr>
<tr>
<td>P-Nitroaniline</td>
<td>P-Ethylphenol</td>
</tr>
<tr>
<td>O-Toluidine</td>
<td>0-Cresol</td>
</tr>
<tr>
<td>O-Anisidine</td>
<td>M-Cresol</td>
</tr>
<tr>
<td>P-Anisidine</td>
<td>P-Cresol</td>
</tr>
<tr>
<td></td>
<td>2, 3-Xylenol</td>
</tr>
<tr>
<td></td>
<td>2, 5-Xylenol</td>
</tr>
<tr>
<td></td>
<td>3, 5-Xylenol</td>
</tr>
</tbody>
</table>
POLYNUCLEAR AROMATICS (PNAs)

- Naphthalene
- Quinoline
- 2-Methylnapthalene
- 1-Methylnapthalene
- Acenaphthylene
- Acenaphthene
- Fluorene
- Phenanthrene
- Anthracene
- Acridine
- Carbazole
- Fluoranthenes
- Pyrene
- Benzo(a)Fluorene
- Benzo(b)Fluorene
- Benz(a)Anthracene
- Chrysene
- Triphenylene
- Dimethylbenz(a)Anthracene
- Benzo(e)Pyrene
- Benzo(a)Pyrene
- Dibenz(a,j)Acridine
- Dibenz(a,i)Carbazole
- Indeno (1,2,3-c,d)Pyrene
- Dibenzoanthracene
- Benzo(g,h,i)Perylene
- Anthanthrene
- Coronene
- Dibenzyrene
### BREAKDOWN OF COMPOUNDS IDENTIFIED AT FACILITIES I AND II

<table>
<thead>
<tr>
<th>FACILITY</th>
<th>ALIPHATIC</th>
<th>AROMATIC</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>81</td>
<td>86</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>67</td>
<td>68</td>
</tr>
</tbody>
</table>
The predominance of the aromatics among the identified compounds is believed to be due to the greater stability of the aromatics relative to the aliphatics at the operating temperatures and pressures of the process. This pattern of relative stability is also apparent among the aromatics as can be seen in Slide 6 which gives a breakdown of the aromatics by the number of aromatic rings within the compound. It is apparent that the more stable class of aromatics, the single-ring and fused two-ring compounds, account for a majority of the compounds identified at the two facilities with an average distribution of 52.3% and 66.8%, respectively, for Facilities I and II.

These findings suggest that compound stability is a factor in determining the types of compounds being formed under the liquefaction process. Other factors would be the type of process, operating parameters, catalyst, etc.

The benzene-soluble fraction has been used as an indirect measure of PNA levels in the working environment. For each sample cassette the cyclohexane soluble fraction was determined separately for the filter and adsorbent to evaluate the applicability of this method to the coal liquefaction environment. These results are given in Slide 7 along with measured PNA levels for these samples. The PNA values represent the sum of the concentration of the 30 PNAs under study. As can be seen some consistency is suggested among the soluble fraction values but a high variability was noted within the PNA values. No apparent relationship was noted between the cyclohexane-soluble fraction
## SLIDE 6

% BREAKDOWN OF AROMATIC COMPOUNDS AT

FACILITY I AND II

<table>
<thead>
<tr>
<th>STRUCTURE</th>
<th>FACILITY I</th>
<th>FACILITY II</th>
<th>REPRESENTATIVE COMPOUNDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonfused Rings</td>
<td>37.2 (32)</td>
<td>2.9 (20)</td>
<td>Indene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Toluidine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anisidine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thiophene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Benzene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Substituted Benzenes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Toluene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Xylene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aniline</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Substituted Aniline</td>
</tr>
<tr>
<td>One Ring</td>
<td>31.4 (27)</td>
<td>26.1 (18)</td>
<td>Biphenyl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Substituted Biphenyls</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bipyrazole</td>
</tr>
<tr>
<td>Two Rings</td>
<td>4.6 (4)</td>
<td>2.9 (2)</td>
<td>Substituted Biphenyls</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bipyrazole</td>
</tr>
<tr>
<td>Three Rings</td>
<td>1.2 (1)</td>
<td>0</td>
<td>Triphenyl Ester</td>
</tr>
<tr>
<td>Fused Ring</td>
<td>56.9 (49)</td>
<td>69.6 (48)</td>
<td>Naphthalene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Substituted Naphthalenes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Quinoline</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acenaphthalene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acenaphthylene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fluorene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Azulene</td>
</tr>
<tr>
<td>Two Rings</td>
<td>20.9 (13)</td>
<td>40.7 (28)</td>
<td>Phenanthrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Substituted Phenanthrenes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anthracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Benzidine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Carbazole</td>
</tr>
<tr>
<td>Three Rings</td>
<td>17.4 (15)</td>
<td>13.0 (9)</td>
<td>Phenanthrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Substituted Phenanthrenes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anthracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Benzidine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Carbazole</td>
</tr>
<tr>
<td>Four Rings</td>
<td>12.3 (11)</td>
<td>7.2 (5)</td>
<td>Benzanthracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Triphenylene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chrysene</td>
</tr>
<tr>
<td>Five Rings</td>
<td>5.8 (6)</td>
<td>8.7</td>
<td>Benzanthracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Triphenylene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chrysene</td>
</tr>
</tbody>
</table>

**808**
### SLIDE 7

**CYCLOHEXANE-SOLUBLE FRACTION vs. TOTAL PNAs**

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Cyclohexane-Soluble Fraction (mg/m³)</th>
<th>Total PNAs (µg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Filter</td>
<td>Adsorbent</td>
</tr>
<tr>
<td>001</td>
<td>0.2</td>
<td>1.6</td>
</tr>
<tr>
<td>002</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>003</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>004</td>
<td>0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>016</td>
<td>0.3</td>
<td>1.2</td>
</tr>
<tr>
<td>021</td>
<td>0.07</td>
<td>0.8</td>
</tr>
</tbody>
</table>

**Correlation Coeff.**

- Filter: 0.05  
- Adsorbent: 0.01  
- Total: 0.12

*n = 6*
VALUES AND THE MEASURED PNA LEVELS INDICATING THAT THE SOLUBLE FRACTION MAY NOT BE AN EFFECTIVE TOOL FOR MEASURING WORKER EXPOSURE TO PNAs.

On the basis of these qualitative results, Stage 2, Quantitative sampling at the three facilities was limited to benzene, toluene, xylene, aromatic amines, and PNAs; compounds and compound classes identified in Stage 1. A panel of NIOSH researchers had previously prioritized the list of chemicals. The ones listed above were in the highest priority grouping.

Stage 2 Sampling Protocol

Three major job categories were identified in which workers may be exposed to process contaminants because of assigned duties. These categories include the plant operators, laboratory technicians, and the maintenance staff. The sampling program emphasized sampling to determine exposure of the plant operators because their exposure is believed to be more representative of the process than the other job categories. The results reported here for the three facilities are therefore primarily concerned with the field operators.

In Stage 2, operator exposure was determined for each of the contaminants identified in Stage 1. These contaminants are listed in Slide 8. Samples taken were of 8-hour duration with flow rates of 100 ml/min for charcoal and silica gel tube samples and 1.5
### SLIDE 8

#### CONTAMINANTS STUDIED IN STAGE II

<table>
<thead>
<tr>
<th>Benzene</th>
<th>Aniline</th>
<th>Naphthalene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>N,N-Dimethylaniline</td>
<td>Quinoline</td>
</tr>
<tr>
<td>Xylene</td>
<td>2,4-Dimethylaniline</td>
<td>2-Methylnaphthalene</td>
</tr>
<tr>
<td></td>
<td>P-Nitroaniline</td>
<td>1-Methylnaphthalene</td>
</tr>
<tr>
<td></td>
<td>O-Toluidine</td>
<td>Acenaphthalene</td>
</tr>
<tr>
<td></td>
<td>O-Anisidine</td>
<td>Aceñaphthene</td>
</tr>
<tr>
<td></td>
<td>P-Anisidine</td>
<td>Fluorene</td>
</tr>
<tr>
<td></td>
<td>Alpha-Naphthylamine</td>
<td>Phenanthrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anthracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acridine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carbazole</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluoranthene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pyrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benzo(a)Fluorene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benzo(b)Fluorene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benzo(a)Anthracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chrysene/Triphenylene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dimethylbenz(a)Anthracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benzo(e)Pyrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benzo(a)Pyrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perylene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dibenzo(a,j)Acridine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dibenzo(a,i)Carbazole</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Indeno(1,2,3-cd)Pyrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dibenzoanthracene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benzo(g,h,i)Perylene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anthanthrene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coronene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DibenzoPyrene</td>
</tr>
</tbody>
</table>
LPM for the personal PNA sample cassette. Personal monitoring samples were supplemented at each facility with 8-hour area samples. The area samples are unit exposures.

A different sampling device has been designed to monitor workers for personal PNA exposure. This sampling device is shown in Slide 9. It consists of a 37mm cassette with a silver membrane filter sandwiched between two gaskets and a 7mm inner diameter glass tubing (boron silicate) containing 150 mg of Chromosorb 102. The two sections were joined with inert ½-inch Tygon tubing. This change was necessary due to pump weight restrictions.

Samples taken in Stage 2 were analyzed using the procedures described for Stage 1; that is gas chromatograph for charcoal and silica gel tube samples, and GC/MS and high performance liquid chromatography using a fluorescent detector for the PNA sample cassettes.

