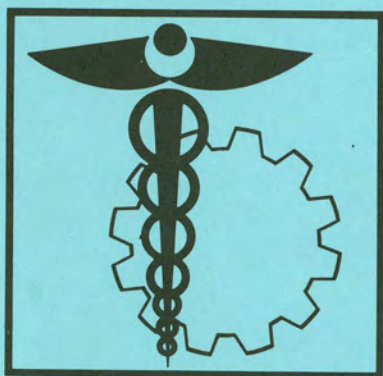


NIOSH GRANTS

RESEARCH and DEMONSTRATION PROJECTS



ANNUAL REPORT
FISCAL YEAR 1986

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health

NIOSH
RESEARCH AND DEMONSTRATION GRANTS
FISCAL YEAR 1986

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health
Atlanta, Georgia 30333

June 1987

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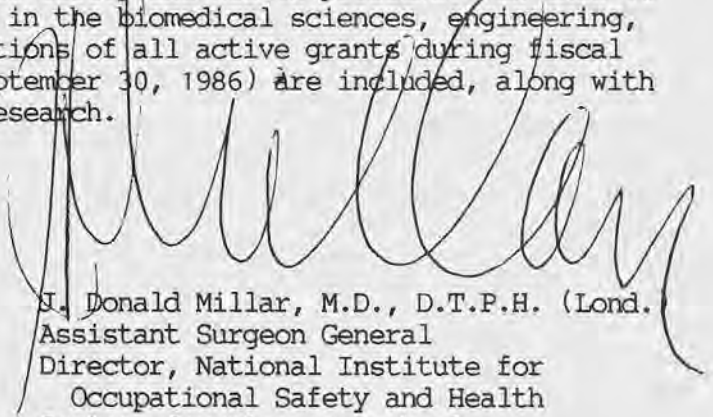
DHHS (NIOSH) Publication No. 87-109

FOREWORD

The National Institute for Occupational Safety and Health (NIOSH) plans, directs, and coordinates a national program of research, training, and related activities to prevent occupational diseases and injuries among workers in the United States. Under the provisions of the Occupational Safety and Health Act of 1970 and the Federal Mine Safety and Health Amendments Act of 1977, research investigations constitute a principal responsibility of NIOSH. In turn, the support of outstanding extramural research is a major component of this research program.

Since becoming Director of NIOSH, I have placed high priority on directing the Institute's activities toward the most significant problems in the field. To focus our intramural efforts, we developed a suggested list of the Ten Leading Work-Related Diseases and Injuries. Two national symposia were held to refine strategies for the prevention of these occupational concerns. Professionals from all sectors of occupational safety and health identified information needs and intervention approaches for a joint effort to deal with this immense national concern. Priorities associated with these leading workplace issues are also reflected in our extramural research program, and a listing of the areas of interest is given in the program announcement included in this report.

This report is published to provide a readily available source of information on the status and scope of the research grants program of NIOSH as a means of stimulating the development of proposals for research of high quality on significant occupational safety and health problems. In this we invite the interest of scientists in the biomedical sciences, engineering, and related disciplines. Descriptions of all active grants during fiscal year 1986 (October 1, 1985, to September 30, 1986) are included, along with publications resulting from the research.



J. Donald Millar, M.D., D.T.P.H. (Lond.)
Assistant Surgeon General
Director, National Institute for
Occupational Safety and Health
Centers for Disease Control

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ANNOUNCEMENT

RESEARCH AND DEMONSTRATIONS RELATING TO OCCUPATIONAL SAFETY AND HEALTH

DEPARTMENT OF HEALTH AND HUMAN SERVICES

CENTERS FOR DISEASE CONTROL

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH

Application Receipt Dates: New applications (February 1, June 1, October 1);
Exceptions: Career Development, Small Grants, and Competing renewal
applications (March 1, July 1, November 1).

The Centers for Disease Control (CDC), National Institute for Occupational Safety and Health (NIOSH) announces that competitive grant applications are being accepted for research and demonstrations relating to occupational safety and health. These include innovative methods, techniques, and approaches for dealing with occupational safety and health problems in the general industry and in mining industry.

Support in the form of individual (research, demonstration, and program) project grants will be awarded for annual budget periods, within a project period not to exceed five years.

I. AUTHORITY

These grants will be awarded and administered by NIOSH under the research and demonstration grant authority of Section 20(a)(1) of the Occupational Safety and Health Act of 1970 (29 U.S.C. 669(a)(1) and Section 501(c) of the Federal Mine Safety and Health Amendments Act of 1977 (30 U.S.C. 951). Program regulations applicable to these grants are contained in Part 87 of Title 42, Code of Federal Regulations, "National Institute for Occupational Safety and Health Research and Demonstration Grants." Except as otherwise indicated, the basic grant administration policies of the Department of Health and Human Services and the Public Health Service are applicable to this program.

II. ELIGIBLE APPLICANTS

Eligible applicants include non-profit and for-profit organizations. Thus universities, colleges, research institutions and other public and private organizations including State and local governments and small, minority and/or woman-owned businesses are eligible for these research and demonstration grants. For-profit organizations will be required to submit a certification as to their status as part of their application.

III. PROGRAM REQUIREMENTS

A. Research Project Grants

A research project grant application should be intended and designed to establish, discover, develop, elucidate, or confirm information relating to occupational safety and health, including innovative methods, techniques, and approaches for dealing with occupational safety and health problems. These studies may generate information that is readily available to solve problems or contribute to a better understanding of underlying causes and mechanisms.

B. Demonstration Grants

A demonstration grant application should address, either on a pilot or full-scale basis, the technical or economic feasibility or application of: (1) a new or improved occupational safety or health procedure, method, technique, or system, or (2) an innovative method, technique, or approach for preventing occupational safety or health problems.

C. Special Emphasis Research Career Award (SERCA) Grants

The SERCA is designed to enhance the research capability of individuals in the formative stages of their careers who have demonstrated outstanding potential for contributing as independent investigators to health-related research. Candidates must have had two or more years of relevant postdoctoral experience prior to the submission date. The application must document accomplishments in this period that demonstrate research potential; it must also present a plan for additional experience in a productive scientific environment at domestic institutions that will foster development of a career of independent research in the area of occupational safety and health. The SERCA is not intended for untried investigators, or for productive, independent investigators with significant numbers of publications of high quality, or for persons of senior academic rank (above associate professor or tenured). Moreover, the award is not intended to substitute one source of salary support for another for an individual who is already conducting full-time research, nor is it intended to be a mechanism for providing institutional support. The application must demonstrate that the award will make a difference in and enhance the candidate's development as an independent investigator.

Candidates must indicate a commitment of at least 60 percent time (not necessarily 60% salary) devoted to research under the SERCA grant, although full-time is desirable. Other work in the area of occupational safety and health will enhance the candidate's qualifications but is not a substitute for this requirement. While working closely with one or more advisers, the awardee is expected to develop capabilities in fundamental, applied, and/or clinical research in one of the areas in section IV. At the end of the award period, evidence of independent investigative capability should be present such that the individual is better able to compete in traditional NIOSH research grant activities.

The total grant award may comprise direct costs of up to \$30,000 per year and up to eight percent additional indirect costs. Direct costs may include salary plus fringe benefits, technical assistance, equipment, supplies, consultant costs, domestic travel, publication, and other costs. If the awardee already holds a small grant on the same research topic, the amount of the SERCA may be reduced up to the amount of the small grant. Awards may be up to three years and will not be renewable.

D. Small Grants

A small grant application is intended to provide financial support to carry out exploratory or pilot studies, to develop or test new techniques or methods, or to analyze data previously collected. This small grant program is intended for predoctoral graduate students, post-doctoral researchers (within three years following completion of doctoral degree or completion of residency or public health training) and junior faculty members (no higher than assistant professor). If university policy requires that a more senior person be listed as principal investigator, the application should specify that the funds are for the use of a particular student or junior-level person and should include appropriate justification for this arrangement. Though biographical sketches are required only for the person actually doing the work, the application should indicate who would be supervising the research. Small grant applications should be identified as such on the application form.

The total small grant award may comprise direct costs of up to \$15,000 per year and additional indirect costs, as appropriate. The grants may be awarded for a project period of up to two years and are thereafter continuable by competitive renewal as a regular research grant. Salary of the principal investigator as well as that of the junior investigator, if university policy requires a senior person to be listed as the principal investigator, will not be allowed on a small grant, though salaries can be requested for necessary support staff such as laboratory technicians, interviewers, etc.

E. Program Project Grants

NIOSH will also accept applications for program project grants, but only after discussion with the individuals listed in this announcement. A program project grant is intended to support a broadly-based, multidisciplinary, often long-term research program which has a specific major objective or a basic theme. It should be directed toward a range of problems having a central research focus in contrast to the usually narrower thrust of the traditional research project. This type of grant generally involves the organized efforts of a group of established investigators, each of whom is conducting research projects designed to elucidate the various aspects or components of the overall objective.

IV. PROGRAMMATIC INTEREST

NIOSH program priorities, listed below, are applicable to all of the above types of grants. The conditions or examples listed under each category are selected examples, not comprehensive definitions of the category. Investigators may also apply in other areas related to occupational safety and health. Applications responding to this announcement will be reviewed by staff for their responsiveness and relevance to occupational safety and health. Assignment to NIOSH for funding consideration will be according to established referral guidelines. Potential applicants with questions concerning the acceptability of their proposed work should contact the individuals listed in this announcement under "FOR FURTHER INFORMATION CONTACT."

1. Occupational lung diseases: asbestosis, byssinosis, silicosis, coal workers' pneumoconiosis, lung cancer, occupational asthma
2. Musculoskeletal injuries: disorders of the back, trunk, upper extremity, neck, lower extremity: traumatically induced Raynaud's phenomenon
3. Occupational cancers (other than lung): leukemia; mesothelioma; cancers of the bladder, nose and liver
4. Severe occupational traumatic injuries: amputations, fractures, eye loss, and lacerations
5. Cardiovascular diseases: hypertension, coronary artery disease, acute myocardial infarction
6. Disorders of reproduction: infertility, spontaneous abortion, teratogenesis
7. Neurotoxic disorders: peripheral neuropathy, toxic encephalitis, psychoses, extreme personality changes (exposure-related)

8. Noise-induced loss of hearing
9. Dermatologic conditions: dermatoses, burns (scalding), chemical burns, contusions (abrasions)
10. Psychological disorders: neuroses, personality disorders, alcoholism, drug dependency
11. Engineering control systems: new technology performance evaluation, preconstruction review, equipment redesign, containment of hazards at the source, fundamental dust generation mechanisms, machine guarding/avoidance methods, explosion control, removal of emissions after generation, dispersion models, monitoring and warning techniques, technology transfer
12. Respirator research: new and innovative respiratory protective devices; techniques to predict performance; effectiveness of respirator programs; physiologic and ergonomic factors; medical surveillance strategies; psychological and motivational aspects; Effectiveness of sorbents and filters, including chemical and physical properties

V. CRITERIA FOR REVIEW

Applications will be evaluated by a dual review process. The primary (peer) review is based on scientific merit and significance of the project, competence of the proposed staff in relation to the type of research involved, feasibility of the project, likelihood of its producing meaningful results, appropriateness of the proposed project period, adequacy of the applicant's resources available for the project, and appropriateness of the budget request. A program project application will also be evaluated for adequacy of methods for coordinating activities toward the central focus.

Demonstration grant applications will be reviewed additionally on the basis of the following criteria:

- o Degree to which project objectives are clearly established, obtainable, and for which progress toward attainment can and will be measured.
- o Availability, adequacy, and competence of personnel, facilities, and other resources needed to carry out the project.
- o Degree to which the project can be expected to yield or demonstrate results that will be useful and desirable on a national or regional basis.

- o Extent of cooperation expected from industry, unions, or other participants in the project, where applicable.

SERCA grant applications will be reviewed additionally on the basis of the following criteria:

- o The review process will consider the applicant's scientific achievements, evidence of demonstrated commitment to a research career in occupational safety and health, and supportive nature of the research environment (including letter(s) of reference from advisor(s) which should accompany the application).

Small grant applications will be reviewed additionally on the basis of the following criteria:

- o The review process will take into consideration the fact that the applicants do not have extensive experience with the grant process.

A secondary review will also be conducted. Factors considered in the secondary review will include:

- o The results of the initial review.
- o The significance of the proposed study to the research programs of NIOSH.
- o National needs and program balance.
- o Policy and budgetary considerations.

VI. APPLICATION AND AWARD

Applications should be submitted on Form PHS-398 (revised May 1982) or PHS-5161-1 for State and local government applications. Forms should be available from the institutional business offices or from:

Office of Grants Inquiries
Division of Research Grants
National Institutes of Health
Westwood Building - Room 449
5333 Westbard Avenue
Bethesda, Maryland 20205

The original and six copies of the application must be submitted to the address below on or before the specified receipt dates in accordance with the instructions in the PHS-398 packet:

Division of Research Grants
National Institutes of Health
Westwood Building - Room 240
Bethesda, Maryland 20205

In developing the application please note that the conventional presentation for grant applications should be used and the points identified under "CRITERIA FOR REVIEW" must be fulfilled.

An applicant organization has the option of having specific salary and fringe benefit amounts for individuals omitted from the copies of the application that are made available to outside reviewing groups. If the applicants organization elects to exercise this option, use asterisks on the original and six copies of the application to indicate those individuals for whom salaries and fringe benefits are being requested; the subtotals must still be shown. In addition, submit an additional copy of page four of Form PHS-398, completed in full with the asterisks replaced by the amount of the salary and fringe benefits requested for each individual listed. This budget page will be reserved for internal PHS staff use only.

The instructions in the Form PHS-398 packet should be followed concerning deadlines for either delivering or mailing the applications. The application should be sent or delivered using the mailing label in the Form PHS-398 packet.

The proposed timetable for receiving applications and awarding grants is as follows except for the SBIR Program, which has a separate Public Health Service announcement and a separate receipt date. Applications for the SBIR Program have a receipt date of December 15.

New and competing renewal applications:

<u>Application Deadline*</u>	<u>Primary Review Group Meeting</u>	<u>Secondary Review Meeting</u>	<u>Expected Start Date</u>
February 1	June	September	December 1
June 1	Oct./Nov.	January	April 1
October 1	Feb./Mar.	May	July 1

*Competing renewal deadlines are one month later.

Exceptions: Career Development and Small Grants

<u>Application Deadline</u>	<u>Primary Review Group Meeting</u>	<u>Secondary Review Meeting</u>	<u>Expected Start Date</u>
March 1	June	September	December 1
July 1	Oct./Nov.	January	April 1
November 1	Feb./Mar.	May	July 1

Awards will be made based on priority score ranking and emphasis area, as well as availability of funds.