Stage 2 Results and Discussion

The results for benzene, toluene, xylene and the eight aromatics studied are summarized in Slide 10. The ranges given represent a composite of the results for the three major job categories. As can be seen measurable quantities were obtained primarily at Facility 1 with the higher end of the range being associated with the performance of maintenance activities. At Facility II only toluene was found at

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Slide 9. PERSONAL MONITORING DEVICE FOR PNA
RESULTS IN PPM FOR BENZENE, TOLUENE, XYLENE, AND EIGHT AROMATIC AMINES AT THREE LIQUEFACTION FACILITIES

<table>
<thead>
<tr>
<th>Compound</th>
<th>FACILITY I</th>
<th></th>
<th>FACILITY II</th>
<th></th>
<th>FACILITY III</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of</td>
<td>Range</td>
<td>No. of</td>
<td>Range</td>
<td>No. of</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>Samples</td>
<td></td>
<td>Samples</td>
<td></td>
<td>Samples</td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>16</td>
<td>0.01 - 0.03</td>
<td>3</td>
<td>0.02 - 0.04</td>
<td>10</td>
<td>ND</td>
</tr>
<tr>
<td>Toluene</td>
<td>16</td>
<td>0.01 - 0.4</td>
<td>3</td>
<td>0.02 - 0.03</td>
<td>10</td>
<td>ND</td>
</tr>
<tr>
<td>Xylene</td>
<td>16</td>
<td>0.01 - 0.07</td>
<td>3</td>
<td>0.01</td>
<td>10</td>
<td>ND</td>
</tr>
<tr>
<td>Aniline</td>
<td>21</td>
<td>0.02</td>
<td>27</td>
<td>ND - 0.05</td>
<td>12</td>
<td>ND</td>
</tr>
<tr>
<td>N, N-Dimethylaniline</td>
<td>21</td>
<td>0.01</td>
<td>27</td>
<td>ND - 0.05</td>
<td>12</td>
<td>ND</td>
</tr>
<tr>
<td>2,4-Dimethylaniline</td>
<td>21</td>
<td>0.01 - 0.04</td>
<td>27</td>
<td>ND - 0.05</td>
<td>12</td>
<td>ND</td>
</tr>
<tr>
<td>P-Nitroaniline</td>
<td>2</td>
<td>-</td>
<td>27</td>
<td>ND - 0.05</td>
<td>12</td>
<td>ND</td>
</tr>
<tr>
<td>O-Toluidine</td>
<td>21</td>
<td>0.01 - 0.02</td>
<td>27</td>
<td>ND - 0.05</td>
<td>12</td>
<td>ND</td>
</tr>
<tr>
<td>O-Anisidine</td>
<td>21</td>
<td>0.02</td>
<td>27</td>
<td>ND</td>
<td>12</td>
<td>ND</td>
</tr>
<tr>
<td>P-Anisidine</td>
<td>21</td>
<td>0.02</td>
<td>27</td>
<td>ND</td>
<td>12</td>
<td>ND</td>
</tr>
<tr>
<td>1-Naphthylamine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
</tbody>
</table>
MEASURABLE LEVELS while none of these contaminants were detectable at Facility III. The detection limit for benzene, toluene and xylene was 0.01 ppm and for the aromatic amines ranged from 0.02 to 0.05 ppm depending on the compound and on sample volume. The highest value recorded was for toluene at 0.4 ppm at Facility I indicating that worker exposure to these contaminants were well below current occupational health standards.

Worker exposure to the PNAs is summarized in Slide 11. Values given are for total PNAs and represent the sum of the measured concentration of the 30 PNAs studied. As can be seen worker exposure to the PNAs was in the microgram-per-cubic-meter range. A comparison of the three facilities shows that Facility I had higher exposure levels than the other facilities. It is anticipated that using appropriate industrial hygiene techniques and control technology, these values will be quite low.

An evaluation of the results indicates that the two- and three-ring PNAs accounted for more than 97 percent by weight of the total PNAs identified at the three facilities as shown in Slide 12. These included naphthalene, quinoline, 2-methylnaphthalene, 1-methylnaphthalene, acenaphthene, fluorene, phenanthrene and anthracene. The remaining three percent was composed primarily of four-ring PNAs.
SLIDE 11

FIELD OPERATOR EXPOSURE TO TOTAL PNAs IN $\text{mg/m}^3$

<table>
<thead>
<tr>
<th></th>
<th>FACILITY I</th>
<th>FACILITY II</th>
<th>FACILITY III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of Samples</strong></td>
<td>12</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>62.8</td>
<td>0.2</td>
<td>9.9</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>3.5-264.3</td>
<td>0.02-0.3</td>
<td>0.05-21.4</td>
</tr>
<tr>
<td><strong>Standard Dev.</strong></td>
<td>72.2</td>
<td>0.1</td>
<td>8.8</td>
</tr>
<tr>
<td><strong>Geometric Mean.</strong></td>
<td>35.6</td>
<td>0.1</td>
<td>3.1</td>
</tr>
<tr>
<td><strong>Geometric Std. Dev.</strong></td>
<td>3.3</td>
<td>22.8</td>
<td>16.0</td>
</tr>
</tbody>
</table>
### Breakdown by Weight of PNA's Found at Three Liquefaction Facilities

<table>
<thead>
<tr>
<th>Two Ring Compounds</th>
<th>Facility I</th>
<th>Facility II</th>
<th>Facility III</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN (μg/m³)</td>
<td>27.2</td>
<td>52.9</td>
<td>7.2</td>
</tr>
<tr>
<td>(PERCENT)</td>
<td>(49,3)</td>
<td>(97,5)</td>
<td>(68,8)</td>
</tr>
<tr>
<td>RANGE (μg/m³)</td>
<td>0.12 - 72.7</td>
<td>14.8 - 72.2</td>
<td>0 - 18.4</td>
</tr>
<tr>
<td>(PERCENT RANGE)</td>
<td>(20 - 78.4)</td>
<td>(89.3 - 99.8)</td>
<td>(0 - 94.1)</td>
</tr>
<tr>
<td>NO. OF SAMPLES</td>
<td>8</td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Three Ring Compounds</th>
<th>Facility I</th>
<th>Facility II</th>
<th>Facility III</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN (μg/m³)</td>
<td>26.5</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>(PERCENT)</td>
<td>(46,0)</td>
<td>(1.7)</td>
<td>(28,1)</td>
</tr>
<tr>
<td>RANGE (μg/m³)</td>
<td>0.4 - 119.</td>
<td>0.03 - 1.5</td>
<td>0.01 - 4.6</td>
</tr>
<tr>
<td>(PERCENT RANGE)</td>
<td>(18.7 - 73.3)</td>
<td>(0.3 - 7.2)</td>
<td>(2.9 - 100)</td>
</tr>
<tr>
<td>NO. OF SAMPLES</td>
<td>8</td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Four Plus Ring Compounds</th>
<th>Facility I</th>
<th>Facility II</th>
<th>Facility III</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN (μg/m³)</td>
<td>0.7</td>
<td>0.9</td>
<td>0.1</td>
</tr>
<tr>
<td>(PERCENT)</td>
<td>(2.1)</td>
<td>(3.3)</td>
<td>(2.6)</td>
</tr>
<tr>
<td>RANGE (μg/m³)</td>
<td>0.02 - 2.4</td>
<td>0.01 - 1.0</td>
<td>0 - 0.8</td>
</tr>
<tr>
<td>(PERCENT RANGE)</td>
<td>(0.7 - 4.7)</td>
<td>(0.02 - 3.5)</td>
<td>(0 - 16.7)</td>
</tr>
<tr>
<td>NO. OF SAMPLES</td>
<td>8</td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>
CONCLUSION

Based on the results of surveys conducted at the three facilities, a preliminary assessment can be made with regard to the coal liquefaction workplace environment. The Stage 1 results indicate that the spectrum of organic contaminants found in the liquefaction workplace environment for that plant on those days under those conditions is not as diverse as would be expected, consisting of the low-molecular weight aromatics with one to three rings apparently predominate in this environment. However, not all low-molecular weight species were present. Notably absent were the phenols and cresols which, although believed to be present in the liquid effluents were not detected in any of the airborne samples.

Stage 2 personal monitoring results indicate that exposure to these low-molecular weight aromatics was in the parts per million range and for the PNA, in the microgram-per-cubic-meter range. At the ppm level, comparison of benzene, toluene, xylene and the aromatic amines with current occupational health standards indicate that these compounds are well below the OSHA limits.

There is an absence of toxicological data to assess the health hazard of prolonged PNA exposures at the microgram-per-cubic-meter range. However, toxicologic studies have shown that process stream extracts obtained from the process streams of different coal conversion processes exhibited carcinogenic properties which were attributed to the PNA constituents (2-7). Since a number of PNA's have exhibited
Carcinogenic properties in animal toxicology studies (8,9), it could be assumed that the PNA constituents found in these processes may be potential carcinogenic hazards as well. This suggests that workers within these facilities may have an added risk of developing cancer because of PNA-induced cancer relative to workers without such exposure. What remains undefined is the quantification of the risk factor.

Comparison of NIOSH Contractor and DOE Industrial Hygiene Data

The data collected by our contractor at the comprehensive survey was obtained during operation of the SRC-II process. For meaningful evaluation this data is being compared with DOE results obtained during SRC-II operations. Because of differences in sampling and analytical procedures, the comparison of data from the two surveys is concerned with magnitude of the observed concentrations for each process area.

Benzene, Toluene, and Xylene

A comparison of the levels for benzene, toluene, and xylene in the coal preparation, mineral separation, and solvent recovery areas showed similar levels for both area samples and personal samples. However, it should be noted that only one sample was taken in each area by our contractor.
THE DOE DATA TAKEN OVER A PERIOD OF 12 MONTHS SHOWED LITTLE VARIATION AMONG SAMPLES WITHIN PROCESS AREAS TESTED: MORE THAN 90 PERCENT OF THE SAMPLES HAD VALUES BETWEEN 0.01 AND 0.05 PPM FOR BENZENE, TOLUENE, AND XYLENE. OUR CONTRACTOR'S DATA TAKEN 8 MONTHS LATER FALLS WITHIN THIS RANGE, WHICH SUGGESTS A UNIFORMITY IN PROCESS EMISSIONS FOR THESE COMPOUNDS IN THE SRC-II PROCESS.

PNAs

WITH REGARD TO THE PNAs, DOE SAMPLES WERE ANALYZED ONLY FOR BENZO(a)PYRENE (BaP). AN EVALUATION OF CONTRACTOR PERSONAL SAMPLES SHOWED SIMILAR LEVELS FOR BaP IN ALL TESTED PROCESS AREAS EXCEPT THE PRODUCT SOLIDIFICATION AREA. FOR THE TWO SURVEYS, LEVELS RANDED FROM NONDETECTABLE TO LESS THAN 0.01 µG/M³, WHEREAS DOE HAD LEVELS ONE TO TWO ORDERS OF MAGNITUDE HIGHER (0.13 - 0.99 µG/M³). THIS DISCREPANCY IN THE PRODUCT SOLIDIFICATION AREA CANNOT BE PROPERLY EVALUATED BECAUSE PLANT OPERATING STATUS INFORMATION DURING THE DOE SURVEY WAS NOT AVAILABLE.

THE DATA ARE COMPARABLE. BOTH DOE AND OUR CONTRACTOR RESULTS AGREE, OR AT LEAST ERR IN THE SAME DIRECTION.
REFERENCES

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Dr. Weisburger (NCI): I have one or two comments. First, what was this coal-derived liquid that was used for the process you mentioned?