VII. REPORTING REQUIREMENTS

Performance reports on awarded grant projects are required annually as a part of the continuation application and a final report is due within ninety days of the end of the project period. The final performance report should include, at a minimum, a statement of original objectives, a summary of research methodology, a summary of positive and negative findings, and a list of publications resulting from the grant. Research papers, project reports, or theses are acceptable items in the final report. The report should stand alone rather than citing the original application. Three copies of reprints of publications prepared under the grant should accompany the report.

VIII. FOR FURTHER INFORMATION CONTACT:

For Technical Information Contact:

Roy M. Fleming, Sc.D.
Associate Director for Grants
National Institute for Occupational Safety and Health
Centers for Disease Control
1600 Clifton Road, N.E., Bldg. 1, Room 3053
Atlanta, Georgia 30333
Telephone: (404) 329-3343

For Business Information Contact:

Leo A. Sanders
Grants Management Officer
Centers for Disease Control
255 E. Paces Ferry Rd., NE, Room 321
Atlanta, Georgia 30305
Telephone: (404) 262-6575

(This program is described in the Catalog of Federal Domestic Assistance Program No. 13.262, Occupational Safety and Health Research Grants. It is not subject to Health Systems Agency nor E.O. 12372 review.)

HHS, PHS, CDC - 2/87

GLOSSARY

Grant Number - The identification number assigned to a grant application

EXAMPLE: 1 R01 OH02183-01A1

1	R01	OH	02183	-01	A1	
						Signifies a first resubmission with
						modifications
						Year of funding
						Sequence number for NIOSH grants
						Institute with primary assignment
						Grant Activity
						Grant Type

Typical Codes

Grant Type

- 1 - New Competing application
- 2 - Competing renewal application
- 5 - Noncompeting continuation application

Grant Activity

- R01 - Research Project Grant
- K01 - Special Emphasis Research Career Award (SERCA) Grant
- R03 - Small Grant
- R13 - Conference Grant
- R18 - Demonstration Grant
- R43 - Small Business Innovation Research (SBIR) Grant

Institute

- OH - NIOSH

Program Areas - Research categories of particular interest to NIOSH

Occupational Lung Diseases	(Lung)
Musculoskeletal Injuries	(Muscu)
Occupational Cancers	(Cancer)
Traumatic Injuries	(Trauma)
Cardiovascular Diseases	(Cardio)
Reproductive Disorders	(Repro)
Neurotoxic Disorders	(Neuro)
Noise-Induced Hearing Loss	(Noise)
Dermatologic Conditions	(Derma)
Psychological Disorders	(Psycho)
Control Technology Research	(Contr)
Respirator Research	(Respir)
Other Occupational Concerns	(Other)

AEROSOL DEPOSITION IN THE HUMAN RESPIRATORY SYSTEM

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Department of Mechanical and Aerospace
Engineering
State University of New York at Buffalo
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Amherst, New York 14260

Occupational Lung Diseases
5 R01 OH00923-05
09/30/83 - 11/30/86
\$58,351 (\$215,528 Cum)

Objectives

This research is directed toward determining the amount and site(s) of deposition of inhaled particles and their subsequent fast clearance in the human respiratory system with the use of theoretical and computational models. Special efforts will be made to examine (i) the effects of various particle factors such as size, shape, charge, mass density and hygroscopicity on deposition and (ii) the extent and causes of intersubject variabilities.

Methodology

Analytical and statistical methods will be employed to achieve the goals with extensive reference to available experimental data. Computer programs are to be developed for predicting deposition under various exposure conditions.

Progress & Accomplishments

At this time, we have completed the following major studies:

1. Modeling of head deposition for spherical and fibrous particles.
2. Development of lung deposition models involving:
 - a. derivation of deposition efficiency formulas in an airway due to various mechanisms;
 - b. study of particle charge effects;
 - c. deposition of ultrafine particles;
 - d. deposition of fibrous particles;
 - e. deposition of hygroscopic particles;
 - f. deposition in young age groups.
3. Development of probabilistic deposition models for:
 - a. deposition of polydisperse aerosols;
 - b. lung deposition in different anatomical models;
 - c. statistical deposition models with random airway structures.
4. Determination of mucociliary transport rates in bronchial airways and the effect of sulfuric acid on such rates.

Significance

Airborne toxic particulates, inhaled into the respiratory system, will deposit there and produce detrimental biological effects. Since the complete removal of these particulates from the industrial environment is practically impossible and economically unfeasible, it is necessary to establish certain exposure standards for occupational protection. It is hoped that within these standards, any biological damage would be unlikely even after long periods of exposure. The results of this research will lead to a better fundamental understanding of the dose-response relationship for occupationally-induced

lung diseases and thus may serve as a basis for developing an effective prevention strategy.

Publications

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HEALTH RISKS IN THE VERMONT GRANITE INDUSTRY

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Occupational Lung Diseases
5 R01 OH01035-04
04/01/83 - 03/31/87
\$287,892 (\$1,042,934 Cum)

Objectives

The overall aim of this study is to carry out a comprehensive assessment of health risks in the Vermont Granite industry, which involves exposure to low levels of quartz. The major specific objectives are three:

1. To perform a longitudinal study of pulmonary function losses in the current workforce. Previous estimates of losses suggested that the loss of forced vital capacity and FEV₁ in this group of workers was excessive and was related to occupational dust exposure. Certain doubts were raised about these previous studies and the current effort is an attempt to confirm, deny, or qualify the suggestion that excessive loss occurs independent of smoking or aging.

2. The second objective is to carry out an industry-wide radiographic survey to ascertain the prevalence of abnormalities consistent with pneumoconiosis in workers who have been exposed to low levels of granite dust since 1940. Radiographic surveys of the industry have been done by the Vermont Industrial Hygiene Division since 1940, suggesting as of 1965 that silicosis was being controlled, but studies published in the 1970's stated that a 30% prevalence of abnormalities existed. The current effort is to assess whether radiographic silicosis or silicotuberculosis exist and to what degree. Many of the workers studied will have a prolonged exposure to granite dust (more than 30 years). Those workers showing abnormalities will be compared with their normal colleagues in terms of smoking history and dust exposure to ascertain the significance of the radiographic changes.

3. The third aim is to make systemic measurements of dust levels in different size stone sheds and in different occupational categories within the industry.

A subsidiary objective includes a study of retired granite workers both with and without radiographic silicosis who have been followed longitudinally both by x-ray and pulmonary function testing. The purpose of this aspect of the project is to ascertain the rate of which pulmonary function changes occur in men who are no longer occupationally exposed to dust and to compare rates of pulmonary function decrement in those with and without silicosis.

Methodology

For spirometric testing, we have used a Collins 13 liter water-sealed Stead-Wells type apparatus since 1979. Testing is carried out at the workplace. Guidelines set down by the American Thoracic Society are being followed in terms of evaluating the results and the acceptability of tracings.

Collection of 8-hour dust samples has utilized personal size selective samplers running at 2 liters per minute for gravimetric estimation. The samples are being analyzed for percentage quartz using x-ray defraction techniques.

Chest x-rays are being read by three certified B readers using the ILO/UC classification system. The definition of abnormality we propose to use is based on a profusion reading of 1/0 or greater of densities considered

to be consistent with pneumoconiosis by either two or three of the readers. These are of course analyzed as to type of abnormality and location as prescribed by the format.

Progress & Accomplishments

Spirometric surveys have now been carried out four times, including two industry-wide surveys which antedated the beginning of the project, which were done in 1979 and 1981. Subsequent surveys have been done in the Spring of 1983 and 1985. Participation of the work force appears to be high. These results have not been analyzed as yet longitudinally, though the data has been entered in the computers and is nearly ready for analysis. We anticipate carrying out a final survey in the Spring of 1987 which will provide an 8-year period of observation of this group of workers.

Approximately 450 dust samples have been collected in various sheds and assorted occupations. The average dust level is 581 mcg/M^3 ; the range of dust exposure varies from 10 to over 2000 mcg/M^3 . These values are comparable to those observed by previous investigators and by the Vermont Occupational Safety and Health Administration. Depending on occupational category, the average values for total dust varied from a low of 389 mcg/M^3 for boxers to a high of 912 mcg/m^3 for maintenance workers (N-6). The roentgenographic survey carried out in 1983 numbered approximately 970 workers and this material has been interpreted and analyzed. Only 26 films were considered to be abnormal as defined by 2 or 3 readers seeing opacities consistent with pneumoconiosis in a profusion of 1/0 or greater. However, 20 of these were abnormalities of the irregular or reticular type and appeared to be predominantly lower lobe.

In addition, we have either x-rayed recently or found films on an additional 1000-2000 former or retired workers who were no longer employed during the survey of 1983. These films are being interpreted and coded and will be analyzed in a comparable manner. The total x-ray analysis should include close to 90% of all Vermont stone-shed workers employed since 1940 and exposed to low dust levels.

Significance

The usefulness of this information pertains mainly to the issue of a safe occupational level of quartz exposure. Currently, the Department of Labor accepts a level of 100 mcg/M^3 of quartz, whereas NIOSH has recommended a level of 50 mcg/M^3 . The levels of dust that we have measured are certainly above the NIOSH recommended level and may be considerably higher than the current levels, based on our estimate of 18% quartz using x-ray diffraction classification. Based on the data so far, it appears that the level of radiographic silicosis is extremely low and there appeared to be no large opacities suggesting complicated disease nor any tuberculosis. The issue whether contemporary dust levels are leading to an excessive loss of pulmonary function should be possible to extract from the data since we will have internal controls. Thus, we will be able to compare granite workers exposed to a given level of dust and of comparable lengths of exposures among smokers and non-smokers.

Publications - None to date

FIBER DEPOSITION IN HUMAN UPPER AIRWAY MODEL

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Occupational Lung Diseases
5 R01 OH01605-03
09/30/83 - 08/31/86
\$69,061 (\$190,558 Cum)

Objectives

The hypothesis to be tested in this study is that the human upper respiratory airways play a significant role in the mode of exposure to airborne fibrous materials. Fibers removed in the nasal or oral airways are not accessible to the deep lung and this can play no role in chronic respiratory disease. Thus sampling of airborne fibers for assessment of risk of respiratory diseases ought to include those lengths and diameters accessible to the non-ciliated airways. The overall aim of the study is to assess the effects of fiber physical characteristics, mode of breathing, and breathing parameters on upper respiratory tract fiber deposition and compare these effects with those for non-fibrous particles.

Methodology

Since no experimental measurements of fiber removal in the human upper airway have been reported, the approach of this study is experimental in nature. While it would be ideal to carry out the studies in human subjects, this is not possible for fibers because of the unknown toxicity of low exposures of glass fiber. Thus, the approach adopted for the experimental measurement of overall and regional removal of fibers in the human upper respiratory airways is to employ an accurate physical model through which fiber aerosols are passed under flow conditions simulating various conditions of breathing. The fibers used in the study are grouped according to narrow ranges of diameter and lengths adjusted by pretreatment. Fibers are labeled with a short-lived radioisotope so that small fiber masses may be measured both within and in the effluent stream of the model. Experiments with monodisperse spherical aerosols have been carried out in similar models for local nasal deposition for comparative purposes.

Progress & Accomplishments

Experiments have been carried out with aerosolized, radiolabelled glass fibers to measure the overall and regional deposition in an upper airway nasal inspirational physical model. The experimental studies were carried out at several flow rates to simulate rest and states of exercise ranging from 20-60 lpm. The model of the upper airway began at the nasal entrance and terminated at the mid-trachea. It included nasal hair in the nostril area which was simulated by tufts of false eyelashes located in the anatomically correct location. The density of the simulated hair was determined by counting nasal hair in one nostril of an adult which yielded a total of approximately 300 hairs. Parallel experiments without nasal hair were carried out to determine the filtering capacity of the nasal hair.

The aerosolized fibers were generated by a rotating blade which removed fibers from a compressed plug which was continuously fed into a chamber and

cleared by a constant flow of dry air. As the fibers were drawn through the model at a constant flow rate, a sample of the fiber aerosol was collected on a membrane filter to determine fiber concentration and size distribution. Simultaneously, a membrane filter sample of the fibers which penetrated the nasal model was collected from which was determined the fiber mass percent penetration.

The results of the experimental studies showed significant nasal deposition percent, ranging from 10-90% of inspired aerosol depending on flow rate, fiber size, and presence of nasal hair. There was a weak dependence on flow rate for some fiber sizes which indicated that the primary mechanism of deposition was interception, while other fibers showed flow dependent deposition dependence more consistent with inertial deposition.

The method for labelling glass fiber with Tc^{99m} which was developed in the laboratory proved to be an efficient and simple method which could readily be used for other studies of fibers in the respiratory tract. The proposed studies of fiber deposition in the oral passage were not performed because the oral passage model was judged to be a poor simulant of the oral airway during breathing in contrast in the nasal passage model.

Significance

Major efforts are being made to identify, quantify, and control exposure to airborne fibers. A primary component of this exposure is inhalation, and the respiratory diseases associated with inhalation require deep lung deposition and lengthy retention. For fiber particles to cause such respiratory diseases, they must penetrate the upper airways. In order to correctly assess the risk of exposure, the fibers most likely to reach the deep lung must be known. With the information from this study, it will be possible to predict which airborne fibers will most effectively reach the deep lung and which fibers will deposit in the upper airways where they can be cleared to the G-I tract and may be involved in other disease process. Any fibrous aerosols, such as glass fiber, amphibole asbestos, or carbon fiber should be subject to the same empirical equations for predicting deep lung delivery as a function of fiber diameter and length.

Publications

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EXPERIMENTAL PNEUMOCONIOSIS OF SANDBLASTING SUBSTITUTES

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Occupational Lung Diseases
5 R01 OH01970-03
04/01/84 - 03/31/87
\$41,644 (\$214,128 Cum)

Objectives

The use of sandblasting is associated with severe and rapidly progressive silicosis. Recognition of these hazards led to the prohibition of sandblasting in Europe and Australia; in the U.S., sand substitutes are now increasingly being used. They are derived from coal ash residues and slag from smelting furnaces, and are comprised of amorphous silicates of Fe, Al, Ca, Mg, and Ti. The free silica content is only 0.03%. The objective of this project is to develop a rat model to assess the pulmonary fibrogenic potential of sand substitutes. Both normal and silicotic rats are investigated after prolonged exposure to the substitutes. After exposure, both groups are followed for an additional 12 months. Control rats are maintained for comparisons. Rats are sequentially sacrificed for histologic and tissue mineral content evaluations as well as biochemical assessment of lung collagen content.

Methodology

The first group of rats is exposed by inhalation to a ball-milled dust of the substitute at a concentration of 10 mg/m³ for 6 hrs/day, 5 days/week for 12 months. Silicosis is induced in a second group by 3 intratracheal instillations, each consisting of 0.833 mg silica dust suspended in 0.3 ml saline (total dose of 2.5 mg). Nine months after instillation, these animals are also exposed by inhalation to the substitute dust (same dust concentration as the first group) for 6 months. Rats are evaluated for: (1) lung mineral burden; substitute dust determined by ICP and silica dust by X-ray diffraction, (2) hydroxyproline content of lung tissue; and (3) histology; evaluation by light microscopy of sagittal sections of right and left lung lobes using hematoxylin & eosin, Gomori's silver impregnation, Masson's trichrome and Harrow Viche stains.