Dr. Berardinelli: It is a mixture of aromatics, i.e., anthracene oil in which the coal is then slurried. At facility number 2, I think, methyl-naphthalene is probably one of the biggest constituents.

Dr. Weisburger (NCI): Also, you have compared the extent of exposure to the polycyclics with -- I mean, between these people and people, say, who are out standing on the street corner in some busy city, you know, where there are lots of automobiles going by and lots of combustion taking place; what is the relative extent of exposure?

Dr. Berardinelli (NIOSH): Okay, that is a very valid question. This is something that I think we will pursue further. The big problem here is analysis. I think EPA was the first one to point out that we cannot use just a silver membrane filter. If you look at the NIOSH methods of sampling analysis for PNA's, a silver membrane filter is recommended. Well, we know that this is not entirely suitable because we are missing the whole vapor phase. A sorbent resin is needed to catch the vapor phase PNA's.

But what I am saying is that some of the old data really cannot be compared to our data because the techniques are different. In addition, we have some problems as to analytical capabilities. Most people have used the cyclohexane-soluble fraction and tried to use that as a PNA indicator. EPA has done work where they have looked extensively at BAP.

I would say that BAP is in the high nanogram per cubic meter range for ambient levels, I think in the Pittsburgh area. I believe more important would be to compare PNA's to other work places. One thing we can do there is to look at some Scandinavian data, which I do not have with me. However, they looked at coke oven emissions using an analytical scheme which analyzed 40 PNA's, and found total PNA's in the milligram per cubic meter range.

So to answer your question fully. I really have to kibitz a bit, because I will say that PNA's seem to be worse than the rural environment.
To assist in carrying out its assigned mission in the field of occupational health, NIOSH is developing a trade name ingredient data base. This data base is intended to provide NIOSH with a more realistic perspective of the problems in the workplace, particularly hazards. As a result, effective programs and policies are designed and implemented.

This data base grew out of the first National Occupational Hazard Survey (NOHS I). Ingredient information on approximately 60,000 products has been compiled through the trade name ingredient clarification (TNIC) process which involves contacting manufacturers, editing incoming information, automating the data, and safeguarding confidential formulas.

To fully appreciate the complexities encountered in obtaining this data, a brief description of the TNIC process will be presented with special emphasis on obtaining information through administrative subpoena action. This action and authority for such action, i.e. Section 15 OSHAct 1970 and the McGee decision, will be described briefly. The involvement of foreign manufacturers provides a special case in the authority to obtain this information. This and other problems unique to the development of this data base will be presented. The current status of the data base will be included in the summary of this report.
In carrying out its assigned mission in occupational safety and health, NIOSH requires a reliable data base on the nature and extent of potentially hazardous exposures in the workplace. The National Occupational Hazard Survey (NOHS) of 1972-1974 developed potential exposure information in a sample of nearly 5,000 plants. Almost 70% of the exposures noted by the surveyors occurred in the form of trade name products. In an effort to clarify those exposures, NIOSH is developing a trade name data base from information gathered in the National Occupational Hazard Survey. To date, ingredient information on approximately 60,000 products has been compiled through the Trade Name Ingredient Clarification (TNIC) process. This data base will be enlarged by the addition of resolved trade name data to be gathered in a second survey. This survey is tentatively scheduled to begin early in fiscal year 1981.

The uses of the NOHS data base are numerous and varied and include the development of estimates of numbers of workers potentially exposed to hazards, cohort location, and development of lists of potential hazards associated with industry types. The utility of the NOHS data base is directly affected by the accuracy and completeness of the trade name product ingredient information.

The trade name resolution process currently being used consists of the following steps:

1. Identify the manufacturer or distributor of the product.
2. Contact the manufacturer by certified mail requesting the information.
3. Edit responses to ensure accuracy and conformance with the required format.
4. Code and automate the data.
5. Input the data to the NOHS data base.

In practice, however, the process of obtaining this information from product manufacturers is not a simple matter of going through the steps described. Numerous problems requiring clarification or further assistance are encountered. Briefly, some of the more common problems are:

1. The manufacturer is unable to identify a product by the name or number on file. Custom formulations, especially in paint and ink manufacturing can be particularly difficult. Several sources of error are possible here:
a. The product name or manufacturer was incorrectly recorded.
b. There was an error in keypunching the information.
c. The product name has been changed.
d. Insufficient descriptive information was recorded e.g. need a batch or customer order number was needed.

2. A second major problem occurs when we cannot readily identify the correct manufacturer of the product; usually for one of the following reasons:
   a. The manufacturer is no longer in business, at least not by the name recorded.
   b. The original manufacturer has merged with another company, usually involving a name change.
   c. Product formulation rights have been sold to another company, usually involving a product name change.

3. There have been cases where the manufacturer chooses to give us no response or refuses to accept certified mail.

4. If a request is sent to the wrong division or department within a large corporation, the recipient of the forms may not be able to respond.

5. In some cases, there is no definite formula for an ingredient in a product. This often occurs in pigments, dyes, oils, fats, and polymers. In some instances boiling point ranges or length of carbon chains may be of some help. This problem is further complicated when ingredient composition varies because of variation in feed stock used in its manufacture. Efforts to resolve petroleum based products is a good example of this problem.

6. Manufacturers occasionally refuse to respond because the information requested is considered a trade secret.

Approximately 30% of the trade name products in the current data base have been claimed as proprietary formulas by manufacturers. Obtaining and handling trade secret information presents unique problems. There is considerable reluctance by some manufacturers to provide trade secret information to NIOSH because of fear of accidental release of such information to competitors. This is particularly true of small chemical manufacturers who market one or a very few products and may also be true with larger manufacturers who market relatively unique products.

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In a number of cases the impact on manufacturers providing this information is considerable in terms of time, money, and manpower. This is especially true for those manufacturers who market hundreds or even thousands of products for which information is requested.

Unfortunately this impact may be largely duplicated when information similar to that requested has already been provided to another Federal agency, e.g. Food and Drug Administration, Environmental Protection Agency, Consumer Product Safety Commission, but cannot be accessed by NIOSH for the purpose of the development of the trade name database.

Trade secrecy also becomes an issue when one or more of the ingredients of a product are manufactured by another company which considers their ingredients to be proprietary information. Nondisclosure agreements between manufacturers are honored by NIOSH by re-routing the request for information directly to the primary manufacturer.

Under Section 15 of the OSHAct of 1970 NIOSH is given the authority to obtain trade secret and other confidential information. This section also requires NIOSH to maintain and safeguard this information. This legislated authority, as described in the OSHAct, is explained to manufacturers when necessary.

This authority was challenged in the case of the United States of America versus McGee Industries, Inc. in January 1977. In this case, NIOSH was seeking the enforcement of an administrative subpoena (duces tecum) issued by NIOSH to McGee Industries, Inc. to obtain trade secret information that McGee Industries had refused to provide. The decision in this case, and a subsequent appeal, was ruled in favor of NIOSH. This case has since served as a precedent for NIOSH in obtaining trade secret information.

The use of administrative subpoenas to obtain information from noncooperative manufacturers is used when other measures fail. Although this is not a court action, it does require the manufacturer to either comply with the request for information or show just cause as to why it should not have to comply. If this subpoena is not observed, court action is required. To date, only the McGee case has gone to court and a decision rendered.

We have attempted to minimize some of the problems involved in obtaining ingredient information through the careful design of the materials which are sent to the manufacturers. The initial letter and directions for completing the response forms must be carefully worded. The letter must be explicit and sufficiently comprehensive in describing precisely what information is requested and why without confusing or intimidating the recipient. Similarly, the directions for completing the forms must be simple and direct, without being perceived as threatening. Clarity of the response form is also important. In some instances, copies of the relevant portions of the OSHAct and/or the McGee decision are sent along with the letter to provide information. It has become apparent to us
that communicating with the manufacturers of trade name products requires a well-designed package of basic letters and forms, with considerable flexibility to accommodate the unforeseen.

In addition, NIOSH has developed an unpublished Trade Name Product Security Policy for the TNIC process which includes background information of the National Occupational Hazard Survey, applicable legal statutes, physical security measures, data access procedures, and restrictions on data use. Copies of this policy are made available to manufacturers upon request. Despite these measures, several manufacturers have drafted up their own security agreements. These often take the form of an affidavit requiring the signature of a NIOSH representative as a precondition for compliance with NIOSH requests. NIOSH has taken the position that such agreements are not necessary, and has further stated that the Trade Name Product Security Policy provides appropriate protection for trade secret data. The penalty for disclosing trade secret information by a federal officer or employee is spelled out in 18 United States Code, Section 1905.

NIOSH's authority to obtain ingredient information on products manufactured by a foreign manufacturer rests with the fact that foreign-made products do in some cases involve exposures to American workers. Compliance to our request by foreign manufacturers has so far been on a voluntary basis. Nondisclosure agreements between foreign and domestic companies, or between foreign parent companies and American subsidiaries are honored.

The current status of the trade name data base (as of February 1980) is given in the accompanying table. Note that various code letters are assigned to a category of products depending on the status of resolution. These symbols are as follows:

- E = trade name entered into system, to be resolved
- 1 = first request letter sent out
- 2 = second request letter sent out
- N = referred to NIOSH surveillance staff by contracted clerical personnel for assistance
- S = satisfactorily completed response
- H = holding for additional information or decision
- R = response received containing chemical information
- Ø = mail returned by post office

No discussion followed this paper.
TABLE

CURRENT STATUS OF THE RESOLUTION OF PRODUCT TRADE NAMES IN THE NIOSH PRODUCT TRADE NAME DATA BASE

<table>
<thead>
<tr>
<th>CODE</th>
<th>NUMBER OF PRODUCTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>553</td>
</tr>
<tr>
<td>1</td>
<td>1,069</td>
</tr>
<tr>
<td>2</td>
<td>2,683</td>
</tr>
<tr>
<td>N</td>
<td>6,431</td>
</tr>
<tr>
<td>S</td>
<td>61,599</td>
</tr>
<tr>
<td>H</td>
<td>2,145</td>
</tr>
<tr>
<td>R</td>
<td>4,882</td>
</tr>
<tr>
<td>Ø</td>
<td>174</td>
</tr>
</tbody>
</table>

In closing, I would like to reiterate that the validity and reliability of the trade name data base and the larger NIOSH data base is directly related to the accuracy and completeness of the trade name resolution effort. Subsequent use of this data base by NIOSH and other users will only be as effective as the data base itself. Although the file on trade name ingredients was initially compiled to complete the NOTIS data base, it has emerged as an important resource in its own right. Our staff receives 600-700 requests for product ingredient information each year from field industrial hygienists, researchers, and other members of the occupational safety and health community. We continue to regard the identification of trade name products, which are potential occupational exposure agents, and the subsequent clarification of those products into their respective ingredients to be an important function of hazard surveillance.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCUPATIONAL CANCER STUDIES

Thursday Afternoon, May 8

PLENARY SESSION

SESSION CHAIRPERSON

Dr. Gregory T. O'Conor
National Cancer Institute

PANEL MEMBERS

Chairpersons from Working Group Sessions A, B and C

Dr. Joseph Fraumeni, NCI
Dr. Richard Marland, EPA
Dr. Norbert Page, EPA
Dr. Kenneth Bridbord, NIOSH
Dr. Nelson Leidel, NIOSH
Dr. O'Conor (NCI): Because we have lost a number of participants, the organizing committee as well as most of the chairmen of the working groups agreed that instead of repeating the working groups of yesterday, we would be better to proceed directly to the plenary session. But if there are specific questions relating to some of the papers this morning which anybody wants to bring up, I think there will be ample time to cover virtually every subject that any individual would like to mention.