Progress & Accomplishments

A laminar flow whole body (64 rats) inhalation exposure system was designed, constructed and tested. The coefficient of variation of the amount of dust deposited in rats' lungs was about 18% (10 rats). A new, simple and consistent method for intratracheal instillation of silica dust was developed and evaluated. The amount of silica dust retained in the lungs was 80% of the injected amount with a coefficient of variation of 13.5% (6 rats). The amount of silica dust retained in the lungs was 80% of the injected amount with a coefficient of variation of 13.5% (6 rats). Analysis of original substitute granules and ball-milled dust by the ICP method indicated that the ball-milling procedure did not change the chemical composition of the starting material.

Histologic examination of lungs obtained from rats exposed to Stanblast dust for 6 and 12 months displayed small, discrete, cellular accumulations

profusely scattered throughout the parenchymal field. They were most often noted in peribronchiolar, perivascular and subpleural location. The majority of these cellular collections were composed of large alveolar macrophages and, less frequently, lymphocytes. Polymorphonuclear leukocytes were rarely seen. Stanblast particles were readily apparent in these locations both intra- and extracellularly. In many instances, the particulates were especially numerous and appeared to completely obliterate the cellular components within the foci. Examination of the alveolar walls surrounding these nodules revealed individual interstitial macrophages containing granules of Stanblast material. Stanblast burdened cells were also frequently seen within the peribronchiolar lymphoid tissues. In addition to these benign appearing lesions, a very small proportion of the foci, observed in the 12-month exposed rats, were dominated by a lymphocytic infiltrate (rather than alveolar macrophages), and displayed an apparent increase in eosinophilic ground substance, suggestive of early granulomatous formation. Assessment of collagen deposition within these foci utilizing the Harrow Viche stain did not, however, reveal a significant increase in interstitial collagen. These variants of Stanblast lymphocyte collections were noted in four of the ten animals exposed for 12 months. The biochemical evaluations corroborated the light microscopic findings, and did not reveal a statistically significant difference in the hydroxyproline contents of the control and exposed rat lungs for both the 6- and 12-month group.

Significance

This study will assess for the first time the safety of these substitutes. It is believed that this rat model will have application in delineating and predicting the potential occupational hazard for abrasive blasters with and without pre-existing silicosis. Occupational exposure standards are based on the assumption that the risk of any particular agent is not influenced by exposure to other environmental factors. This study will provide important information on the risk of lung fibrosis from biologic interactions between different inhaled agents, which could affect the future development of occupational standards. More specifically, this project may demonstrate increased risk of progression of silicosis if silicotic sandblasters use the substitutes.

Publications - None to date

RESPIRATORY HAZARDS OF POULTRY WORKERS

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Occupational Lung Diseases
5 R01 OH02029-03
09/01/84 - 08/31/88
\$232,099 (\$531,688 Cum)

Objectives

We propose the first indepth medical and environmental study to investigate possible occupational hazards of poultry workers. Our research has four aims:

To characterize the operations within the various components of the poultry industry on a national basis, relative to occupational health risks.

To identify the significant subgroups of poultry workers at potential risk.

To evaluate the air in the work environment of selected establishments for potentially harmful vapors and dusts.

To make any indicated recommendations to the poultry industry for improvement of the workplace and worker protection.

Methodology

The proposed investigation is a four-year study consisting of 3 phases. Phase I is an effort to develop an industry-wide worker exposure profile by performing a series of walkthrough evaluations of typical turkey-growing, chicken-growing, hatcher, egg producing, and poultry processing operations. We intend to identify and quantify the subgroups of poultry workers with the greatest potential for occupational health risks for comprehensive medical and environmental studies in phase II.

In phase II (years 2 and 3) we will recruit 400 workers for medical studies, (100 workers from each of the following 4 subgroups: turkey, broiler, layer, and processing operations). Medical assessment of these subject will include administration of an ATS questionnaire (modified for occupational history), spirometry before and after a minimum of 2 hours of work, analysis of serum precipitins, and allergy skin testing. This data will be compared to similar data from two reference populations: 1) regionally proportioned unexposed blue collar workers, and 2) a blue collar reference population previously studied by NIOSH. Environmental assessment of the estimated 116 work-sites and 20 control work-sites will include measurements for ammonia, carbon dioxide, carbon monoxide, total and respirable dust, and endotoxin.

Phase III, data analysis, will occur during year 04 of the study. We will examine the relationships of environmental exposures to respiratory symptoms and pulmonary function. We will also examine relationships of environmental control systems and levels of environmental contaminants. We will determine what recommendations are needed, if any, to improve the work environment, to protect workers from potential health hazards, and for possible future research.

Progress & Accomplishments

Phase I of the project includes both data collection and data generation activities. A comprehensive literature review has been underway and is continuing. This review involves production figures, processes, structures,

work practices, a general assessment of potential occupational exposures, and geographical influences on these characteristics. The goal of this phase is to determine the size of the population at risk and to get an idea of air contaminants that may be encountered in phase II.

Walkthrough assessments of poultry operations in Iowa, Minnesota, Arkansas, and California have been completed. Data has been collected concerning concentration of airborne dust and ammonia. Types of building design with the inherent variables of ventilation and bird handling have been ascertained. From this, a risk index was being developed to qualify those groups with greatest exposure and potential occupational hazards. Phase II of the study had begun, and nearly 100 workers from 55 laying farms have been tested to dated, along with 27 turkey confinement workers from 11 farms. Cooperation with local poultry and egg producers has generally been quite good, and recruitment is being continued for the remaining exposure groups and blue collar controls.

Significance

Agricultural workers are a large and understudied population. Over the past few years we have recognized an emerging occupational respiratory disease hazard to farmers and farm workers within swine confinement buildings. Since most of the poultry in the U.S. are now raised in confinement buildings, we anticipate similar occupational problems may be present in poultry workers. Our pilot studies suggested they experience exposures and respiratory symptoms similar to swine confinement workers.

Publications - Abstract(s) available

PULMONARY TOXICITY OF THE SEMICONDUCTOR, GALLIUM ARSENIDE

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Occupational Lung Diseases
1 R01 OH02076-01
11/01/84 - 10/31/87
\$129,194 (\$241,433 Cum)

Objectives

The objective is to investigate the lung toxicity and potential systemic toxicity of particulate gallium arsenide (GaAs), a semiconductor in industrial use. Previous work has shown a dose-dependent increase in rat lung weight after intratracheal GaAs that was not caused by aqueous edema. These lungs showed histological changes after 14 and 28 days but fibrosis was not evident from the histology nor were 4-hydroxyproline levels elevated.

The aims of this study are to define the lung changes induced by GaAs and to choose an animal model other than the rat for these studies. Another animal model will be chosen based on its sensitivity to indicators of systemic arsenic toxicity. The lung toxicity will be examined at times up to 6 months after exposure to intratracheal GaAs to determine if significant changes have occurred in lung lipids, collagen, protein or DNA. These changes will be correlated with tissue and blood levels of gallium and arsenic.

Methodology

The response of the rat to arsenic will be compared to the mouse, hamster and guinea pig. The systemic toxicity parameters examined include: hemoglobin, blood and fecal arsenic, plasma glucose, creatinine and urea nitrogen, urinary porphyrin and kidney pyruvate dehydrogenase. The sensitive parameters will be chosen and dose-response relationships determined.

The lung toxicity of GaAs after intratracheal administration of 100, 30 and 2.5 mg/kg will focus on the changes in collagen and histology. Lung collagen will be examined by using stains specific for collagen in addition to the analysis of 4-hydroxyproline in the lung. Fixed slides are examined after Masson's trichrome and silver argyrophilic stains and frozen slides were examined by immunofluorescence for fibronectin and collagen III. Any changes observed will be compared with those induced by 200 mg/kg silica and a maximally tolerated dose of As₂O₃. Lipid changes will be studied by measuring cholesterol and phospholipids. Other parameters will be examined to determine the overall lung response: lung wet weight, lung dry weight, protein and DNA. Arsenic and gallium levels in lung, blood and excreta will be measured. Arsenic will be determined by atomic absorption spectrophotometry after arsine generation and gallium will be determined by fluorescence spectrophotometry after chelation or flame emission methods.

Progress & Accomplishments

The rat, mouse, hamster and guinea pig were examined for their sensitivity to systemic arsenic toxicity. Although all responded, the mouse was the most sensitive using the parameters of pyruvate dehydrogenase activity depression and urinary porphyrin level elevation. The rat was much less sensitive to the systemic effects of arsenic but was sensitive to the lung toxicity of GaAs.

Intratracheal administration of 100 mg/kg GaAs to rats showed chronic strong fibroproliferative inflammation in the lung, often localized possibly due to non-uniform distribution of the test agent. Administration of 30 mg/kg GaAs to rats showed less severe changes than the higher dose and the magnitude of the pathological changes, which mainly included cellular infiltrate and edema, were more evident at seven days exposure than at 28 days.

For silicia, every parameter examined (cell infiltrate, edema, abnormal cells, fibrosis and reticulin) showed excessive scores. The accumulation of collagen at four weeks exposure was more than seven days, although at both time periods, the collagen content was excessive. The nodules, loaded with cells and collagen, were a predominant feature.

Saline treated control animals had normal lung morphology. Changes in the biochemical parameters measured were consistent with the above pathological changes.

Histology and biochemical measurements are being completed on animals held for a 3 and 6 month recovery period after the exposure. Rats treated with 2.5 mg/kg GaAs and mice treated with GaAs are presently under study.

Significance

The pulmonary toxicity after GaAs exposure that was seen in previous experiments has been confirmed in these studies. The initial effects of 100 mg/kg GaAs are about as severe as 200 mg/kg silica but, unlike the silica treated rats the GaAs treated animals appear to be recovering. The time course and dose-response effects of the GaAs are being defined under the single dosing conditions being tested. We hope to determine the important parameters that affect this pulmonary toxicity and to define the role of systemic toxicity in future studies.

Publications - Abstract(s) available

Carter DE, Bellamy WT: Toxicology of the Group III-V Intermetallic Semiconductor, Gallium Arsenide. Accepted for publication in "Biological Monitoring of Toxic Metals", Proceedings of the 17th Rochester Conference. In press.

EPITHELIAL SURFACE PROTEINS: MARKERS OF CANCER RISK

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Occupational Lung Diseases
1 R01 OH02114-01
01/01/86 - 12/31/88
\$132,884 (\$132,884 Cum)

Objectives

We planned to study alterations in the bronchial epithelium caused by exposure to cigarette smoke and asbestos. We have chosen to compare histologic evidence of mucosal injury (metaplasia) with immunochemical evidence of metaplasia. We will relate alterations in these markers to smoking history, asbestos exposure and dietary retinoid ingestion. We will compare asbestos workers to subjects with a variety of other lung diseases and to normal control subjects. We hypothesize that:

1. The attack rate of metaplasia should be very high in asbestos workers.
2. That smoking history should correlate directly and retinoid ingestion inversely with evidence of metaplasia.
3. That smoking cessation may be important in modulating these markers.

Methodology

Fourteen asbestos workers received one bronchoscopy each. Twelve subjects were Connecticut residents, while one each traveled from Florida and Virginia at their own expense to participate. The workers included eight smokers and six subjects who had discontinued smoking three months to five years prior to the bronchoscopy. The average age was 57 years (range 43-70). All are or were insulators with over 20 years exposure to asbestos. The bronchoscopic procedure was smooth. One minor complication was noted. An episode of presyncope which was controlled by terminating the procedure prematurely. We changed the protocol to eliminate codeine premedication. We have had no recurrence. Additionally, we have studied four subjects with mild COPD, nine subjects with bronchiolitis obliterans and seven subjects with IgA deficiency and recurrent lung infections.

Each patient received an occupational history, dietary history, social history (tobacco and alcohol use). Pulmonary functions tests and chest x-ray provided clinical evidence of asbestos- or smoking-induced lung disease. Nasal wash and parotid fluid were obtained and a bronchoscopy was performed. During the bronchoscopy, I obtained four to six biopsies from subcarinas of large airways. Bronchial brushings were suspended in saline. Finally, a lavage was obtained from an unbiopsied airway.

Progress & Accomplishments

Pulmonary functions tests of asbestos workers showed a restrictive ventilatory defect. Total lung capacity was reduced to 75% of predicted and diffusion capacity was decreased to 77%. Chest x-rays revealed pleural plaques in all subjects and parenchymal fibrosis in 4 of 14 workers.

Bronchial biopsies disclosed metaplasia in seven of eight subjects. Tissue is being processed for the remaining 6 patients. We plan to stain these biopsies to study the distribution of keratins immunohistochemically. Furthermore, we have performed in situ hybridization of c-DNA probes for keratins (provided by Dr. Elaine Fuchs) and for c-ras and c-myc oncogenes.

Grain counts of these preparations are in progress. Bronchial brush cells (79% epithelial cells) will be lysed, cytosol proteins will be recovered and eletrophesed into polyacrylamide and western blots will be stained for keratins.

Bronchial lavage cells contained 90% alveolar macrophages, 8% lymphocytes and 2% neutrophillic polymorphonuclear leukocytes.

Bronchial lavage fluid from workers (n=12) has shown detectible keratins in 75%. This contrasts with the frequency of keratins in other diseases: none of 4 COPD patients; none of 7 with acute infection and three of 9 with bronchiolitis obliterans (chronic bronchial inflammation).

Significance

We have noted a high frequency of metaplasia in this population of asbestos workers who are current or former smokers. Although we are unable to say the best way to assess this lesion (histologic or biochemical analyses), we are confident that this group represents an excellent target population for attempts to modify pharmacologically epithelial growth and differentiation. In the coming years, we will collect more patients, assess the data to evaluate the utility of various markers (biopsy, lavage, saliva, nasal wash) and recruit some of these patients with metaplasia for an intervention study.

Publications - Abstract(s) available

TANNIN AS A BYSSINOTIC AGENT-EFFECT ON ENDOTHELIAL CELLS

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Occupational Lung Diseases
5 R01 OH02116-02
06/01/85 - 05/31/88
\$54,569 (\$114,674 Cum)

Objectives

Byssinosis is a significant occupational health problem among cotton mill workers. The source of the byssinotic agent appears to be the cotton bract, although the specific causative bract component or components are unknown. This project comprises an evaluation of the effect of condensed tannin, a purified component of the cotton bract, on vascular endothelial cells. The overall goal of this proposal is to characterize the effects on vascular endothelial cells mediated both by purified tannin and by aqueous extracts of the cotton bracts from which the tannin was isolated. We believe that an understanding of the cellular toxicity of tannin may offer insights into the pathophysiology of byssinosis.

Methodology

Specific Aim 1: Characterization of time and dose dependencies of endothelial cellular toxicity mediated by tannin and bracts extracts.

The experiments addressing this specific aim consist of assays of cellular toxicity using ^{51}Cr release from endothelial cells exposed to varying doses of tannin for varying periods of time. These experiments yield toxicity curves of tannin effects on the endothelial cells.

Specific Aim 2: Determination if tannin alters paracellular endothelial permeability prior to the onset of cell death.

Experiments in this specific aim are intended to determine if the barrier function of endothelial cells is affected by tannin, that is, if an endothelial monolayer might be affected by tannin in other ways than simple cell death. The methods to accomplish this aim involve measurement of paracellular permeability using exclusion of vital dye by endothelial cell monolayers.

Specific Aim 3: Determination if tannin promotes detectable changes in endothelial cell structure or function prior to cell death.

The experiments in this specific aim will determine if significant sublethal injury is induced in the endothelial cells by tannin, and whether such injury significantly affects cellular function. These experiments assess several parameters of cellular function, including cellular protein synthesis, serotonin uptake, and pinocytotic rate.

Specific Aim 4: Characterization of the effects of chronic, sublethal exposure to tannin on endothelial cell function.

This last specific aim builds upon the data accumulated in the first three specific aims. We will determine the minimum tannin dose and extent of exposure required to induce the various specific toxic effects delineated in the first three specific aims. This is of major importance in regard to cumulative toxicity that a cotton mill worker could experience over a many year exposure to cotton bracts. An important aspect of these experiments is the reversibility of any of the observed tannin-induced effects described above.

Progress & Accomplishments

Specific Aim 1: The experiments proposed in Specific Aim 1 have been accomplished and are described in the publication listed below. We have defined the time and dose dependency of tannin toxicity to endothelial cells, and compared these data to tannin effects on peripheral skin fibroblasts. These data show that the endothelial cell appears to be particularly, perhaps uniquely, susceptible to tannin-mediated effects.

Specific Aim 2: We have not as yet performed experiments that address paracellular endothelial permeability, although we have now succeeded in consistently growing the endothelial cells on the cytodex beads. Therefore we expect that these experiments of Evan's blue dye inclusion can be accomplished in the next six to nine months.

Specific Aim 3: We have performed experiments of cellular protein synthesis during the initial, prelethal, period of exposure to tannin as described in the grant proposal. Although cellular release of ^{51}Cr does not occur until after three hours of tannin exposure and protein synthesis continues for several hours following the initiation of a toxic tannin dose, synthesis of all proteins is profoundly diminished within the first half hour of exposure. We have determined this using endogenous labeling experiments of serial pulses of ^{35}S -methionine in tannin exposed cells. We are presently performing experiments of cellular transport of various amino acids to determine if this toxicity is mediated by, among other things, an interference with transmembrane uptake of amino acids and glucose. Our morphologic observations accomplished in Specific Aim 1 lead us to believe that the primary toxic effect of tannin is on cellular membrane function.

Significance

We believe that the major significance of the project so far is in describing the dose and time dependencies of endothelial cellular toxicity to cotton bract tannin. We find it of great interest that skin fibroblasts, a mesenchymal cell remote from the vascular tree, is relatively resistant to tannin-mediated toxicity. Further, there are obviously profound membrane effects provoked in the endothelial cells by cotton bract tannin. In addition, we have shown that the toxicity to endothelial cells by crude cotton bract extract is primarily accounted for by the tannin in the extract. Since bract extract is a complicated mixture of materials, this observation supports our original hypothesis that tannin may be an important byssinotic agent.

Publications - Abstract(s) available

Johnson CM, Hanson MN, Rohrbach MS: Toxicity to Endothelial Cells Mediated by Cotton Bract Tannin: Potential Contribution to the Pathogenesis of Byssinosis. *Amer J of Pathology* 122:399-409, March 1986.

Johnson CM, Hanson MN, Rohrbach MS: Endothelial Cell Cytotoxicity of Cotton Bracts Tannin and Aqueous Cotton Bracts Extract: Tannin is the Predominant Cytotoxin Present in Aqueous Cotton Bracts Extract. *Environ Health Perspect* 66:97-104, 1986.

210 POLONIUM RADIATION AND DITHIOLS

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Occupational Lung Diseases
1 R01 OH02185-01
01/01/86 - 12/31/87
\$75,132 (\$75,132 Cum)

Objectives

The overall aim of this project was to discover compounds that would protect rats from the lethal effects of the radon decay product, polonium-210 and to extend any findings to determine whether such compounds will result in decorporation or excretion of polonium-210 from the animal.

Methodology

Polonium-210 was given ip for the protection studies and SC for the decorporation studies. For the decorporation studies, after 21 days of potential therapy, tissues were removed and the alpha- emitting radioactivity determined. These experiments are very hazardous to perform and because protection devices to protect the experimenter must be worn. Thus they are slow and expensive.

Progress & Accomplishments

So far it appears that at this stage of the investigation we have one of the most potent agents known for the decorporation of 210-polonium. DMPS, DMSA, DMPS and N-acetyl-L-cysteine have been investigated as to their activity for protecting rats against the lethal effects of 210-Po and their mobilization of tissue 210-Po. Rats given 210-Po (40uCi/kg) ip had a median survival time (mst) of 39 days. The mst was increased to 106 days when any of the three dimercaptans were administered sc. ($p < .002$). After 21 days, kidney levels of 210-Po in rats given 210-Po (0.4uCi/kg) sc, followed one hour later by DMPA, were only 28% of those of the untreated controls and significantly lower than those receiving DMSA, DMPS, N-acetyl-L cysteine, or WR2721 under the conditions of the present experiments. After DMPA treatment, the 210-Po levels of the spleen, a very radiosensitive tissue, was 25% of the saline treated control. DMPA may have some unique properties as compared to the other water soluble dimercapto metal binding agents.

These experiments with DMPA were performed with suboptimal doses of DMPA since the material is very difficult to obtain. The results will be even better after using more DMPA. DMPA has great potential for becoming the decorporating agent for polonium isotopes, which are decay products of radon-222.

Significance

There has been recent concern about radon and its decay products as major environmental health hazards. Polonium-210, an alpha emitter, is one of the longer life decay products of radon-222. Radon-222 is a gas that decays through a sequence of radioactive elements that includes the alpha emitting polonium isotopes, 218-Po, 214-Po and 210-Po. This radioactive noble gas and its daughters contribute 40% of the annual dose of radiation to which most humans are exposed. The production of radon and its radioactive decay products occurs continuously in the ground from the decay of radium.

High concentrations of this radioactive gas are often carried in ground water and it diffuses readily through the soil and into the atmosphere. The concern about radon and its daughters has been stated succinctly as follows: "It is not so much radon itself that is of concern, but rather the short-lived daughters of the gas, which are radioactive isotopes of solid elements such as polonium. These solid particles form a radioactive aerosol; when inhaled, they irradiate the lung tissues and increase the risk of lung cancer".

Polonium-210 occurs widely in nature and is an important component of the natural radiation background to which man is exposed. It enters the human body via two routes, by ingestion in the food and by inhalation.

These studies can help evaluate the damage done to a living body by the presence of alpha emitters. Polonium-210 is found in mining environments, in some poorly ventilated work places and houses, in nuclear facilities and other environments. In addition, our studies can make experimental information available to physicians who have to treat humans after exposure to alpha emitters.

Publications - None to date

MECHANISM AND TREATMENT OF PHOSGENE POISONING

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Occupational Lung Diseases
7 R01 OH02264-01*
08/01/84 - 07/31/87
\$86,126 (\$213,918 Cum)

*Previously supported under Grant Number R01 OH02023.

Objectives

The purpose of this research project was to establish a model of acute toxic gas inhalation from an oxidant gas, study its pathophysiology and determine if a method of treatment could be developed which might be used immediately after accidental exposure to attenuate lung injury. We chose phosgene as the model gas because it is a potent oxidant, is widely used in the synthetic chemicals industry, and produces profound lung injury analagous to that seen from other oxidants, including methyl isocyanate, the agent responsible for Bhopal.

Methodology

Male New Zealand white rabbits (3-4kg) were exposed to phosgene in a specially constructed facility. Phosgene (200 ppm in ultrapure air at 1800 psi) was reduced to 10 psi using a corrosion resistant regulator and delivered to a sealed plexiglass exposure box at 5 l/min through stainless steel tubing. The exposure box containing the rabbit was housed inside an exposure hood. Effluent from this hood and the box was detoxified by the scrubber which exposes phosgene to an aqueous mist of 0.2% sodium hydroxide, converting the phosgene into CO₂ and water.

Dose of phosgene was varied by increasing the duration of exposure and expressed as the product of concentration x time (ppm-min). Lung perfusion was performed 4 hours after exposure. After pentobarbital anesthesia, the left chest was opened and pulmonary artery and left atrium were cannulated. The lungs were perfused in a recirculating manner with blood and protein-free Krebs Henseleit solution at 50 ml/min. The lungs were ventilated through a tracheostomy at a rate of 18 breaths/min and a tidal volume of 7 ml/kg with 2 cm H₂O positive end expiratory pressure. Pulmonary arterial, left atrial and maximal inspiratory tracheal pressures were monitored with pressure transducers and a polygraph. The perfusate reservoir was suspended from a force displacement transducer so that rate of lung weight gain from edema formation could be monitored as the converse of weight change of the reservoir. Left atrial pressure was maintained at zero. Following perfusion, lungs were weighed, then dried 72 hours in a vacuum oven for determination of wet/dry lung weight ratio.

Progress & Accomplishments

In initial studies, the dose of phosgene necessary to produce profound lung injury was established by exposing rabbits to 1000, 1500 and 2000 ppm-min. We determined that 2000 ppm-min phosgene (P) consistently produced severe lung injury and used this dose in subsequent experiments. As shown in the table below, rabbits exposed to phosgene had a marked elevation maximal

inspiratory tracheal pressure (Pt), an increased rate of lung weight gain (AW) and elevated wet/dry lung weight ratio (W/D), but pulmonary arterial pressure remained normal. Development of lung edema despite normal perfusion pressure indicate that phosgene causes an increase in alveolar capillary membrane permeability. The absence of pulmonary vasoconstriction in rabbits exposed to phosgene is in contrast to the marked pulmonary vasoconstriction observed in perfused lungs from vascular administration of oxidants such as hydrogen peroxide.

The currently recommended treatment for acute toxic gas inhalation is high dose corticosteroids. We hypothesized that agents which increase cyclic AMP such as the beta-2 adrenergic agent terbutaline or the phosphodiesterase inhibitor aminophylline might attenuate lung injury from phosgene, since these agents have been shown to prevent increased vascular permeability in systemic vessels from a variety of stimuli. Rabbits were exposed to phosgene, then treated with methylprednisolone (30 mg/kg IV), terbutalin (20 ug/kg SQ, then 10 ug/kg hr) or aminophylline (16 mg/kg IV, then 8 mg/kg IP every 2 hours) immediately after exposure. Lungs were perfused 4 hours later. Methylprednisolone failed to protect from lung injury, but both terbutaline and aminophylline markedly attenuated the increase in Pt, AW and W/D ratio from phosgene.

	Pp mm Hg	Pt mm Hg	W/gm/min	W/D
Unexposed	14 ± 0.8	7 ± 0.4	.08 ± .01	7.9 ± 0.4
Phosgene 200 ppm-min	15 ± 0.9	22 ± 2	1.20 ± .39	14.2 ± 1.4
P+methylprednisolone	14 ± 1	27 ± 4*	1.41 ± .44	17.6 ± 7.2
P+Terbutaline	13 ± 1&	14 ± 1&	.21 ± .08*	9.2 ± 1.7*
P+Aminophylline	12 ± 0.4@	16 ± 0.2*	.24 ± .01*	8.5 ± 1.2@

*p<.05; @p<.01; &p<.001 compared to untreated lungs

Significance

Our results indicate that agents increasing cyclic AMP provide substantial protection from lung injury after toxic gas inhalation. These agents might ultimately prove to be a safe and effective therapy which could be administered emergently in the workplace. We hypothesize that elevating cyclic AMP prevents contracture of lung endothelial and epithelial cells in response to injury, thus attenuating injury-induced widening of paracellular spaces, but other mechanisms might also account for their beneficial effect. Corticosteroids provided no protection from injury when given after exposure and should no longer be recommended as a therapy for victims of toxic gas inhalation without further documentation of benefit.

Publications- Abstract(s) available.

Kennedy TP, Hoidal JR, Michael JR, Sciuto AM, Hopkins C, Tolley E, Gurtner GH. Ibuprofen and agents increasing cyclic amp protect against lung from inhaled toxic gas. Submitted.

LUNG COLLAGEN IN SILICOSIS: FIBROSIS MECHANISMS

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Occupational Lung Diseases
5 K01 OH00002-03
09/28/84 - 08/31/87
\$32,400 (\$97,200 Cum)

Objectives

There are four specific aims to be accomplished in this project:

1. To test the hypothesis that collagen crosslinking in silicotic rat lungs differs from that found in normal lungs in one or more of the following aspects: types and amount of crosslinks per collagen molecule, time course of crosslink formation, turnover of crosslinks, reducibility of crosslinks, and location of crosslinks.

2. To test the hypothesis that crosslinking in silicotic rat lungs differs from that found in bleomycin-exposed rat lungs.

3. To determine whether elastin crosslinking can be measured in the lungs of either normal adult rats or rats exposed to fibrogenic stimuli (silica, bleomycin), and if so, to determine if there are differences among the groups.

4. To develop simple screening assays of urine or blood, based on data obtained in Specific Aims #1-3, indicative of early silicotic changes.

Methodology

We have developed techniques for separation and quantification of both conventional amino acids and collagen crosslinks using a flexible reverse-phase HPLC system. The equipment, reagents, and elution programs used for such analyses have been described by us in detail (Reiser and Last, Liquid Chromatography 1:498-502, 1983).

Silicosis was induced in rats by intratracheal instillation of 50 mg of crystalline silica. Fibrosis was also induced by intratracheal instillation of 1-1.5M of bleomycin. In vivo labelling of crosslinks was accomplished by implanting Alzet minipumps containing 800 μ Ci (3 H) lysine.

Progress & Accomplishments

During the second year of the project, we began analysis of the in vivo labelled tissue. Hydroxyproline values were measured in the diaphragmatic lobes and were consistent with previous experiments. We have started measuring label incorporation into hydroxylysine and crosslinks. Although our pilot studies indicated that lung tissue could be directly analyzed with prior fractionation, label incorporation was lower than we had anticipated. The procedure for analysis has therefore been changed somewhat. Whole lungs are washed and digested with collagenase. More than 98% of the collagen is digested (based on hydroxyproline content of the supernate). The supernate is then analyzed for label incorporation into lysine and hydroxylysine using an isocratic HPLC program; the use of collagenase thus allows us to determine the percent hydroxylation of lysine in both total and newly synthesized collagen. The remainder of the supernate is then chromatographed on a Bio-Gel P-2 column, using a pyridine-acetate buffer, to separate the crosslinks from the bulk of the amino acids. The appropriate fractions are pooled and chromatographed by HPLC. Preliminary data indicate increased label incorporation into hydroxypyridinium in silicotic lungs as compared with control lungs at the later time points. These data are consistent with

our findings on unlabelled tissue (Reiser and Last, "Collagen crosslinking in lungs of rats with experimental silicosis," Coll. Rel. Res., in press, 1986).