This is the windup of what I think has been a very successful and productive meeting. Instead of saying it at the end, I will say it now. I think on behalf of all of us we would like to thank Dr. Kraybill and the other members of the organizing committee for taking the initiative to plan and organize this meeting at this time. It is a first and has become a very effective program. I think we are all glad that this meeting has taken place and hope that it will be the first of many more to come, each one more effective and productive than the last.

The way I thought we might start this is to have one member of each of the working groups to summarize the events of yesterday and to make any further comments he might like to make. We can open that for limited discussion and then have the reports discussion after that. So may I start by asking Dr. Bridbord to report on the working group and his reflections on the meeting in relation to epidemiologic studies.

Dr. Bridbord (NIOSH): Thank you. I think we had a very interesting and interactive session dealing with epidemiology needs and opportunities for collaboration that span both the workplace as well as the general environment. I tried to condense the discussion into roughly a dozen areas and perhaps Dr. Fraumeni might also come up here and add any additional points that come to mind that I may not have touched on.

One of the most intriguing areas for discussion, which relates to both the workplace as well as the general environment, is follow-up studies secondary to the cancer maps that NCI has developed, which have generated many hypotheses and suggestions for new avenues of studies, both looking at communities around specific point sources as well as particular working groups. These opportunities include, for example, case control studies and additional studies using hospital records. But certainly I think there was a general consensus that some follow-up studies should be given a high priority.

In this regard, we spent some time talking about the need to pay more attention linking indices of exposure to particular substances, to
specific end health effects, be they based on either indices of mortality or morbidity for that matter. Here we had a fairly lively discussion about the role of people in the area of chemistry and how important it is for the chemist to be interacting with the people who are more health oriented and for the physical scientist and the biological scientist to understand the strengths of each approach and some of the limitations that go along with those approaches. We collectively felt that the National Death Index offers a particularly important opportunity for studies looking at the risks that may be associated with community exposures as well as workplace exposure. Here the existence of the National Death Index might actually allow a much more aggressive posture with respect to prospective studies, at least forming registries of exposed populations that might be assessed a number of years from now. These registries might help to clarify either some existing situations or may help to clarify risks in newly emerging technologies, particularly where we can start to define groups exposed to specific materials at the onset.

Some of the examples that were suggested included the possibility of looking at the population in Duluth, Minnesota exposed to asbestos in drinking water and using the National Death Index to follow a very large number of people exposed to that common source.

Three other examples of emerging technologies that might be particularly important for occupational safety and health, as well as perhaps in some cases for populations residing in communities, include the emergence of coal gasification and liquification as an additional source of petroleum products, the emergence of the recombinant DNA industry which is likely to grow at a very rapid rate, and some of the dramatic changes that occur in the electronics industry. These are just three examples, in addition to Duluth, of situations that might be looked at from the point of view of registries and more prospective studies.

While the main point of our discussion centered around risks of cancer, there was also a fair recognition that there was an opportunity to gain some insights in the area of reproductive effects, effects that might be mediated through exposure of men as well as women to toxic agents, and the need to look at that area in its own right and also to consider how that related to the whole problem of cancer risk. Here it was felt that we should be a bit more flexible in the future to consider those types of studies in this collaborative program. I know that has importance in the case of OSHA and in the case of EPA as well. In the case of NIOSH, we frequently get questions about those kinds of risk.

Speaking of OSHA and EPA, there was also a recognition of the need for the health researchers to be as responsive as possible to the needs of the regulatory agencies, in this case OSHA and EPA, but also other regulatory agencies as well. The issue of asbestos was raised again as one which is perhaps worthy of some additional study. The one very concrete suggestion was to consider the possibility of a mesothelioma
registry and that this may give us some insights in terms of populations exposed that may be at risk though we do not currently understand precisely what that risk status might be. From the EPA perspective, the issue of indoor air pollution was raised. There is concern about exposures to many toxic substances in the indoor environment, particularly as we move toward energy conservation in cutting down the air turnover rates within buildings. This would tend to accentuate and exacerbate any potential indoor exposure situation.

A number of points were raised, including the problem of asbestos inside buildings, as well as some of the problems with urea formaldehyde insulation, particularly in trailers.

The area of control technology was also discussed. It was recognized that there needs to be some consideration given to looking for solutions to problems as well as identifying problems. Up until now, we have not had major emphasis in the control technology area. This is one that I think is particularly important. I know it is important to NIOSH and I know it is important to OSHA. It applies to the general environment as well as to the working environment.

A very specific suggestion which would be particularly helpful in the case of cohort mortality studies looking at risks in the workplace would be to spend some of our energies developing a fairly comprehensive control population with which to compare specific working populations in order to assess potential risks associated with cancer or deaths of certain types in specific industries. Here, in general, in workplace studies we tend to use the general population of the United States. To the extent that workplace hazards represent an important part of the overall occupational and environmental cancer problem, then we underestimate what those true risks might be by looking at the United States population instead of a more comparable control group, that is, a more healthy working population but not a population exposed to toxic agents. This is something that would have potential long-term benefits, if we could develop that.

Other ideas for studies that were mentioned, and these do not foreclose other opportunities either, include the point that farmers appear to be an increased risk for leukemia and multiple myeloma. This has recently received some attention. Another idea is in the area of nitrosamine exposure, where we have a fairly good amount of data from animal studies but not a lot of corroborating evidence in terms of specific exposed human populations, largely because I do not think we have had the opportunity to do additional follow-up studies and to try to correlate some of the animal data with human observations.

I think there was a strong sentiment raised that it would be important for the federal agencies that depend upon the data from the Social Security Administration to conduct epidemiology studies to be as consistent as possible in requests to the Social Security
Administration, but also to make sure that our collective requests get across the importance of having further access to those particular types of data for epidemiological studies.

Additional opportunities for study were suggested in terms of foreign countries, particularly the Scandinavian countries. Sweden is as outstanding an example of that as any country, where many registries of exposed populations exist and provide an opportunity to link health and exposure data that do not exist in most countries. Here it was also mentioned that in the area of occupational safety and health, there has been a start to have better collaboration between the United States and the Swedish researchers.

Finally, we did note some words and suggestions on where to go from here. One of the suggestions would be that we have a briefing for each other on our proposed new starts for FY 1981. This is among EPA, NCI and NIOSH. We should make sure that in looking to future fiscal years we do not inadvertently duplicate some of our work, but as important, we should also look for opportunities for future collaboration. We should try to get scientists from each of the agencies to work closely together and to help solve some of these important problems.

Dr. O'Conor (NCI): Thank you, Dr. Bridbord. Dr. Fraumeni, do you have anything you would like to add to Dr. Bridbord's remarks?

Dr. Fraumeni (NCI): I think Dr. Bridbord has summarized very nicely the meeting that took place yesterday. I do not have very much more to add, except that I think we should be mindful that there are federal agencies other than NCI, EPA and NIOSH with experiences and resources that may be indispensable for collaborative projects. For example, Dr. Caldwell from CDC is part of a very active group working in the field of environmental cancer. He and others should be brought into the total picture when we talk about federal interagency efforts.

Dr. Bridbord talked about a special priority being given to certain types of studies. These should also include efforts to clarify the effects of widespread population exposures, to help resolve matters of public health, public policy, or regulatory concern; and to help contribute toward a better understanding of carcinogenic mechanisms. It is important, for example, not only to know that nitrosamines in the workplace may give rise to cancer, but if we were to discover a relationship, it would have much wider ramifications toward an understanding of the role of nitrosamines in human cancer generally.

Dr. Bridbord has discussed the obvious importance of analytic studies in cancer epidemiology, particularly case-control and cohort studies. Perhaps more emphasis should be given to the utilization and integration of routinely collected data at the federal level. This includes data resources of the Social Security Administration, the National Center for Health Statistics, the Bureau of Census and the Internal Revenue
Service. We need to collaborate with investigators at these agencies. For instance, we need a system of occupational mortality statistics in selected states, which would be carried-out with the National Center for Health Statistics. We should make special efforts to preserve and utilize the data from private industry, labor unions, insurance plans, health care delivery systems, federal employee records, and, of course, death certificates.

Great emphasis should be given to multidisciplinary studies between epidemiologists and experimentalists. This will help resolve several issues. More efforts should be given to measuring chemical and physical agents in the environment in the context of an epidemiologic evaluation of cancer risk. We should strive to identify the subclinical effects of environmental agents in body tissues and fluids and attempt to measure most susceptibility through laboratory markers in the hope of elucidating environmental interactions. Here again, the effective conduct of these types of studies will require the coordination of resources and talents in the various agencies.

Coordination, of course, is critical in these interagency efforts. At the very simplest level, this may involve, as suggested by Dr. Spirtas, an exchange of organizational listings of epidemiologists and the routine exchange of information that may help stimulate collaborative research to fill gaps and also prevent any wasteful duplication of effort. We might consider periodic interagency meetings to review fast-breaking developments in epidemiology and to uncover special opportunities for further collaboration.

Although our focus has been environmental and occupational chemicals, we should take account of pressure that is mounting to evaluate the effects of radiation. We need studies of sufficient power to evaluate populations exposed to ionizing radiation at various dose levels that can be reasonably quantified in order to provide risk estimates that are required in setting radiation protection guidelines for occupationally exposed groups and for persons exposed to medical or environmental radiation.