An important goal of the project was to determine the location of the crosslinking sites at which the observed changes occurred, as such information would help us to define the pathology of silicosis at the molecular level. We have accomplished a great deal in this area and a manuscript is in preparation. Briefly, we have isolated a tryptic peptide from $\alpha_1(I)CB-7$ that shows an increase in DHLNL in silicosis (Ref. 3).

In Specific Aim #3 we proposed to examine elastin crosslinking in control and fibrotic rat lungs. We found no differences in lung desmosine (an elastin specific crosslink). Thus, this aim will not be pursued further.

The 4th Specific Aim was the development of assays for measuring crosslinks excreted in urine to determine if changes in the lungs were reflected in the excretion of collagen breakdown products. We have done considerable work in this area. Our initial protocol included precipitation with acetone. However, we found that a large proportion of the major collagenous crosslinks were present in the supernate, apparently bound to small peptides. In our revised protocol, we now reduce an unfractionated aliquot with $NaBH_4$, hydrolyze, and treat with charcoal. Analysis of the radiolabelled difunctional crosslinks can then be performed as previously described. However, we encountered problems in the analysis of the non-reducible crosslinking OHP. The hydrolysate contained a number of fluorescent compounds with excitation and emission maxima similar to that of OHP. Since OHP shifts its excitation maximum to 330 at neutral or alkaline pH, we added 0.1 M NaOH to the effluent stream. We have begun analysis of urine obtained from rats 6 months after silica exposure. Preliminary data indicate that there may be a slight increase in OHP excreted in silicotic rats.

Significance

In previous studies we have observed that lung collagen appears more "normal" metabolically in silicosis than in other types of fibrosis. In order to investigate the metabolic derangements that cause this apparently "normal" collagen to become deposited in fibrotic nodules, we decided to analyze collagen crosslinks as a way of tracking the metabolic fate of lung collagen from tropo-collagen to mature collagen fibers. Our preliminary data thus far have suggested that site-specific alterations in the level of lysine hydroxylation in silicosis may be a key event in determining whether collagen is destined to become fibrotic. Identification of the derangement at the molecular level should provide insight into the still poorly understood mechanisms of this disease, and may lead to the development of rational strategies for both intervention and early diagnosis.

Publications

Reiser KM, Tryka AF, Lindenschmidt RC, Last JA, Witschi HR: Changes in collagen crosslinking in bleomycin-induced pulmonary fibrosis. *Journal of Biochemical Toxicology* 1:83, 83-91, 1986.

Reiser KM, Last, JA: Early cellular events in pulmonary fibrosis. *Exp. Lung Res.* 10:331-335, 1986.

Geismar LS, Hennessey S, Reiser KM, Last JA: D-penicillamine prevents collagen accumulation in lungs of rats given bleomycin. *Chest* 89:1535-1545, 1986.

OXYGEN FREE RADICAL IN PULMONARY FIBROSIS

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Occupational Lung Diseases
5 K01 OH00007-03
09/28/84 - 08/31/87
\$32,400 (\$97,200 Cum)

Summary

Exposure to asbestos and silica is associated with a variety of pulmonary disorders including pulmonary interstitial fibrosis. Although asbestosis and silicosis have been recognized as occupational lung diseases for nearly a century, the mechanism(s) by which these fibrogenic minerals induce lung cell injury and fibrosis is speculative, particularly at the cellular level.

In this proposal we hypothesize that oxygen free radicals may be one important mediator of lung cell damage and/or fibrosis in vitro and in vivo after exposure to fibrogenic minerals. To test this hypothesis, we will undertake the following studies:

1. Cytotoxicity induced by exposure in vitro to various concentrations of asbestos fibers and silicon dioxides will be determined in lung fibroblasts, alveolar macrophages and epithelial cells.

2. Production of superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH) by these cells after exposure to minerals at various concentrations will be monitored and correlated with results of #1 above.

3. Levels of superoxide dismutase (SOD) in lung fibroblasts, alveolar macrophages and epithelial cells after exposure to minerals in vitro and in lung sections of rats after inhalation of asbestos in vivo will be measured using quantitative biochemical assays and immunocytochemical techniques.

4. We will determine whether mineral-induced injury to alveolar macrophages, fibroblasts and epithelial cells in vitro can be prevented by the addition of scavengers of O_2^- and OH.

These studies will be important in elucidating the importance of oxygen free radicals in mineral-induced lung cell injury, asbestosis and silicosis. In addition, they will contribute information on possible preventative and therapeutic approaches to these serious occupational diseases.

Publications - None to date

SILICOSIS: IMMUNOLOGICAL ABNORMALITIES

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Occupational Lung Diseases
5 K01 OH00018-03
09/28/84 - 08/31/87
\$32,400 (\$97,200 Cum)

Objectives

To compare immunologic findings in silicotic subjects, identified in an epidemiologic study of respiratory disease among Leadville, Colorado, residents, with those in community controls and in nonsilicotic miners having substantial past silica exposure.

Methodology

Fifty silicotics, 43 community controls, and 68 dust-exposed nonsilicotic controls have been interviewed by a local public health nurse. They have also had chest radiographs taken, performed spirometry, given a sputum sample, and given a blood specimen for complete blood and differential counts, immunoglobulins, immune complexes, autoantibodies, and separation of mononuclear cells. Detailed immunologic studies on peripheral blood mononuclear cells being performed include lymphocyte proliferation to three mitogens (phytohemagglutinin, concanavalin A, and pokeweed mitogen) and two antigens (purified protein derivative and candida); helper cells, suppressor cells, and natural killer cell phenotypes; lymphokine production, assayed for interleukin-2 and interferon; and evaluation of natural killer cell activity. Immunologic differences found for silicotic subjects in comparison to dust exposed and unexposed controls will be related to profusion of small and large opacities on chest radiographs and to the presence of mycobacterial infection due to *M. tuberculosis* or atypical mycobacteria. Thus far, seven silicotics have had bronchoalveolar lavage in an independent study which is comparing constituents of lavage fluid to T-cell phenotypes in peripheral blood.

Progress & Accomplishments

The 161 participants have been interviewed and field-tested. Laboratory work and computer data entry are in progress.

Significance

Preliminary data suggest that silicotic subjects, in comparison to their wives, have decreased percentages of helper T-cells and increased percentages of lymphocytes of the natural killer cell phenotype. Through this study effort, we will seek to identify immunologic factors which may predispose dust-exposed individuals to silicosis and which may predispose silicotic subjects to mycobacterial infections. Further understanding of the immunology of silicosis may suggest primary and secondary prevention measures which can be confirmed in future prospective studies.

Publications - None to date

EFFECT OF MINERAL EXPOSURE ON MACROPHAGE FUNCTION

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Occupational Lung Diseases
5 K01 OH00019-03
09/28/84 - 08/31/87
\$26,524 (\$86,261 Cum)

Objectives

The effects of in vitro and in vivo (inhalation) exposure to minerals found in association with coal mine dust on function of individual alveolar macrophages will be studied. Comparison between in vitro and in vivo measurements will permit evaluation of the feasibility of using these in vitro techniques to predict in vivo effects.

Methodology

Dose-response and particle size dependent studies on functional alterations will be conducted using exposure levels which are sublethal to the cells. Two separate techniques will be used to evaluate toxic effects on cells lavaged from male Long Evans hooded rats. Electrophysiological voltage clamp experiments will be used to determine the effect of exposure on plasma membrane permeability to ions and electro-optical techniques will be used to measure the effect on production of superoxide anion, an antibacterial agent produced by the cell. In order to correlate functional alteration with exposure to minerals, backscattered electron imaging and X-ray microanalysis will be performed on individual cells to identify those cells which have phagocytized the materials.

Progress & Accomplishments

An electro-optical method has been developed to measure superoxide (SO) release from single pulmonary alveolar macrophages (PAM) collected from lungs of rat by tracheal lavage. PAM were washed and resuspended and aliquots placed in HEPES buffer in a culture dish (37° C) on the stage of an inverted microscope. During adherence to the plate surface, cells released SO which was measured by the reduction of nitroblue tetrazolium (NBT), contained in the medium, to a formazan precipitate.

Cells contained in a microscopic field were transilluminated at 550 nm (absorbance peak for formazan), televised and video recorded for 40 minutes. The cells became progressively darker as the formazan precipitate was produced. Temporal changes in light intensity over the cells was measured by playing the recorded video images through a photometric analyzer. The light intensity data were converted to a relative mass of formazan produced using the Beer-Lambert law.

Formazan production was represented by a first order model with time delay indicating that PAM are self-regulating in that they reached a new steady state and stopped releasing superoxide. Heterogeneity was apparent since under the same conditions different cells released different amounts of SO at different rates. Changes in light intensity were found to be due to formazan production since removal of NBT from the medium resulted in no change in light intensity over the forty minute measurement period. Several blockers of SO release were tested to determine the specificity of NBT reduction for SO release. Production of and rate of release of SO were reduced 46% to 76%

demonstrating that the majority of formazan production is due to SO release. The method developed is the first to quantify SO release from single cells and should be useful for studying single cell dynamics, heterogeneity and activation of superoxide producing system.

The effects of in vitro and in vivo exposure to minerals on macrophage ability to produce SO were examined. In vitro exposure to 0.025 or 0.05 mg/ml kaolin for 40 min resulted in no significant differences in either total formazan produced or the rate of formazan production. However, in vitro exposure to 0.025 mg/ml of quartz significantly reduced both the maximum and the rate of formazan production. In contrast to the in vitro results, PAM lavaged from animals that had inhaled quartz dusts for 2 or 4 weeks exhibited increased formazan production. However, only PAM analyzed from animals sacrificed 3 days after the end of exposure showed significant increases while cells from animals sacrificed 10 or 31 days post-exposure were not significantly different from controls. These results might suggest that there is an initial acute response to exposure of quartz that decreases the ability of PAM to produce SO, followed by recruitment or production of PAM with increased SO producing capability. It appears that removal of animals from the source of exposure permits PAM to return to control levels of SO production.

Preliminary measurements have also been made in the electrophysiological portion of the study. Successful whole cell patch clamping has been performed and to date we have measured the resting membrane potential and stability in a number of cells. Cells have stayed attached to the electrode for up to 30 minutes and exhibit membrane potentials in the range of -55 to -80 mV. In addition, standard current-voltage curves have been run on several cells. The effect of minerals on the resting membrane potential and on the current-voltage characteristics of PAM will be tested in the next year of the grant period.

Significance

Comparison between in vivo and in vitro exposure will permit evaluation of the feasibility of using these in vitro techniques to predict in vivo effects. This interdisciplinary approach should yield important information on the cellular sites of toxicity of minerals and should provide data to aid in assessing risks following inhalation.

Publications

Digregorio K, Cilento E, Lantz RC: The development of an electro-optical technique to measure superoxide release from pulmonary alveolar macrophages exposed to coal dusts. Proceedings of Coal Mine Dust Conference, Oct. 8-10, 1984, Morgantown, WV p 222-226.

Degregorio K, Cilento E, Lantz RC: Superoxide release from single pulmonary alveolar macrophages. Fed Proc 45:309, 1986.

Digregorio K: Development of an electro-optical method to measure superoxide release from single pulmonary alveolar macrophages. Masters Thesis, Department of Chemical Engineering, West Virginia University, Morgantown, WV, 1986.

GAS AND PARTICLE DEPOSITION IN TRACHEOBRONCHIAL AIRWAYS

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Occupational Lung Diseases
5 K01 OH00022-03
09/28/84 - 08/31/87
\$32,400 (\$97,200 Cum)

Objectives

The research is the first stage of a program focused on the development and critical evaluation of quantitative predictive models for gas and aerosol deposition efficiency in lung airways in humans and animals under a variety of respiratory modes and rates. The initial objective is to develop realistic physical models of the tracheobronchial airways of humans and experimental animals. The second objective is to use the models to examine the intrabronchial dose distribution of both gases and particles. The ultimate objective is to develop predictive models for deposition using both theory and experimentally derived coefficients, including models that account for the pronounced nonuniformity of epithelial deposition on the airways.

Methodology

Single path hollow airway casts of human and dog tracheobronchial airways are built, including a cast composed of all major branches, a cast composed of minor branches, and a cast containing approximately equal numbers of major and minor branches. Flow distribution will be measured prior to cast pruning, and each branch removed will be replaced with an equivalent flow resistor. The single pathway casts will be fabricated so as to permit repeated assembly for experimental deposition measurements.

Aerosol deposition in the small airways will be examined for the distinctly nonuniform deposition patterns that have been previously observed in the major human airways and, more recently, at alveolar duct bifurcations in rodents. The utility of the casts for quantitative detailed gas dosimetry will be demonstrated using NO_2 as a model air pollutant and triethanolamine (TEA) as an absorbing surface. Overall deposition efficiencies at each airway segment and the pattern of deposition within airways will be defined for various constant and cyclic flow rates.

Progress & Accomplishments

Morphometric and flow distribution measurements have been carried out in full human and canine lung casts from which single pathway casts are being produced. The casts extended to a level near the terminal bronchioles. The number of terminal airways on each cast is estimated to be well over one thousand. Morphometric measurements were made on the solid negative wax casts through the sixth generation for all branches and to the terminal airways along three single paths of our models (major, minor, and alternating major-minor path). The human and canine casts had nearly the same number of airway generations for each of the single-path models. Following the morphometric measurements the hollow positive casts of the airways were prepared.

The distribution of air among the airways of the cast was then measured for steady inspiration of 15, 30, 45, and 60 L/min supplied to the trachea for the human, and 7.5, 15, 30, and 45 L/min for the canine. Distribution of

the flow to the lobes of the lung was consistent with what would be expected considering typical lobar volumes if the in vivo distribution of the airflow approximates the volume fraction of lung served by the airways.

In a coordinate study on regional particle deposition, deposition of 0.20, 0.15, and 0.04 μm diameter particles was studied at cyclic inspiratory flow rates of approximately 18 and 34 liters/minute in a central cast of the human tracheobronchial tree. These ultrafine particles are of particular interest for the calculation of the dose to the bronchial epithelium from the short lived decay products of radon-222. The data suggest that the dose from rapid acting toxic ultrafine particles will be highest at the bifurcations and could be about 20% greater than that predicted by a factor of about 2. Until better models are developed the correction factors we have derived are useful for estimating deposition of inhaled particles in the size range to which the decay products of radon are attached.