We also need to evaluate the potential hazards of the depletion of the ozone layer in special surveys to monitor trends in skin cancer, including melanoma, along with analytic studies to measure the impact of ultraviolet radiation and other risk factors.

Dr.bridwara mentioned the importance of international studies, particularly in countries that have unique data resources, or environmental exposures that are unusually heavy or early. An example would be the exposure of workers in coal gasification plants in Europe and South Africa that started many years ago. Of course, the Atomic bomb Casualty Commission in Japan may be the most valuable radiation resource in the world.
We need more epidemiologic inputs into preventive measures. Further, epidemiologic and biometric research is needed on programs aimed at primary and secondary prevention of cancer, including intervention studies in high-risk groups. This includes, for example, the assessment of work practice and industrial process changes and screening programs that may enable the early detection of environmental cancer. Here again, the utility of laboratory indicators of preneoplastic states and the effectiveness of chemoprevention on the natural history of these lesions should receive more emphasis.

Also needed is the further development of biostatistical methodology to assist the area of risk assessment, including methods for extrapolating carcinogenic response in laboratory animals to man, especially at low-dose levels.

Finally, in studies that are primarily designed to study cancer, it is crucial that we not limit our attention to cancer but also search for other potential effects of environmental exposures. The interrelationships between cancer and other effects may shed light on important biological mechanisms.

Dr. O'Conor (NCI): Thank you.

I think it is clear from those two summaries that the epidemiologic studies will continue to play a very large and important role in this program. I was interested to note that in the last few areas Dr. Fraumeni mentioned there was interest among the epidemiologists in some of the very specific areas that were discussed in the second working group. So I think this is a healthy sign of the recognition of the need for interdisciplinary studies.

Before we go on to the next summary, I wonder if there are any specific questions that any of the participants have now relating to either the epidemiology papers or the epidemiology program. Let's keep the discussion at this time fairly specific, because we will get into a broader general discussion later. Are there any specific points that anybody would like to clarify or raise at this time?

Dr. Morris (EPA): I have a question. In the IRLG (Interagency Regulatory Liaison Group), there is a working group on data formatting and standardizing data presentations at least in the testing area. Is there any thought or is it even reasonable to consider this in the epidemiology area? Because you are getting a variety of data from a lot of different sources, is there any need for us to consider any kind of standardization in this area?

Dr. O'Conor (NCI): Do either Drs. Bridbord or Fraumeni want to answer that? It sounds like something that is going on in NTP. I believe that Dr. Chu was associated with that effect but I do not see him here.
Dr. Orme (NCI): I am not sure if that was the question, which really has to do with epidemiology standards. As you know, this is a somewhat controversial area, and I have not been involved in the IRLG discussions. However, I think that the major emphasis of the interagency program that we are discussing here should be in pushing back the frontiers of knowledge in the field of environmental cancer, rather than trying to rehash what are acceptable practices of epidemiologic research.

Dr. Morris (NCI): I do not believe I was suggesting standards in epidemiology. I was referring more to the reports that are presented and the development of data. It should be put into common format that can be utilized by a number of agencies and sources. That is what I was addressing.

Dr. Fraumeni (NCI): We did not discuss this, but my feeling is that this particular issue should not be a major priority for our group.

Dr. Galbraith (EPA): I would like to address the comment regarding standards, because regulatory agencies run into problems regarding standards for epidemiologic studies, particularly those sponsored by non-regulatory agencies. There have been instances within the last six months in which I have discussed epidemiologic studies with various regulatory groups within the EPA and found that they did not want to be involved in the planning of studies because they felt that involvement in the planning of studies to be conducted in foreign countries may create a situation in which they might have to endorse the conclusions of the study. It was feared the study might lack proper controls. Would you have any comment to make in that regard?

Dr. Fraumeni (NCI): Each new study can be evaluated on its own merit by epidemiologists at each of the agencies or collectively. Already at hand are standard methods for designing, conducting and interpreting epidemiologic studies. Each study can be examined separately regardless of any new standards that are promulgated. We do this all the time.

Dr. Galbraith (EPA): Should there be minimum standards for epidemiology studies?

Dr. Fraumeni (NCI): I am rather flexible on how epidemiology is conducted, and favor a wide variety of approaches. Therefore, it is important to ensure that any guideline or standards do not inhibit fresh approaches in etiologic research. I am not sure anyone is opposed in the general sense to standards of epidemiologic practice, so this may simply be a semantic problem.

Dr. O'Connor (NCI): One can consume a tremendous amount of energy and time in standardization and development of methodology. I think what Dr. Fraumeni is saying, and I certainly would agree with him, that although a certain amount of that is important, the major thrust of this
program should be innovative and should be concerned with research in the broadest context in terms of etiology and prevention.

Are there any other specific points?

Dr. Alavanja (NIOSH): Just as a point of information, it seems that the discussion did not get around to it, but there is a working group in epidemiology for the IRLG. It does have a set of guidelines, not for standardizing epidemiology, but to sort of set forth guidelines for the documentation of epidemiological studies. It was not felt that standards for epidemiology or methodology should be created. It should not be standardized in any fashion, but the full documentation of the study may be important. In cases where studies simply do not have all the information, where information may not have been incorporated, there may be design features that have simply not been reported which are vital to the interpretation of the studies. So this is an activity that is ongoing. In fact, it was out for public comment and now it is at the stage of revision. So these guidelines for documentation are out and it is going to be something which, if it holds to its current timetable, will be published as a series of guidelines -- not standards -- before the end of the year.

Dr. O'Conor (NCI): Thank you. Any other points?

If not, Dr. Page will present the summary for Working Group B, which dealt with toxicology and methodology.

Dr. Page (EPA): Thank you, Dr. O'Conor. My report will be a little bit shorter than what I anticipated since many of the areas that have been described already in the Epidemiology Working Group were identified also by the Toxicology Working Group as high priority areas. However, I will go back into those shortly to explain where we feel maybe the animal toxicology effort can correlate well with the epidemiological efforts.

As an overview, however, it was felt that this collaborative program has gotten off to a pretty good start. The impression I received, however, is that it can even be better focused towards areas that may be truly important to the regulatory agencies and to the research agencies. The term technology transfer has been used quite a bit. I think the EPA sees the role of research organizations as helping to identify fruitful research findings to be translated into aspects of applied research which the regulatory agencies can use to go about their business.

It is my observation that there may be a need for a little closer coordination perhaps between the agencies and between the project officers within the various programs. In some cases, I think it has been very close and in some cases it may not be quite as close as we might desire.

Again, I am going to give some of my own personal observations, since I
have not been that involved with the program. But it also seems to me that we are talking about a program which is rather small compared to the total biological program in cancer research. It occurs to me that there is a great amount of research going on in the same areas that we are doing just a little bit of in this program. I think it is essential that we be aware of all the research under way. I am not saying that we are not, but one of the difficulties in presenting a program, at least as I saw it, is that we are talking about small pieces of research in a particular area.

In some cases, the other related research is not brought in so that we know the scope of this activity in relation to a particular area.

Another observation is that we are a small closed group. I think it would benefit the future meetings to at least invite observers from some of the other program areas and other agencies, perhaps even some of the people in the contracts that are doing this work. This has probably already been thought of, so I am sure that I am not providing any new thoughts for you, but there is a fair amount of research under way of a more applied nature by the Army, some under the National Toxicology Program and the Food and Drug Administration. I would recommend that, as you advance in this program and at future meetings of this nature, you bring in some of these observers from the other organizations. I think that would help to cement coordination with the other research programs.

As far as the identification of research areas in toxicology, there is a great overlap with those that have been presented. It seemed to me that the number one concern expressed by at least those having regulatory responsibility, and I am including NIOSH in this category because much of the work that NIOSH does is of a regulatory nature even though they are not a regulatory agency, was the issue of assessment of carcinogenic hazard or carcinogenic risk. How do you quantitate potency? This was asked. Here was an area where we often do not talk the same language when we talk about potency. Some talk about strictly the chemical reactivity, the biological activity at the molecular level, and others are talking about the summation of the total events that take place which include the metabolism, the kinetics of the transport and the disposition of the chemical.

It was identified as a crucial area for the regulatory agencies. However, it is one that the research agencies like NCI can contribute to in a very meaningful way. So, it is felt that there is a crucial need to improve our methods of carcinogen risk assessment.

Another area that was identified as high priority was that of research on methods to reduce the carcinogenic hazard to populations that are exposed to "known" carcinogens, to chemicals which have been identified by an animal test or even in vitro tests as being potential carcinogens. There are a number of suggestions representing monitoring
aspects including diagnostic tests. We heard of some immunodiagnostic tests that may be a fruitful area for research in a collaborative effort. The methods of reducing hazards and control technology were identified as high priority for this collaborative effort.

Another major area which the Toxicology Group felt was very important was the further improvement and development of the shorter term tests, the in vitro screening tests. I am singling out the in vitro tests at this time even though there was interest in improving the routine in vivo bioassay systems. But inasmuch as this seems to be now more in the purview of the National Toxicology Program or other efforts at the NCI, we did not deal much with the in vivo area. However, in the in vitro systems, the emphasis was placed on the cell transformation systems, being as they represent a unique system for bridging the gap between the bacterial systems and then the longer term in vivo tests in a battery or a tier scheme. There was considerable interest in seeing further research on the validation or improvement of the cell transformation systems.

However, along the line of the in vitro tests, I think it also came out that we really need to take a close look at all the in vitro tests and prove the predictability of these tests. We heard at this meeting of the variability in the S-9 fraction and how this can influence the results from some of the bacterial tests. I think for a regulatory agency to utilized these test systems, we have got to have some assurance that they are predictable and that there are laboratories which can perform these tests in a reliable manner and, of course, we want to know that the results are of relevance to the human situation. I will put it that way. We are always on line to justify actions in the regulatory agencies. Therefore, test systems have to be first utilized as a basis of predicting the human effects. In vitro systems will play a major role, certainly for the EPA, in an assessment scheme. They will have a role in the tier scheme or a battery approach to assessment of potential carcinogens.

A fourth area that was identified as important and perhaps underfunded was that of assessing effects of mixtures or additive effects. A good example was presented also at this meeting on the co-carcinogen effects of disulfuram and ethylene dibromide. That was just one example presented, but I think there are probably many that could be mentioned. In the real work situation, exposure is usually to a mixture of chemicals and not to a single pure chemical. So I think there is certainly a priority that should be given to research on the effects of multiple exposures rather than pure exposure.