Significance

The proper development of dose-response relationships for occupational respiratory diseases requires understanding of the determinants of the dose to critical respiratory tract tissues from inhaled gases and aerosols. Knowledge of the deposition patterns and efficiencies within airways is essential for the production of reliable dosimetric models. Predictions of aerosol deposition in the central airways based on uniform deposition are clearly unsatisfactory. Present models are not able to deal with the inhomogeneity of deposition demonstrated in the cast system. It is anticipated that as this work progresses, more realistic theoretical models will result from better understanding of the airflow patterns in the airways. In addition the differences and similarities between deposition patterns in the human dichotomous branching tracheobronchial tree and the canine monopodial branching will be documented. This is important if results obtained in experimental animal models are to be properly extrapolated to humans.

Publications - Abstract(s) available

RESPIRATORY DISEASE IN BERYLLIUM WORKERS

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Occupational Lung Diseases
5 R03 OH01684-02
08/01/84 - 07/31/86
\$13,132 (\$32,257 Cum)

Objectives

This project is an investigation of the respiratory effects of long-term, low-level exposure to beryllium (Be). Beryllium refinery workers have been studied and beryllium exposure data, chest x-rays, and measures of lung function were collected over a period of six years. These data are being analyzed to quantify exposure-response relationships for beryllium, while exploring techniques which may be useful for studying other occupational respiratory disease as well. The project has the following objectives:

1. The characterization of the type and degree of lung dysfunction in workers with chronic exposure to beryllium.
2. The development of exposure-response models of beryllium's effects using both continuous (pulmonary function and blood gas data) and discrete (x-ray and symptom reports data) measures of respiratory effects studied cross-sectionally and longitudinally.
3. The quantification of exposure-response relationships for beryllium exposure and pulmonary function among different population subgroups.
4. The investigation of time trends in disease development, including the possibility of the reversibility of the disease.

Methodology

Beginning in 1977, health examinations of the production workers in a large beryllium refinery have been conducted every three years. Industrial hygiene data were collected from a variety of government and management studies dating from 1947 onward. These data were used to estimate the exposure levels in each job in the plant and the changes in those levels since 1940. Each worker was then assigned an estimated beryllium exposure history based on which jobs he/she held and in which years. The health examination consisted of pulmonary function measurement, a P-A chest radiograph, determination of arterial blood gases, a physical examination, and a questionnaire for the recording of respiratory symptoms, smoking history, and other pertinent information.

Progress & Accomplishments

Statistical analyses were conducted on the data from the 297 white male workers seen in 1977, who comprised approximately 85% of the total production workforce at that time. The population, with an average age of 43 years, had worked an average of 17 years at the plant, and had accrued an estimated median cumulative exposure to beryllium of 65 micrograms/m³-years. After adjusting for age, height and smoking by multivariate linear models, significant associations between beryllium exposure and decrements in forced vital capacity (FVC) and forced expiratory volume in the first second (FEV₁) were observed. In contrast, no association between beryllium exposure and mid-maximum expiratory flow was found. Workers with high exposures to beryllium also tended to have higher alveolar-arterial oxygen gradients

(AaDO₂) and more small and irregular opacities on radiograph than those with lower exposures, again after controlling for age and smoking.

The associations between beryllium exposure and decrements in FVC and FEV₁ were strongest when only exposures more than twenty years prior to the survey were considered. These associations persisted when analyses were restricted to the subset with normal chest radiographs. In contrast, increases in AaDO₂ and in small and irregular opacities on radiograph, were not at all associated with exposures of the distant past, but were associated with recent exposures -- those received in the five to ten years prior to health examination. These data suggest that there may be an effect of beryllium on lung function that is distinct from radiographically diagnosed chronic beryllium disease.

Significance

At least 30,000 workers in the United States are exposed to beryllium. Most of these workers are widely dispersed in small factories, making epidemiologic study of beryllium's health effects difficult. This project represents one of the few opportunities to follow a well-defined cohort of beryllium-exposed workers to study the chronic, subclinical effects of exposure to the metal.

The methods being developed in this project to estimate exposure parameters to represent lifetime exposure histories to a toxin may be useful in studies of many other occupational health hazards.

Publications - None to date

LUNG PROTEASES AND ANTIPROTEASE IN COAL DUST EXPOSURE

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Occupational Lung Diseases
5 R03 OH01715-02
05/01/84 - 04/30/86
\$24,750 (\$50,250 Cum)

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Objectives

Extended human exposure to respirable coal mine dust is associated with the development of coal workers pneumoconiosis (CWP). Focal emphysema is a part of simple CWP. The lesion has been ascribed to contraction of connective tissue fibers deposited in this locus with consequent enlargement of adjacent airspaces. Alternatively this lesion may result from elastin lysis from elastolytic enzymes produced in either polymorphonuclear leukocytes (PMN) and/or alveolar macrophages (AM). The purpose of this proposal is to study the hypothesis that elastase secreted by alveolar macrophages following *in vivo* exposure of laboratory animals to bituminous coal will create focal emphysema and simple CWP. The ability of macrophages which have phagocytized coal dust to produce and secrete enzymes in concentrations which exceed the capacity of natural protease inhibitors α_2 -macroglobulin (α_2 -M) and alveolar α_1 -protease inhibitors will be studied to evaluate the role of coal on lung protease anti-protease balance.

Methodology

Bituminous coal of known composition was processed by differential centrifugation in sucrose solution to remove large particulate matters (20u) and to minimize submicronic particles. Coal suspensions in quantities of 2 to 4 mg in 0.2 ml saline were introduced intratracheally into male Syrian Golden hamsters. Control hamsters each receives a similar volume of sterile saline. The inflammatory response of the lung following a single intratracheal (IT) instillation of coal was studied by bronchoalveolar lavage (BAL) 3 days after instillation. Total and differential cell counts were performed on each of the hamster BAL fluid following centrifugation. Total elastolytic potential of the lavaged leukocytes and alveolar macrophages were determined by elastolytic measurements using a radiolabelled soluble elastin substrate. The α -PI and α_2 -M levels in the BAL fluid were determined by radioimmunoassays (RIA) using specific anti-sera developed for each of the purified hamster α_1 -PI and α_2 -M proteins. The functional activities of the α_1 -PI and α_2 -M in the BAL fluid were also measured. These parameters were compared and related stoichiometrically to determine the effect of coal instillation on the protease and anti-protease balance within the alveolar environment of the coal hamsters and the saline controls. The effect of multiple exposure to coal particles on the protease and anti-protease balance was also performed by repeated IT instillations of 40-62 mg of coal at 5-7 day intervals over a 4-5 week period. The stiochiometric balance of the BAL fluid protease and anti-protease were similarly determined at 3 days and at 90 days after the final instillation. These biochemical parameters were compared with the pathological studies including histological evaluation of hematoxylin and eosin, trichrom, elastin, reticulum and connective tissue stains on the corresponding lung sections of the coal and control hamsters.

Progress & Accomplishments

In the single instillation studies, the alveolar macrophages in the alveolar fluid (AF) were cultured and the conditioned media (CM) analyzed for elastolytic activity. α_1 -PI and α_2 -M plasma values were also determined. The total cells, AMs and PMNs in the AF of hamsters receiving 4mg of coal were significantly greater than that of controls whereas in the group receiving 2mg only the PMN showed significant increase. Elastase activity in the CM of cultured AM was increased in animals receiving both 2 and 4 mg of coal (Control: 59.0 ± 10.5 ng; 2mg: 62.6 ± 7.6 ng; 4mg: 81.1 ± 12.9 ng per animal). However, the only significant change was noted in the 4mg group. This increase in elastolytic activity in the conditioned media appears to correlate with the number of AMs in the AF. No significant alterations in elastolytic activity of AF of 2 or 4mg coal groups were observed. The total concentration of α_1 -PI and α_2 -M of the AF and plasma as well as their functional activity did not differ significantly between control, 2mg and 4mg coal groups. These data suggests the potential for increased secretion of elastolytic activity by the AM exposed to coal dust was enhanced without a concomitant alteration in AF concentrations of α_1 -PI or α_2 -M.

With multiple coal exposure, the alveolar fluid PMN was increased on day 3 ($12.7 \pm 4.1 \times 10^6$ vs $0.11 \pm 0.04 \times 10^6$ in controls (C) and declined to $5.1 \pm 0.8 \times 10^6$ at day 90. The alveolar macrophages were greatest at day 3 ($22.7 \pm 0.8 \times 10^6$) and decreased at day 90 ($13.2 \pm 1.3 \times 10^6$, control $5.1 \pm 0.3 \times 10^6$). The total elastolytic activity of the leukocytes was greatest on day 3 (97 ± 45 ng) and decreased on day 90 to 35 ± 12 ng, control 0. Plasma values of α_1 protease inhibitor (α_1 PI) and α_2 macroglobulin (α_2 M) in coal dusted hamsters were not significantly different from control at day 3 and 90. However, the AF α_1 -PI and α_2 -M values were significantly greater than controls at 3 and 90 days. The Elastase Inhibitory Capacity (EIC) of the AF at both periods coal was significantly greater than control but the AF specific activity (SA) was not altered. These data indicate a simultaneous increase in the elastolytic burden and the protease inhibitor activity of the AF following coal dust instillation. Emphysema is not observed histologically indicating the dominance of the antiprotease response in the protease-antiprotease balance.

Significance

The present proposal is directed specifically to the study of coal dust on the level of proteases and anti-proteases found in the lung. While pathological changes in coal dust exposed animal lungs have been documented, biochemical changes on proteolytic activities in response to long term coal dust challenges has never been reported. If the overall amount of PMN or AM elastase is increased in coal dust exposed hamsters, one would assume that proteolytic damage is an important determinant for coal induced emphysema, provided that the level of antiproteases (in the lavage fluid) does not change. A decreased anti-proteolytic activity will, by similar interpretation, also produce an emphysema-like lesion even if the proteolytic destruction of lung connective tissue is not augmented. This study will provide needed information on the role of the protease anti-protease balance in the onset and progression of CWP and may lead us to a better understanding of the development of coal workers pneumoconiosis.

Publications - Abstract(s) available

RESPIRATORY ABNORMALITIES OF HARDROCK MINERS

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Occupational Lung Diseases
5 R03 OH01865-02
09/01/83 - 04/30/86
\$22,966 (\$45,766 Cum)

Objectives

To perform a population based study of a random sample of residents of Leadville, Colorado, to compare miners and nonminers for prevalence of chronic bronchitis and other respiratory symptoms, measurements of subclinical small airways abnormalities, and measurements of obstructive and restrictive lung disease, controlling for smoking status. Any health abnormalities found for miners will be related to indices of dust exposure.

Methodology

Subjects were invited to participate, on the basis of randomly-chosen block of residence, by a local outreach worker who conducted a census of eligible adults. An interviewer recorded respiratory, smoking and occupational histories with a questionnaire for the 769 participants. Physiologic evaluation included single breath nitrogen test (closing capacity, closing volume, and slope of the alveolar plateau), diffusing capacity of the lung, and plethysmographic measurements (total gas volume, airways resistance, total lung capacity, including forced expiratory volume at 1 second, peak expiratory flow, flow rates at 25, 50, and 75% of the vital capacity, and maximal mid-expiratory flow rate). Quality assurance for questionnaire and physiological data was based on retesting a 10% random sample. Work histories from 240 male miner subjects were spot-checked against Climax Molybdenum Company personnel records and used to calculate an index of occupational dust exposure from industrial hygiene measurements for job classifications and dustiness rank score.

Progress & Accomplishments

Data analysis is still being conducted. We are focusing on the 77 percent of male participants who had worked in the mining industry and are attempting to relate their pulmonary function test results to hardrock dust exposure while controlling for smoking history. Initial analyses were conducted using a cumulative dust exposure index for Climax Molybdenum Company employees developed from work histories compiled with a dustiness score for 371 job titles, the latter being the mean of scores by four independent raters familiar with Climax operations. Having recently discarded our analyses using the cumulative dust index because of its nonlinearity, we are now developing a dust exposure measure based as much possible on industrial hygiene measurements. We will then use the dust measure, along with packyears of smoking, age, and height, as an independent variable in liner regression analyses of measurements of obstructive and restrictive lung disease.

An examination of respiratory symptoms associated with lengthy mining experience showed the only one related in these data was exertional dyspnea. The odds ratio for persons with more than 10 years in underground mining or in the crushers was 3.3 in comparison to persons with no dust exposure, and 3.9 in comparison to persons with 10 or fewer years underground or in the crusher.

Significance

Through this study we hope to:

- 1) assess whether symptom prevalence and pulmonary function abnormalities seem out of proportion for a miner group at high altitude where minute ventilation is increased, and
- 3) evaluate the field use of sophisticated pulmonary physiology evaluation, such as plethysmography and single breath nitrogen tests, for occupational epidemiology.

Publications - None to date

INTERACTION OF QUARTZ WITH BIOLOGICAL MEMBRANES

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Occupational Lung Diseases
5 R03 OH01905-02
07/01/84 - 06/30/86
\$8,775 (\$19,495 Cum)

Objectives

The major objective of this project is to further the understanding of the molecular nature of the interaction between the surface of biologically active minerals and cell membranes.

Methodology

Two bioassays widely used in pneumoconiosis research are red cell and macrophage cytotoxicity. In the hemolytic model, the mineral's ability to lyse the erythrocyte membrane is determined quantitatively. The mature erythrocyte is a non-nucleated biconcave disk which contains hemoglobin. Many biologically active minerals have the ability to bind to the membrane surface and alter the permeability of the membrane. The hemoglobin released from the cell is determined using optical spectroscopy after the intact cells, mineral dust and cell stroma are centrifuged down. A dose-response curve is determined showing percentage hemolysis as a function of mineral concentration. The lower part of the curve is linear and from the equation of the line the concentration of mineral dust (usually expressed as mg/ml) required to lyse 50% of the erythrocytes is calculated. The value, called the HC50, is an index of the activity of a given mineral specimen.

Erythrocytes are not known to have any role in the pathogenesis of any of the pneumoconiotic diseases. While membrane damage to the macrophages is thought to play a central role in silicosis and asbestosis. Macrophage viability is usually determined using a staining technique and an enzyme assay. One is a morphological criteria and the other is biochemical. In our preliminary work, we have used a morphological end point. Unstimulated peritoneal macrophages taken from mice have been used. The peritoneal exudate cells are allowed to adhere for one hour and the media is then changed in a way as to remove the non-adherent cells. By definition, the adherent cells are macrophages.

Progress & Accomplishments

The hemolytic model was used most extensively to determine the following variables:

- 1) membranolytic activity of quartz as a function of particle size;
- 2) membranolytic activity of quartz as a function of quartz variety (e.g., rose, milky, amethyst, etc.), holding particle size constant;
- 3) effect of the method of size reduction, hydration, heating, and chemical modification on membranolytic activity;
- 4) the mechanism of quartz membranolytic activity was studied to determine which functional groups on the quartz surface are recognized by the erythrocyte membrane and whether they play a role in membrane damage;

5) the membranolytic activity of the titanium dioxide polymorphs (rutile and anatase) were determined, and the surface properties and character of these materials compared to quartz;

6) A general model developed for properties which impart activity to hydrated metal oxides.

Significance

The hemolytic activity of quartz closely follows the expressed surface area of the mineral. The surface can exist in a number of states of vastly different chemical character and these display different activities. It is possible to have quartz which is biologically inactive. These principles have been extended to the polymorphs of titanium dioxide (rutile and anatase) and the same concept of surface structure, which was found to be important for quartz, holds for the titanium dioxides.

The studies are now being extended to macrophages to determine if the same physicochemical properties of quartz damage both erythrocytes and macrophages.

Publications

Langer AM, Nolan RP: Minerals, rocks and ore bodies: sources of agents in human disease. Proceedings of the Second International Congress on Applied Mineralogy. Park WC, Hausen DM, Hagni RD (eds), The Metallurgical Society of AIME, Los Angeles, p 1159-1177, 1984.

Langer AM, Nolan RP: Physicochemical properties of quartz controlling biological activity. In: Silica, Silicosis, and Cancer: Controversy in Occupational Medicine. Goldsmith DF, Winn DM, Shy CM (eds), Praeger Publishers; New York, p 125-135, 1985.

Langer AM, Nolan RP: Physicochemical properties of minerals relevant to biological activities: state of the art. In Vitro Effects of Mineral Dusts. Third International Workshop. Beck EG, Bignon J (eds). Springer-Verlag, Berlin, 9-24, 1985.

Nolan RP Langer AM: Particle Size and chemically-induced variability in the membranolytic activity of quartz: preliminary observations. In Vitro Effects of Mineral Dusts. Third International Workshop. Beck EG, Bignon J (eds), 39-50, Springer-Verlag, Berlin, 1985.

Nolan RP, Langer AM, Weisman I, Herson GB: Surface character and membranolytic activity of rutile and anatase--two titanium dioxide polymorphs. Br J Indust Med (in press).

OCCUPATIONAL HEALTH FACTORS IN FARMER'S LUNG DISEASE

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Occupational Lung Diseases
5 R03 OH02098-02
04/01/85 - 03/31/87
\$20,723 (\$41,694 Cum)

Objectives

1. Assessment of the association between circulating antibody status and the incidence of farmer's lung disease, the prevalence of respiratory symptoms, and decline in lung function with time.
2. Assessment of the association between environmental, economic and education factors and circulating antibody status.

Methodology

This study is a prospective epidemiologic analysis of a population-based random sample of 1444 farming individuals living in central Wisconsin. One hundred and forty-seven subjects demonstrated positive precipitins by double-immunodiffusion assay in 1977. In the follow-up study, these precipitin-positive subjects were matched by age, sex, and smoking status to 147 subjects who were precipitin-negative in 1977. The American Thoracic Society standardized respiratory questionnaire and pulmonary function tests were administered on participating subjects to determine if precipitin-positive subjects were experiencing more respiratory difficulties than precipitin-negative individuals seven years post-exposure. Additionally, sera were collected from all subjects to determine their current antibody status. Statistical analyses will be performed using SAS.

Progress & Accomplishments

In the first year of the study, the interview process and data collection phase was completed. Ninety-one percent response and 78% participation levels were achieved. Additionally, enzyme immunoassay have been performed on the current sera by Dr. Marx and his technical staff at the Marshfield Medical Foundation. These data have been entered into the computer and "cleaned". Preliminary analyses of these data were reported at the International Symposium on Health and Safety in Agriculture held in Saskatoon, Saskatchewan, October 8-11, 1985.

In the second year of the study, the 1977 data were reanalyzed using the ELISA assay and incorporated into the master data base. Statistical analyses of this data based are in progress in order to examine risk factors and trends in more detail. Publications currently in preparation include: 1) "The Prognostic Significance of Farmer's Lung Disease Antibodies" - a general paper reporting the major results of the study which will be submitted to the American Review of Respiratory Disease; 2) "Occupational Practices of Female Farmers" - descriptive data which is currently non-existent and which will be submitted to the APHA Journal; 3) "Sources of Occupational Health Information for the Agricultural Community" - a more descriptive data which will help health educators in rural areas focus their efforts.

Significance

A test for the presence of antibodies to farmer's lung disease antigen's is a commonly used clinical procedure and a positive result usually denotes recent exposure to thermophilic actinomycete. A population-based prospective analysis of outcome of farmers who have antibodies to farmer's lung antigens has not previously been reported in the literature and, therefore, it is not known whether farmers with antibodies are at increased risk for developing symptoms of interstitial lung disease relative to farmers without antibodies. Complicating this question is the phenomena of pulmonary mycotoxicosis, a syndrome which mimics acute farmer's lung disease with the exceptions that individuals who suffer from pulmonary mycotoxicosis do not have farmer's lung disease antibodies and that it is believed that pulmonary mycotoxicosis does not progress to chronic interstitial lung disease. The role of antibodies in the risk for chronic farmer's lung disease becomes, therefore, an important issue from both diagnostic and pathologic perspectives.

Publications - Abstract(s) available

Guernsey JR: The Prognostic Significance of Farmer's Lung Disease Antibodies Relative to Measures of Respiratory Disease in a Wisconsin Dairy Farming Population. Ph.D. Dissertation, University of Iowa, December, 1985.

Guernsey JR, Morgan DP, Marx JJ, Horvath EP, Pierce WE, Merchant JA: Respiratory Disease Antibody Status. Proceedings, International Symposium of Health and Safety in Agriculture, October 8 -11, 1985 Saskatoon, Saskatchewan, Canada, in press.

Guernsey JR, Morgan DP, Marx JJ, Horvath EP, Pierce WE, Merchant JA: Respiratory Disease Risk Relative to Farmer's Lung Disease Antibody Status. Proceedings, International Symposium of Health and Safety in Agriculture, October 8-11, 1985 Saskatoon, Saskatchewan, Canada, in press.

COAL WORKERS' RESPIRATORY DISEASE PROGRAM EVALUATION

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Occupational Lung Diseases
1 R03 OH02189-01
12/01/85 - 11/30/86
\$24,675 (\$24,675 Cum)

Objectives

The following hypothesis is being tested: Miners enrolled for treatment in a pulmonary rehabilitation program (Pennsylvania Coal Workers' Respiratory Disease Program or CWRDP) will develop lesser respiratory morbidity (as measured by ventilatory function, arterial blood gas levels, and respiratory hospital days) when compared to miners not returning for treatment.

The specific aims include: 1) To observe the outcome variables of pulmonary function (FEV_1 , FVC), arterial blood gas levels (pO_2 , pCO_2), and respiratory hospital utilization in miners returning for treatment using CWRDP 1979 through 1983 clinic records. 2) To compare the paired pre and the post program outcome variables in a subset of the treated and a subset of non-treated miners (stratified by ventilatory function status at initial evaluation) by examining hospital medical records for 5 years pre and 5 years post program enrollment. 3) To contrast the subsets of treated and non-treated miners with regard to the outcome variables obtained from the hospital medical records with adjustment by multivariate analyses for confounding variables such as age and smoking. 4) To validate clinic records by examining hospital medical records on the subset of miners. 5) To analyze data separately for the treated and the non-treated. 6) To compare cost saving (benefits) with program implementation costs if the program results in reduced hospital utilization.

Methodology

This non-current prospective study examines a cohort of approximately 1000 white male miners with 1979 enrollment in the Pennsylvania CWRDP. Of the original cohort, some 300 miners have returned for treatment following initial evaluation in 1979. Using 1979 through 1983 clinic records, the treatment effects on the outcome variables of pulmonary function status (FEV_1 , FVC) arterial blood gas levels (pO_2 , pCO_2), and respiratory hospital utilization is being examined observing patterns of change over time in the returnees.

On a subset of 106 returning miners and 106 non returnees (stratified by ventilatory function at initial evaluation), hospital medical records are being abstracted for a period of 5 years pre and 5 years post program enrollment to compare the paired pre and post outcome variables and to validate clinic records in each group.

The subsets of treated and non-treated miners will also be contrasted with regard to the outcome variables obtained from the hospital medical records with adjustment by multivariate analyses for confounding variables such as age and smoking. Analyses will be done separately for the treated and non-treated miners.

If post program reduction in respiratory hospitalization is shown, a cost-benefit analysis will be done comparing cost savings (benefits) with program implementation costs.

Progress & Accomplishments

The findings are limited to data from the initial evaluation and include characteristics of the cohort. Although 782 patients are in the cohort, analyses are only done on the 775 white males. The cohort size is expected to increase when additional data are verified.

Very preliminary findings reveal a cohort of predominantly retired miners whose mean age is 64 years. The range in age is from 29 to 88 years. The majority of the men are non smokers with 47% being ex smokers and 34% never smoked. Twenty percent are current smokers. The mean FEV₁ for this cohort is 2.32 liters (68.3% predicted). The mean FVC is 3.29 liters or 76.3 percent predicted. The ratio of the mean values of FEV₁ and FVC is 69.3%.

The CWRDP clinics are located in eastern and western Pennsylvania. Differences between these two areas are of interest because anthracite coal is found in east Pennsylvania and bituminous in the west. Anthracite has been associated with a higher prevalence rate of: the number of patients seen by the CWRDP are almost evenly distributed between the eastern and western Pennsylvania clinics (49% of the cohort reside in the east and 51% in the west). The patients in the west tend to be younger. Those in the west (Centerville, New Kensington, Johnstown) have a mean age of 61.6 years. The mean age for the patients in east Pennsylvania (Pottsville, Hazleton, Wilkes-Barre, Scranton) is 66.3 years. The younger population may result from a greater number of working mines in west Pennsylvania.

Of the total, about 50% of the miners are between the ages of 60 and 69 years. Of the remaining 50%, an almost equal distribution is seen between those below 60 years and those above 69 years. Although the exact cause is unknown, two conditions may be responsible for the 60 to 69 year age peak. First, large numbers of men were recruited to work in coal mines following World War II. Few persons were subsequently hired in the intervening years. In the men were in their 20s when recruited to work, they would be in their 60s in 1979. Second, the Department of Labor referred many retirement age miners to the Program for disability evaluation. In all smoking cigarettes, a similar pattern is seen. The proportion of smokers is greatest in the 60 to 69 years age group.

Crapo's prediction formula is used to calculate expected values for the forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC). Respiratory impairment is defined as any value of FEV₁ which falls below Crapo's predicted value less the 95% confidence interval (1).

With this criterion, 56% of the cohort are classified as impaired. A positive correlation exists between age and impairment. With advancing age, the proportion of impaired miners increases. Fifty nine percent of the miners 70 years and older are impaired as opposed to 43% of those less than 50 years.

A positive correlation is also present with the amount of smoking and impairment. As the amount of smoking increases, the proportion of impaired increases. Seventy-two percent of the miners who smoke greater than 40 cigarettes a day are impaired; however, in even the non-smokers, 43% have respiratory impairment.

No real difference is noted between mean FEV₁ percent predicted at initial evaluation in persons with no hospital days (68.6) and those with short stays (69.2) in the year prior to enrollment. Persons whose hospital stays exceed 7 days, however, have a nearly 10% lower mean FEV₁ percent predicted (59.7) than the other two groups. If the treatment program is shown to improve ventilatory function, then a subsequent reduction in hospitalizations may also be expected.

Significance

The underlying hypothesis that prolonged hospital stays is associated with respiratory impairment is supported by the preliminary data. Persons with lengthy hospital stays (i.e. > 7 days) have a nearly 10% lower mean FEV₁ percent predicted than those with short (i.e. < 7 days) or no hospital stays. While final analyses are incomplete, the data are consistent with the study question.

Publications

Ashizawa AE: Coal workers' respiratory disease program evaluation. In the Annals of the American Conference of Governmental Industrial Hygienists, Vol. 14, Wheeler RW, Ed. Cincinnati, Ohio, American Conference of Governmental Industrial Hygienists, p157-162, 1986.

COTTON BRACT INHALATION AND AIRWAY HYPERRESPONSIVENESS

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Occupational Lung Diseases
1 R03 OH02265-01
09/01/86 - 08/31/87
\$23,000 (\$23,000 Cum)

Summary

Byssinosis is an occupational pulmonary disease associated with the inhalation of cotton and other textile dusts. In many healthy subjects never before exposed to cotton dust, the symptoms (e.g., tightness in chest and shortness of breath) and physiologic changes (e.g., changes in pulmonary function) of early byssinosis can be reproduced by the inhalation of an aerosol of a water-soluble extract of cotton bract. The mechanism by which cotton bract extract (CBE) induces obstruction of the airways is unclear. Recent evidence suggests that a generalized inflammatory reaction may be responsible for acute and subacute byssinotic changes. If this is indeed the case, one might expect airway hyperreactivity to result from CBE exposure. Characterization of changes in human airway responsiveness is therefore an important link between our animal models and clinical manifestations in cotton textile workers. We propose to examine changes in airway hyperreactivity from baseline values at four points in time following 300 mg/ml CBE exposure: 2 hours, 8 hours, 24 hours, and 1 week later. This will be achieved by measurement of response to methacholine inhalation at doses of 1, 10, 20, 40, 80, 160, and 320 mg/ml. The effect of normal saline aerosol (placebo) on the response to methacholine dose-response will also be measured and evaluated in a random, cross-over design. Measurements of response to methacholine will be evaluated in both reactors and non-reactors to CBE. This is important since changes in airway reactivity have been shown to be present in the absence of lung function changes resulting from the provocative agent itself, (e.g. ozone). Lung function assessment will be made with maximal and partial flow-volume curves. We will attempt to reduce airway hyperreactivity caused by CBE using pretreatment with the anti-inflammatory drugs indomethacin and prednisone. Characterizing airway reactivity following CBE inhalations is important since it may help explain sustained symptoms in workers and may also help characterize interactions between cotton dust and other irritants such as cigarette smoke. Finally, the data obtained may help explain how repeated acute responses may eventually lead to chronic disease in textile workers.

Publications - None to date

INTERNATIONAL CONFERENCE ON BIOLOGICAL MECHANISMS

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Occupational Lung Diseases
1 R13 OH02117-01
02/01/86 - 01/31/87
\$10,000 (\$10,000 Cum)

Summary

The occupational lung diseases (e.g., the pneumoconioses--asbestosis, silicosis, coal workers' pneumoconiosis--and lung cancer, mesothelioma, and airways disease) constitute a major portion of disability among workers. NIH/NIOSH have labeled occupational lung disease number one among occupational diseases and, even though the epidemiology of these disorders has been well described over the past two decades, research scientists have only begun to address their basic mechanisms.

The International Conference on the Biological Mechanisms of Occupational Lung Disease (ICBMOLD) has the specific objectives of addressing these basic mechanisms. Speakers and contributors of international caliber have been selected and have agreed to participate in this 7th annual research conference that will address the specific areas of biological mechanisms of occupational lung disease.