There was a little thought given to the need for further research into the structure/activity relationships. It was not discussed to any great extent, except I believe that Dr. Fraumeni has already mentioned this as a high priority area also for epidemiological work.
As you see, the main research areas identified in the Toxicology Group are very similar to those which have already been identified in the Epidemiological Work Group.

Dr. O'Conor (NCI): I would only make one comment to supplement what Dr. Page has said about our meeting, since we have already exchanged our ideas and agreed on what we thought was important at the working session, and that relates to the in vitro tests. It was pointed out that the development and the study of in vitro methodology is one of the most important tools that we have in studying the mechanisms of transformation and the mechanisms of carcinogenesis generally. I think a major part of our time was devoted to the whole question of in vitro tests and their use and the different purposes that they serve, and Dr. Page mentioned their use in screening and tier systems. So this particular area and methodology automatically becomes a bridge between research of interest to the fundamental basic researchers and to the regulatory agencies. It is really a natural and convenient meeting ground.

Are there any specific questions or comments in relation to this general area, which is a very broad one?

Dr. Saffiotti (NCI): A point that was somewhat referred to during the discussion of the Toxicology Subgroup might be useful to discuss here as a plenary group. I am referring to the growing applicability of many of these new methods of research, especially the in vitro methods and those applying in vitro techniques to human tissues and metabolic studies, to problems that have a counterpart in epidemiology and clinical medicine. So one can actually introduce laboratory methods into a study that has a basis in epidemiology in terms of selecting populations for study; in terms of correlating levels, which was mentioned in the Epidemiology Report; in terms of determinations of levels of interaction or metabolites in human tissues and body fluids. Take these back to the laboratory site and utilize dynamically the give and take of human observations and laboratory observations, in a sense, in a more tightly planned fashion than we have perhaps done so far. I guess that this type of interaction of the various agencies with their various backgrounds can be very helpful and stimulating. So, I would like to see a greater cooperation between laboratories and the clinical and epidemiological approaches.

Dr. O'Conor (NCI): Thank you. Are there other comments?

Dr. Orme (NCI): I am sorry that I missed the session yesterday, because I had a question that I wanted to pose. If I had been there, I would have advocated the further development of in vivo methodology. I think we have tremendous obstacles to overcome before we have adequate in vivo methodology for identifying carcinogens.

One of the facts which is generally ignored is the variation we see
experimentally in response to exposure to carcinogens. Drs. Griesemer and Cueto have recently put together a summary paper on 192 bioassays that were conducted by the bioassay program. Of these 192 tests which were conducted at the maximum tolerated dose, there were 35 cases of strong carcinogenicity detected in one species and no evidence whatsoever of carcinogenicity in the other species. There were also another ten tests where sufficient evidence for carcinogenicity in one species was obtained, but no evidence in the other species. I am talking mainly about the mouse and the rat in our bioassays.

Not only do we not know at this time how to extrapolate from rodent to man, we also do not know how to extrapolate from mouse to rat or vice versa. Even within a species, in reference to one of the phenomena that I was discussing this morning, we find that certain strains of mice are almost totally resistant to UV irradiation as far as carcinogenesis is concerned and others get tumors in high multiplicity within 24 weeks.

I think the significance of this, as far as various mathematical models that are posed for assessing human risk, should be investigated. We may actually require an additional experimentation step which looks at the strain specific difference or the species-specific differences. This could also be used as a tool which gets at the mechanism of carcinogenesis. We should get out of this game of assessing potential risk and get into the much more interesting game of identifying populations and the size of populations at high risk.

I think one of the biggest problems we are now faced with in going from a methodology of testing based on the Fischer 344 rat or the B6C3Fl hybrid mouse is that our entire resources program is developed toward producing these animals specifically and that we do not have resources to get into multiple strain testing at this time. It is a serious logistical problem to produce the mice required for multiple strain testing.

The second thing that I would like to mention concerns prioritization of work. This EPA/NCI agreement is the last resort for people in experimental photocarcinogenesis. The Department of Transportation, the EPA Bacter Program, and NCI to a large extent, have all pulled out of the photocarcinogenesis area. We were very fortunate that Dr. Kraybill and the other members of the organization of this agreement allowed us to support the residual activities in photocarcinogenesis.

I think it is a very important area. There are 400,000 cases of skin cancer diagnosed each year. They are easy to treat, fortunately, but it is still an amazing number of cancers to receive such little attention. There are several experimental features about skin cancer which I think could be pursued quite easily. It is one of the few cancers that is easy to follow, as far as progression is concerned. The mechanism, the time-to-appearance of tumor, and the dosing with UV can be measured quantitatively with ease. I think it is a very good system to
pursue. I am very happy that we have received some support from the NCI/EPA Program for it. If it is diminished, I am afraid that there will be very little photocarcinogenesis research conducted anywhere in the country.

Dr. O'Conor (NCI): That is a slant that we were not fully aware of, but I think it is an important one.

Dr. Kraybill (NCI): I would like to ask Dr. O'Conor and perhaps Dr. Domanski a question. I quite agree with Dr. Orme on the first point. We need to know more about strain sensitivity and interspecies sensitivity. But will that type of effort not be encompassed to a degree under your interspecies comparison program? Am I correct in assuming this?

Dr. O'Conor (NCI): Dr. Kraybill is referring to what might be considered a new programmatic approach to this general problem which we are taking at NCI in cooperation and under the encouragement of the EPA. Some of the officials at EPA were strongly encouraging us to conduct more research related to extrapolation involving pharmacokinetics and biochemical parameters in different species of animals so that, hopefully, this could lead to better development of regulatory guides.

Under this stimulus, we considered various approaches. A month or two ago, we had a workshop on this subject. It was an interesting day-long meeting, but it certainly was not one where we arrived at a general consensus. Everybody recognized that this was an extremely important but extremely difficult area. As we mentioned in our working group yesterday, we really do not have enough information at hand. So the objective is to encourage more research and get additional information.

So we are going to issue some RFA's within the next few weeks which will cover various aspects of the approach to interspecies comparisons. We will see that all of the participating agencies here get copies of these RFA's. It is a modest start with some commitment of funds, but if the response is good from the scientific community, and the types of proposals seem to be directed to this area of research, then we would hope to expand it. There is so much work to be done in this area that I do not think that what we are doing precludes the collaborative interagency program from also getting into this field. As has been mentioned earlier today by Dr. Bridbord, I think there is room for interagency discussion of the on-going work in each agency which might be related to the program, whether it is supported under the program or not.

Dr. Weisburger (NCI): I have a qualm about some of the work on the benzidine-based dyes, where I think we need another approach to the metabolism of these compounds. All the work has achieved is to look at the metabolic products of the chemicals as if those are responsible for
all of the activities seen in animals. Nobody seems to be thinking of
the fact that the whole molecule, even though it is very complicated and
could be the focal point of a tough problem indeed, should be
considered.

Of course, when one looks at the result in animals, benzidine itself,
which is the split product, is not very effective in rats, much less so
than the benzidine-based dyes. I think a new approach should be taken
to this problem.

Dr. Galbraith (EPA): EPA is sponsoring an aromatic amine program at the
National Center for Toxicology Research. We currently have underway
three carcinogenicity bioassays and a fourth is scheduled to be
initiated. This program was initiated several years ago as a result of
concerns regarding benzidine and aromatic amines in water. We are very
interested in continuing with the program and expanding it in order to
learn more about structure/activity relationships. We are in the
process of determining the program direction. We will welcome any
suggestions in this regard. I am sorry that Dr. Hart, the new Director
of NCTk, was not able to be here, however, I believe that he would be
happy to entertain any suggestions or discuss this program with you.

Dr. Jammer (NIOSH): I would like to strongly support the work that
needs to be done in the area of structure/activity relationships. It is
apparent from even a casual reading of the relevant scientific
literature that only a very few of some highly sophisticated and
computerized techniques identifying structure/activity relationships
are used in the area of mutagenesis or carcinogenesis. These methods,
which are based on N dimensional vector spaces and based on information
theory, have enormous numbers of variables simultaneously, such as you
are faced with in species extrapolation. The power is there. They are
used primarily by drug companies to synthesize new molecules and new
moities.

The second use for such studies, beyond the point of trying to figure
out which neighboring congeners will be active in biological systems, is
their usefulness in pointing out which compounds will be useful
substitutes for carcinogens in terms of their physical properties.
Identification of substitutes could be made much more rapidly using
structure/activity relationships than the trial and error methods.

Dr. ÒConor (NCI): Thank you.

A question was submitted yesterday to our group. It was of a fairly
general nature and we thought it was probably more appropriate for the
plenary session. I think we were right, because the question in fact
covers a number of the areas that have already been discussed in terms
of epidemiology recommendations and the Toxicology Working Group
recommendations, in that it refers not to just interspecies but
intraspecies, particularly as it relates to human beings. It is really
more of a comment than a question and it might serve as a point of discussion at this point in the meeting.

The time is overdue to consider the significance of individual variation in response to carcinogens. Current methodology favors the yes/no approach to carcinogenicity. Not only is this the wrong methodology, but it implies that we are asking the wrong question. The individual who is posing the question would like to suggest that the appropriate question in carcinogenicity testing is what percentage of the human population is in a high risk category with respect to exposure to a chemical and why? He goes on to say that to answer this question we must develop resources not currently available.

I think one could not argue with the need and the desirability of knowing what percent of the human population is, and who these people are, who are at a high risk to exposure to a chemical and why they are at a risk. This really comes back to the whole problem of the etiology and prevention of cancer.

Would anybody like to comment on that statement or proposition?

Dr. Fraumeni (NCI): Well, this is a very challenging issue. Case-control studies of various cancers should collect detailed information not only to identify environmental hazards, but also to help clarify interactions between multiple risk factors, including cigarette smoking and occupational exposures, environmental agents and host susceptibility, and so forth.

For a more complete, comprehensive and precise evaluation of the interplay between these risks factors, we are relying increasingly on the experimentalist to come up with markers of susceptibility. The epidemiologist is waiting for these new developments to emerge from the laboratory that will permit us to characterize high-risk states with greater precision.

Dr. Bridbord (NIOSH): One other point to note, again from an epidemiologic perspective, is that in the existing cohort mortality studies most of those populations represent a diversity of length of exposure and latency with the vast majority of people not having been followed for a long enough period of time to really have a true idea of what the quantitative impact might be on that population. We also probably miss some effects that may otherwise be on the margins. An additional recommendation might be the need to selectively go back and update previous cohort mortality studies perhaps every five or ten years; to at least expend a certain amount of effort to do that, so that eventually we follow enough cohorts pretty much to extinction that we really do understand what that lifetime impact might be.

Dr. O'Conor (NCI): I think it is good that we got that statement and those comments into the record.
Dr. Orme (NCI): I would like to make a very specific suggestion with respect to the resource problem that we are facing. Everytime we have attempted to do a multi-strain test for carcinogenicity, we have come up with dramatically different results in the various strains. I agree heartily with the epidemiologists. The epidemiologists are way ahead of the experimentalists as far as identifying high risk populations. I had the honor of organizing the bioassay of 8-methoxy psoralen, which is used in the treatment of psoriasis. There had been an epidemiology study done on the patients who had received this. It was quite clear from these studies that people who had already received x-ray treatment, people who were in fair skin categories, people who had previously had an excised skin cancer were in a much higher risk category than other populations.

The specific proposal that I would like to make is that the NCI/EPA group consider funding a feasibility trial for introduction of the mouse embryo freezing technique as a potential way to obtain large numbers of different mice simultaneously for multistrain testing approaches to assessment of risk. As I said, this would solve the one big problem that we are faced with now; that is, getting enough of the different animals together at one time to do a coordinated bioassay using different biological material.

Dr. Kraybill (NCI): I would like to ask the experimentalists who are here, Dr. Saffiotti, Dr. Weisburger and the rest of you, is there association, for example, between aryl hydroxylases as a biochemical marker, and the proclivity to develop a carcinoma? Does this enzyme system come into play? If it does, could one look for this kind of parameter and then say that these kinds of people may be at less risk; they can smoke cigarettes and not come down as readily with bronchiogenic carcinoma?

Dr. O'Conor (NCI): I think you have oversimplified the problem.

Dr. Weisburger (NCI): Well, the study from Texas which was reported in the book by Griffin and Shaw, "Carcinogens: Identification and Mechanisms of Action" seemed to indicate that there was not any clearcut correlation between these two factors. Others may show a different aspect, but that is what I have heard so far.

Dr. O'Conor (NCI): I think the question is whether they come into play. The answer is yes.

Dr. Saffiotti (NCI): Yes, Dr. Kraybill has put the question in sort of photographic form by picking an example of one particular enzyme. Obviously, the biological responses are controlled by a much broader spectrum of systems, including a battery of enzymes.
The point is an important one. I think that with the progress of research in these areas, we will gradually learn to recognize at least certain high risk groups or categories or conditions, and then we will have to learn to see how permanent they are, whether they vary with environmental factors, with time, with age, and so on. I mean the approach is right; I do not think we are yet at the time when we can pick one of these as an index of susceptibility. The point that Dr. Fraumeni was making earlier is a point that we also share very much, that is, the epidemiologists are waiting for the laboratory people to come up with markers that could be used. I think that types of markers and methods for studying them, and so on, are in fact, coming out. I indicated this yesterday in the other group. I am optimistic that in this new decade this whole area will really come to some fruition.

Dr. O'Conor (NCI): Well, I think that now might be a good time to turn to the third working group, which is really the subject of the plenary session and which relates to the importance of interagency programs. I think we all agree on that. Fundamentally, we are interested in the development of the future. Dr. Marland will give the report and open the discussion.

Dr. Marland (EPA): Thank you, Dr. O'Conor. Let me first explain the absence of my colleague. He was called to Buffalo, New York as an expert witness.

It is probably fair to say that there was generated yesterday afternoon in Workshop C nothing new, nothing exciting, nothing that is probably not pretty clear to all of us. Therefore, let me indicate that it is likely that the comments that I would make would be more for emphasis than for any startling revelation that may be there.

In the first place, it was clear that the collaboration between NCI and NIOSH is a different form of collaboration than between NCI and the EPA, even though the program is somewhat similar in terms of its description under the statutory and other developmental and programmatic language. That can become important and perhaps I will get back to that a bit later. But I think that the fundamental difference pervades some of the comments that were made.

One of the earlier comments which I think showed an important recognition of the value of collaboration came from one of the NIOSH representatives, in which he expressed hope that the nature of cooperation between the NCI and NIOSH in undertaking some basic studies had provided some insight into some type of intervention process to prevent a cancerous development within an occupational environment and that some of the more applied studies could also be continued on a joint basis. This indeed stimulated some discussion and I think it is indicative of the high value placed by NIOSH on the involvement of personnel from the NCI and from other kinds of researchers involved in assisting in the application of some of the results of the more fundamental studies.
There was a continual commentary that the conversations, communications and exchanges of information among the agencies involved, i.e., NCI, EPA, NIUSH and OSHA are indeed too sparse. There is a quick and broad recognition among all of us that we need to collaborate better and that this one episode that we are celebrating here with a workshop is perhaps something which should be repeated, extended, or at least enlarged upon.

I think there is no question that the kind of collaboration that EPA and NCI have had has been a source of major benefit and has prevented probably some unfortunate glitches. Most important, however, it has brought to bear the kind of input, the kind of collaboration, the kind of communication and conversation which has benefited EPA and, I hope, it has also benefited the NCI.

Now, where are some specific areas, in addition to the one to which the NIUSH representative referred? What are some other ones? One which attracted rather significant discussion was advice as to where one would proceed to deal with chemicals as potential carcinogens. Do we as a regulatory agency or as a nation or as government intend to use our resources and devote our research to the existing "baddies", the arsenic, the benzenes and the dyes, or do we concentrate on those substances which may be entering the marketplace for which use patterns, distribution, manufacturing indeed have not been established, but which are indicated as being on the threshold of being manufactured?

This is an important question. It is a question which EPA has to answer every day. That in itself is enough to make it important to EPA. However, I think it has an important impact on the nature of research activities that could help EPA solve its problems.

For instance, quite clearly, if one is going to address a major part of his work control activity to the new chemical prior to its entry into the marketplace, the dependency on 18 month rat tests as a minimum kind of classification for risk is not possible. You then get to the structure/activity tree as a model of decision-making, as to whether or not that particular compound, as yet a secret material in which the structure is known and very little else, should be further tested. By virtue of its structure, EPA could indeed require a substantial testing. But in the absence of structural kinds of indications of toxicity, such expensive testing on the part of industry may not be warranted.

I think that there was a fairly strong sense coming from the group yesterday that reliable structure/activity prediction models are not presently sufficiently reliable for us to win court cases. Research is needed to improve the kind of quality data that one gets from the use of models. So there is a specific suggestion which does represent some degree of consensus among some of the participants.
Another one, one which I have a personal feeling about and apparently so did the participants in Dr. Bridbord's and Dr. Page's groups, deals with the ability to better quantify risk. Risk assessment by a great many names is a terribly hazardous undertaking. Unfortunately, it is the assignment of a quantified risk that puts EPA into court and it is a feature of our regulation which is mandated. It is necessary. We do not have the luxury of saying, well, our scientists assure us that this compound is bad under almost all circumstances, and then being able to regulate on the basis of what the consensus among scientists would indicate. It is necessary that we quantify. It is necessary that we assign a risk factor. None of us, apparently in any of the three groups, feels satisfied that the state of the art here is where it should be.

Parenthetically, I would like to say something for at least a few of us. I think that Dr. Kraybill and Dr. Saffiotti at least were present at a recent meeting of the Environmental Carcinogenesis Subcommittee of the National Cancer Advisory Board in which this issue was discussed loudly, long, and for days. The consensus there was very clear that the state of the art is in rather poor shape. The issue under debate was not whether more research was needed, but whether or not the National Cancer Institute should come out with a rather strong statement pointing out the weakness of this.

However, that paper has now been converted into a description of the nature of research which would be desirable, which is to strengthen the science base on which risk assessment is made. I believe that at least in EPA, and judging from the other groups also in NCI and NIOSH, we should begin to pay serious attention to recommendations such as that. Even though it is not an official report of the Cancer Board, it is certainly an extremely well thought out document which does describe research needs in the field of risk assessment. I think that constitutes another fairly sincere recommendation from our group.

The next point is probably the most important one and yet it is the one which is most difficult to describe. I do not know how anyone would approach a research project on it. It can be described as the institutional problems inherent in controlling carcinogens in the environment. The institutional problems are those which do not deal with the development of scientific data. They do not even deal with risk assessment, which I allege is not science, risk assessment is policy -- but that is a debate.

When you have a fairly clearly established relationship between a chemical compound or an environmental condition and cancer and you find that society is still unwilling to make any changes so as to deal with the threat, then you find a very strong sense of discouragement on the part of the regulator. I am sure the scientist feels that he is not being heard or appreciated.
The institutional problems brought about by the courts, the law suits, the interminable delays in getting commitments from the affected parts of society to change the environment, and prevent the access of this compound to people are problems that need to be addressed. They need to be attended to. I do not know how one goes about it. But if anyone needs to be told further that smoking is related to cancer, I do not know how it could be said. Why is it that we still have a very significant population of smokers? The same is true with the exposure to a great many other materials. There is the issue of saccharin. Why is it that suddenly not only the Congress but Americans by the tens of thousands rise up in indignation and say that we scientists are crazy because they do not have cancer and they have been using saccharin? This is in effect what they are saying.

In the PHS, we call the institutional problems of translating science and regulation into meaningful action and into an agreeable thing, education. I think it is an impossible chore to assign simply to the word education. I think it has to be a more highly refined kind of problem. Whether it can be researched or not, whether it is a function of collaborative research or not poses a real problem.

That is the nature of the comments which we think might be substantive and which point to the areas in which future collaboration could be of use both to NIOSH and to the EPA participants.

I would like to add a personal commentary. I feel that the representation from EPA should be stated and that is that the EPA is registering a strong degree of satisfaction with the way in which the National Cancer Institute, and particularly Dr. Kaybill and his staff and those whom he has called in to help us, the way in which you are helping us identify superior research activities and projects to help meet our regulatory aims. We think the system is working well in that regard. If there is a way in which it can be improved, it would go along the lines that Dr. Bridbord pointed out. There should be an additional monitoring of the projects in place. In other words, there should be a continuity of effort as these projects develop, mature and begin to produce reports. If there can be an improvement, it could be in that direction.

There must be expressions of sympathy when the regulator is in court. He has been sued. He does not have the luxury of telling the judge, "Well, judge, just cool it for awhile; we are going to turn loose some people who are going to get these numbers and we are going to come here in about two years and we are going to impress the devil of out you."

That does not work when the suit is being brought by a group who feels as though they have been harmed because they have right to due process and they are keeping EPA or OSHA's feet to the fire. Therefore, the kind of data which we need are those that are not currently available. I continue to point out that the twain probably will never meet.
There is the regulator/scientist who says that these data are of
sufficient value and validity to recommend to our lawyers that they go
to court with them. The more traditional and, if you will, the more
appropriate view of the researcher is one who says, "These data may be
all right for what they represent, but they do not represent a
sufficient picture to be absolutely confident of what we are doing."
Now these two will never meet. But I think that through the
collaborative work that we have here we can at least bring about an
appreciation of each other's problems, so that we tend not to embarrass
each other. If we can do that, we have come a long way. I hope that
can serve as possibly a challenge to us for the future.

Dr. O'Connor (NCI): Thank you, Dr. Marland, for those very thoughtful
remarks which reflect the deliberations of the committee. It almost
leaves little to say. Maybe that is a good time to close. Dr.
Kraybill, would you like to make some remarks?

Dr. Kraybill (NCI): In all humility, I appreciate the fine remarks by
Dr. O'Connor and Dr. Marland. However, I would like to deflect attention
away from myself. If you would look in this program, you will see the
people who organized this meeting and this program. Great thanks go to
Dr. Bridbord and Dr. Leidel, who played a big role in getting this
agenda together. Thanks also go to Drs. Cameron, Burton, Lee, Morris
and Galbraith and the advisory levels with the NCI/NIOSH program, Drs.
Saffiotti, Weisburger, Cooper, Fraumeni, and Ms. Blackwood. These are
the people who made this program possible. Above all of this, if we do
not have the support of Dr. O'Connor, and Dr. DeVita, and Mr. Jellinek
and Dr. Gage and Dr. Robbins, we are ineffective. I was listening very
closely to what Dr. DeVita said the other day and I gather that he is
supportive of this sort of effort. We understand that Dr. Richmond, the
Surgeon General, is very keen on collaborative program work.

I would like to make another comment that is not quite as complimentary,
because it comes deep from the heart and I am really concerned. I hope
Dr. Galbraith and Dr. Lee support me. If they do not, well get up and
disagree with me. Dr. Marland touched on it.

This is a collaborative program. We are not yet fully collaborating;
deceive me. When we organized the NCI/EPA projects, we had a lot of
coordination and we had collaboration. What does collaboration mean to
me? One day I asked Dr. Mason, "You have that other chap from EPA on
that project with you. Are you really collaborating? Are you talking
to him?" Dr. Mason said to me, "Oh, yes, I know what is going on."
Now, in some of our projects this is happening, I can point out one as a
shining example. I think the project of body burden of chemicals Cindy
Stroup directs reflects the interplay of many people and I believe the
interaction here is excellent. But for some of the projects that
prevail, the direction and supervision is not collaborative. Tom Orme
mentioned that this was a learning curve for him in his connection or
collaboration with Dr. Wiser.
You have to be really interested in wanting to work together. You can drive a horse to water, but you cannot necessarily make him drink.

As I mentioned in the overview statement the other day, and I guess I am referring to NIOSH here, and Dr. Marland you touched on it too; I would certainly like to see our NCI/NIOSH program represent more in the spirit of true collaboration. By that, I mean that half of our project officers be NCI and the other half be people from NIOSH. When we give a report, we should show that it is mutually shared and directed and we are truly cooperative.

I know that we have made some great strides, Dr. Bridbord, but I think we ought to go a little further in that direction to show a little more NCI participation into the NIOSH program.

Dr. O'Connor (N~): Does anybody want to challenge Dr. Kraybill?

Dr. Marland (EPA): Dr. Kraybill and I used to share an office years ago. I learned then not to challenge him, but you can disagree with him.

It was Dr. Page who made a very specific and useful commentary. Dr. Kraybill was so busy making notes on it, that he did not fully catch it. An improved collaborative monitoring would be one of the things that I noted on my sheet. That is why I did not mention it. But I believe that EPA at least, represented by Dr. Page and me, would like to see it improved and we would recommend it to NIOSH for their consideration. Dr. Page, did I speak fairly?

Dr. Page (EPA): I agree.

Dr. Bridbord (NIOSH): I think we too would like to see this move toward much more true collaboration than has existed in the past. I think we would all agree that this meeting and hopefully future meetings will help achieve that.

Dr. Galbraith (EPA): I have a couple of comments. Being involved with several collaborative efforts for the Office of Research and Development, I would like to say that I think this one is operating the best of all of them. We seem to be accomplishing a great deal more in a collaborative fashion, even though there is a great deal of improvement that is necessary.

One thing that has not been said and I would hate to leave here without saying it, is that no one has commented on how these collaborative agreements or mechanisms come about. I think this is addressed in the preface to the report by Dr. Kraybill. This mechanism did come about as
a result of an initiative by the Office of Management and Budget. Other collaborative agreements or mechanisms are developed as a result of initiatives in the Office of Science and Technology or Congress.

It seems to me that every one of them that I know anything about is a result of a need. Maybe there are times when we should be anticipating these needs. Mechanisms that are already set up, like the one that we currently have in place, should be anticipating future needs so that we do not have other mechanisms developed which may be wasteful of resources and manpower.

In that regard, I had a call last week from a staffer in the Office of Science and Technology. He was very interested in getting some EPA people and some HEW people together to set up a collaborative mechanism to work on what he believes is an area that requires additional collaborative work between the agencies. In this telephone conversation, I told him about this mechanism, the one we have been discussing during the last three days, and I suggested that he look toward this and other mechanisms that are in place in trying to resolve some of the issues concerning him.

Dr. O'Conor (NCI): In terms of how the program came about, as far as NCI is concerned, the program was more or less urged upon us. I can say that when it was urged upon us, it was not received with the greatest of enthusiasm. I think that is the good thing about it. At the upper levels you can lower the boom, as Dr. Kraybill said, but I do not think that works very well. I think what makes a program like this work, particularly among scientists, is that there is some benefit to be gained. You cannot make people collaborate unless they feel that they are getting some scientific benefit and enjoyment and productivity out of it. The people involved have recognized that it is advantageous to work together and it can be fun and useful.

I think this has become a model program in collaboration. I anticipate that it will expand. As Dr. Saffiotti pointed out yesterday, a lot of the areas that are covered under this program really are relatively underfunded in our interest and in support of basic fundamental research. We all recognize the importance of that, but there is also a big need for applied research. I hesitate to use the word "applied" because it is really more than applied research, but it is a certain type of research that is clearly needed.

From the NCI point of view, I think this program will become increasingly important because we are in the process of developing a new format and structure to what was formerly the Division of Cancer Control. I think this Division, which Dr. Sloan is representing at this meeting, will be very much concerned with the more applied aspects of the projects and the subjects of interest to this collaborative program. I look forward to a lot of participation from the members of that Division and more participation from the members of the Division.
which I hear, and hopefully more participation from the various components of EPA and NIOSH. As has been mentioned, we will probably look for ways to bring in appropriate components of other agencies.

Finally, I can speak with a lot of confidence when I say that I know Dr. DeVita is very supportive of this type of effort. The Board of Scientific Counselors for the Division of Cancer Cause and Prevention was extremely impressed when Dr. Kraybill presented his program at the last meeting. They did not realize that it existed and they felt that we have made a mistake in not publicizing more widely the fact that this interesting, extensive and collaborative program was ongoing.

Dr. Bridbord (NIOSH): I would just like to point out that there has been some steps within HEW through the Committee to Coordinate Environmental and Related Programs. A subcommittee of that committee is examining the waste dump issue. NIOSH has already interacted directly with OSHA and EPA to facilitate collaboration on that specific problem.

Dr. Morris (EPA): I have a comment to make. In our discussion here at the end and certainly during the summary meetings, we have concentrated on intergovernmental relations and looking at mechanisms on how we can communicate and collaborate better. I certainly am a supporter of everything that has been said.

One thing that I think we have not gotten into, and I would like to throw out for you to think about in the future, is where do we go from here in terms of the products we produce? There have been so many reports in government where we compliment ourselves on what a great job we have done then we put this on the shelf with another volume of accomplishments that the Federal Government has achieved. The reason I bring this up is because last year, as a part of the Toxic Substances Control activities, we were making a very conscious effort to work with public participation activities, in particular with states and smaller segments of states. The New Jersey group is a good example. I think that was mentioned here the other day. There, the Environmental Protection Agency has been one of the foremost leaders at the state level.

I would like to see some of these products that we have developed transmitted through some interagency mechanisms or intergovernmental mechanism to these individuals, so that they can appreciate the importance of this interaction. I know at least from the applications we receive in our agency that there were a number of activities relating to epidemiology studies that are being done at the state level. So the kinds of things that you do in this area for NIOSH is important to these people. They are getting involved in short term test methodologies. I worry a little bit about who is going to be doing them, what are their particular qualifications, how they are going to assess the data, and how they are going to use that data. That concerns me as well as it concerns all of us.
So, to make a long story short, I think we ought to think about some intergovernmental mechanism by which our products can get down to the working level in the state and local governments for their use. Perhaps that kind of effort can be done through EPA's regional or state programs or through the information programs within NCI.

Dr. Sloan (NCI): Following up on Dr. Morris' comment, I think it might be helpful if I gave a brief outline of what the Division of Cancer Control, as part of the new division, may be able to do to supplement and complement what you are doing. We are on the edge of technology transfer, taking the developments from research and trying to put them into application as rapidly as possible in the field of cancer control. So whatever comes out of the research areas with which you are dealing in the field of cancer should be grist for our mill.

We do have two interagency agreements, one with NIOSH and one with OSHA, which I would like Dr. Galbraith to know about. The one with NIOSH is to support demonstration and education grants on proper methods for the removal and containment of asbestos in schools. We also have an educational program through NIOSH working with the American College of Radiology to develop educational materials for radiologists about asbestos-related disease. Then, through an interagency agreement with OSHA, we are working to support those new directions grants which are concerned with teaching cancer control measures to current workers throughout the country.

As Dr. DeVita said at the beginning of this meeting, through a reorganization we are about to add to the Cancer Control activities the work of the Cancer Centers and their research programs and the organ site programs, which have a very heavy interest in the environmental and occupational causes of the particular cancer with which they deal. So we feel that in the future we will have many more reasons to work very closely with your interagency group.

Dr. O'Connor (NCI): Unless anybody has any other pressing comments, we have reached a period when we can entertain a motion for adjournment.

Again, on behalf of the National Cancer Institute, I would like to thank everyone for their participation at this meeting. I would also like to thank everyone who contributed so effectively to it.
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:  
PROGRESS ON JOINT ENVIRONMENTAL AND  
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