Publications - None to date

ETIOLOGICAL FACTORS IN INDUSTRIAL LOW BACK INJURY

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Musculoskeletal Injuries
5 R01 OH00982-03
03/01/82 - 02/28/86
\$190,230 (\$458,110 Cum)

Objectives

1. To identify premorbid individual physical, demographic, and psychosocial factors associated with industrial back injuries and time loss.
2. To determine whether the perceived or measured physical demands of an individual's job relate to reported back injuries.
3. To determine whether physical exam findings obtained immediately following reported back injuries relate to resultant time loss.
4. To study the reliability and validity of various techniques, such as ultrasonography of the spinal canal and isometric strength testing, which have been proposed for use in identifying individuals at greatest risk for back injury.
5. To assess the validity of retrospective approaches by comparing the prospective data from this study with data generated during a previous retrospective study of the same plant.

Methodology

Blue collar employees (n=3020) of a Northwest aircraft manufacturing plant volunteered to take part in the study. The volunteers underwent cardiovascular risk screening, and a physical exam which included anthropometric measurements, lifting strength, submaximal treadmill testing, ultrasonography of the lumbar spinal canal, reflex testing, and assessment of posture and flexibility. The volunteers were also given demographic, social, and psychological questionnaires to complete. The questionnaires requested information pertaining to: past medical history, past employment history, education level, marital status, family support systems, social support at the worksite, perceptions of job demands, expectancies regarding the locus of control of physical health and well being, exposure to vibration due to riding in motor vehicles to, from, and during work, and frequency of social activities and activities of daily living. The Minnesota Multiphasic Personality Inventory (MMPI) was also included. In addition, job analysis was conducted so that the influence of loads on the spine imposed by various job tasks could be studied with regard to their relationship to back injuries.

Over the past three years since the completion of the initial examinations, the study subjects have been tracked for reported back injuries. The onsite medical clinic staff cooperate in order that standard injury physical exam data is collected at the time volunteers report injuries. The injured volunteer is also given a questionnaire requesting similar information to that gathered premorbidly. In addition, routine injury reports are obtained, which include information relating to such things as the stated mechanism of injury and associated time loss from work.

Progress & Accomplishments

The medical screening and collection of premorbid physical exam and psychosocial data collection was concluded, our efforts were directed toward the completion of the job analysis portion of the study. Presently, we have

collected data on over 300 back injuries and analysis has begun for predicting acute back injury episodes.

The team evaluated the reliability of three evaluation tools used during the collection of premorbid data: strength testing, submaximal treadmill testing, and ultrasonography of the lumbar canal. Analysis of strength testing techniques included: 1) the evaluation of loads imposed on the lumbar spine during different test lifts, and 2) statistical evaluation of testing variations. Our study led to recommendation for improving the safety of the isometric strength testing technique. We have also determined the accuracy of B-scan ultrasonography for spinal canal measurement. In addition, the safety and accuracy of the submaximal treadmill testing technique was established (median error 7.3% of actual VO_2 max), with the cardiovascular fitness of more than 2400 subjects measured without a complication. Our study has also illuminated the relationship of a high hysteria scale on the MMPI, smoking, support systems at home and at work, and history of back problems requiring surgery or back treatment over the previous 10 years to subsequent injuries. Most recently, our extensive evaluation of anthropometric, lifting strength, and flexibility data has provided the most definitive results to date regarding the controversy surrounding the importance of these factors in back injury incidence. Flexibility and lifting strength did not assist in predicting future back problems, and relate more to aging than to back injuries. With completion of our current analyses of premorbid physical exam data with regard to back injury incidence, we will better understand the relationship of many of the other premorbid factors to acute back injuries.

Significance

Back injuries in industrial settings result in significant time loss disability and, as such, constitute a major socioeconomic problem for most modern societies. The cost of back injuries to society and industry is enormous, making this problem the most expensive health care problem in the 30 to 50 age group (Kelsey, 1978). Despite efforts since the 1920's to curb the industrial back problem, its impact has continued to grow.

Completion of the study will yield sufficient information to allow us to determine the relative importance of such factors as loads on the lumbar spine imposed at the worksite, fitness, height, weight, sex, age, spinal canal diameter, medical history, psychological stress, psychological traits, and social background in the development of back injuries and chronic disability.

In the United States, the industrial back problem affects at least 2% of employees and their families each year, increasing the cost of industrial products worldwide. The knowledge generated from the present study will lead to the development and employment of logical strategies to lessen the burden of this significant health problem.

Publications - Abstract(s) available

Hansson TH, Bigos SJ, Wortley MD, Spengler DM: The load on the Lumbar Spine During Isometric Strength Testing. Spine, October 1984.

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OCCUPATIONAL LIFTING AND LOW BACK MUSCULAR FATIGUE

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Musculoskeletal Injuries
1 R01 OH01929-01A1
01/01/86 - 12/31/86
\$68,026 (\$68,026 Cum)

Objectives

This project will investigate muscular fatigue as a causative factor in low back injuries with the long-term objective of better evaluating, treating, and preventing low back disabilities. The aims of this project are: (1) during a continuous, repetitive, fatiguing lifting task, to describe the changes which occur in the instantaneous fatigue state of muscles of the low back and in selected kinematic and kinetic features of lifting; and (2) during this same lifting task, to determine the relationship between fatigability and the fiber composition of the muscles of the low back.

Methodology

Thirty normal subjects will undergo a screening medical examination and lift strength testing. Under direction of computer controlled visual and auditory cues, each subject will then work at a repetitive lifting task having load and frequency parameters which are known (from psychophysical studies) to be fatiguing. During repetitive lifting the following variables will be monitored: (1) flexion/extension motion of the lumbar spine; (2) vertical acceleration of the load; and (3) myoelectrical activity from five locations over the paraspinal muscles. The task will continue until predetermined fatigue criteria are reached (or the subject requests to discontinue the experiment). Following testing, fiber composition of the low back muscles will be determined by muscle biopsy. Data analysis will consist of determining the relationships among the variables as stated in the specific aims.

Progress & Accomplishments

Data have been collected and analysis is underway.

Significance

The significance of this project is two-fold. First, it will provide a description of the changes in selected kinematic and kinetic features which occur as the requirements of a lifting task exceed the capabilities of a worker. Secondly, it will examine whether the relative fiber composition of the low back musculature is a predisposing factor in the onset of fatigue and alterations in lifting technique.

Publications - None to date

LIFTING CAPACITY OF INDUSTRIAL AND STUDENT POPULATIONS

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Musculoskeletal Injuries
1 R01 OH01956-01
09/01/84 - 12/31/85
\$44,558 (\$44,558 Cum)

Objectives

The major objectives of this research were to determine the difference between lifting capabilities (maximum acceptable weight of lift) of industrial and student populations, by collecting experimental data under identical conditions, and to quantify patterns of these differences.

Methodology

Using the psychophysical approach, 37 male and 37 female college students determined (subjectively) the maximum amount of weight they were willing to lift for a given combination of task variables. Three task variables will be studied: box size, lifting frequency, and height of lift. Their levels were identical to those used for industrial subjects in a previous study (NIOSH Grant No. 1 R01 OH01429-02). The experimental protocol used for student subjects was also identical to that used for industrial subjects.

As in the case of the industrial population, five male and five female students (a subset of the sample population) participated in the study to verify the psychophysical approach in manual lifting. The changes in their oxygen uptake and heart rate with the lifting duration were also determined.

Progress & Accomplishments

Project completed. Final report mailed 1/21/86.

Significance

The results of the study indicated that: The psychophysical approach is also not validated in case of student subjects. The decline of responses with time, however, is slower compared to industrial workers. This indicates that industrial workers overestimate their capacities more (compared to inexperienced students) when the psychophysical methodology is used.

Overall, male students lifted 89% of the weight industrial males lifted for 8-hours. For 12-hour duration, male students lifted 98% of the weight industrial males lifted. The corresponding numbers for female were 94% and 98%.

Generally, the student population response to task variables was similar to that of the industrial population.

In addition to the findings: Relationships between the two populations, for various combinations of task variables, are provided.

Data bases for lifting asymmetrical loads are provided for industrial male and female workers.

Publications

"Comparison of Lifting Capabilities of Experienced and Inexperienced Workers,"
Proceedings of the Human Factors Society Annual Meeting, pp. 239-243, 1986.

BACK INJURIES: MECHANICAL STRESSES IN THE HUMAN SPINE

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Musculoskeletal Injuries
5 R01 OH01962-03*
09/01/83 - 08/31/86
\$138,739 (\$369,000 Cum)

*Previously supported under Grant Number R01 OH00514.

Objectives

The objectives of this research are: (1) to analyze the three dimensional force systems that act on the human spine during industrial handling and lifting tasks, (2) to analyze the mechanical stresses imposed on the structures of the trunk by those force systems, and (3) to apply this information to reduce industrial injuries.

Methodology

Basic features of the program are: (1) the use of computer-generated analogs of the entire thoracolumbar spine and of single substructures of the trunk to make the three dimensional force analyses, and (2) the conduct of experiments to provide input data for the models, to determine when the models are valid, and to confirm predictions made from model-obtained results.

Progress & Accomplishments

The progress and accomplishments are documented by the Publications.

Significance

A large proportion of industrial injuries result from manual materials handling and lifting tasks. Back injuries occur with great frequency, often cause acute suffering and serious disability in the workers involved, and are extremely costly to the industrial economy. The results of these biomechanical studies should lead to significant improvements in the ability to reduce the incidence and severity of industrial back injuries.

Publications

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BACK PAIN IN INDUSTRY: A PROSPECTIVE EPIDEMIOLOGIC STUDY

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Musculoskeletal Injuries
1 R01 OH02084-01
12/01/84 - 11/30/87
\$165,058 (\$311,852 Cum)

Objectives

A three-year prospective epidemiologic study of back pain has been initiated among the employees of the Allen-Bradley Company, Milwaukee, Wisconsin. This large scale study seeks to address deficiencies in characterization of individual and occupational risk factors for low back pain and to define rates of occurrence of low back pain in a light industrial population. Managerial, secretarial, clerical and industrial workers have been included. Specific variables to be assessed as potential risk factors include attributes of the individual and characterization of occupational tasks and the work environment as well as a variety of off-job activities. These variables are to be correlated with both initial and recurrent "episodes" of low back pain (resolution without specific treatment), "cases" of low back pain for which treatment is sought and the frequency and duration of time lost from work. Treatments sought by the employee are to be cataloged, and the major descriptive outcomes of the study for both initial and recurrent events are to include period prevalence rates, and annual incidence and recurrence rates for the total population of employees and for various demographic sub-groups in different job categories. Identification of major risk factors may enhance the likelihood of successful implementation of appropriate preventive strategies.

Methodology

Employees at the Allen-Bradley Company voluntarily participated in the study by completion of a questionnaire administered by nurse-coordinators at the work site. The questionnaire characterizes individual demographic characteristics, off-work activities, pertinent medical history relating to low back pain and the employees perception of job tasks. Limited anthropometric measurements are obtained at the time of entry into the study. Rates of occurrence of "episodes" of low back pain are identified by follow-up annual questionnaires, and "cases" are to be identified at the time of presentation to the Medical Department of the Allen-Bradley Company. Characterization of occupational environment and work tasks has commenced and is to continue during the third year of this study. Both cross sectional and longitudinal associations of potential risk factors with outcome variables are included in the protocol for analysis of data. In addition to descriptive statistical analysis, univariate and multivariate techniques are to be applied.

Progress & Accomplishments

To date 2,506 employees have voluntarily participated in the study through response to questionnaires administered at the work site and these individuals have undergone limited anthropometric measurements. 1,306 employees have responded to the first of annual follow-up questionnaires. Short-form questionnaires completed by employees presenting to the Medical

Department with episodes and cases of back pain have been collected, and departmental records have been abstracted. Preliminary statistical evaluation has been carried out and the prevalence of back pain and/or stiffness was noted to be 86%. Rates of occurrence and recurrence were found to vary between sites in the back (upper/lower; midline/paramedian). The group with exclusive midline low back pain has been individually analyzed to exclude possible confounding effect of pain at other sites which is to be analyzed as an independent risk factor. Risk factors identified in the Allen-Bradley population have included the male gender and parameters of body frame size including wrist and elbow breadth. A peak of prevalence in the fifth decade was identified in both males and females and was significantly associated with higher weights in males. The waist/hip ratio was more strongly associated with back pain than was either measure in isolation. Relative risk rates were dramatically higher for recurrence of back pain than for development of an episode without history of back pain. Prevalence rates were not statistically significantly different between white and blue collar workers, and although the recurrence rates of back pain did not appear to vary significantly on the basis of a preliminary classification of job tasks, the likelihood of development of initial episodes was significantly higher in individuals who reported either sitting or standing for more than 6 hours per day. The rate of development of first episodes of back pain appears to vary between divisions of the plant.

Significance

Back injuries are among the most frequent of work-related diseases and injuries, and spinal pain is among the most common of human afflictions. Costs are reflected in both human suffering and immense economic losses to business and to society. Epidemiologic data on low back pain have not been extensively developed in the United States; it is hoped that through identification of risk factors, prevention of back pain and prevention of the progress of back pain to disability may become more likely.

Publications - None to date

ISOMETRIC VS. ISOKINETIC BACK LIFTING PERFORMANCE

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Musculoskeletal Injuries
5 R03 OH01755-02
07/01/83 - 10/31/85
\$22,630 (\$41,700 Cum)

Objectives

Existing knowledge of the biomechanical capabilities of the back during lifting activities is limited to static evaluations of exertions. This has resulted in ergonomic recommendations, models, and evaluative procedures which have produced little impact in the control of the low back pain (LBP) problem. The problem arose from the fact that manual material handling tasks usually require dynamic action of the biomechanical system. The objective of this research is to investigate biomechanical capability of the back in a quasi-dynamic testing procedure. This effort has facilitated the assessment of trunk loading costs when isokinetic trunk motion is included in the evaluation.

Methodology

A laboratory investigation which permits a biomechanical interpretation of the consequences of motion in the sagittal plane of the body was conducted. Forty-five male volunteers ages 17 to 61 served as subjects. In this experiment the trunk motion was controlled using an isokinetic dynamometer. This device permitted the monitoring of both static and isokinetic trunk exertions while the subjects were in back lift positions. Static exertions at 0, 22.5 and 45 deg. of forward trunk bend and isokinetic exertions of 15, 30 and 90 deg/sec of trunk motion through the 60 to 0 deg angle range were monitored. Trunk torque and back muscle electromyography (EMG) were recorded as the subjects performed the exertion.

Progress & Accomplishments

This project has been completed. The results reveal that significantly greater loadings occur under slow dynamic conditions compared with static conditions, indicating that static models are not good indicators of dynamic trunk load. The correlation between trunk torque and EMG in the erector spinae muscles also increased significantly as the trunk velocity increased indicating that EMG may serve as a good indicator of trunk exertions under dynamic conditions.

The relative trunk loading needed to support a constant torque under the various velocity and angle conditions were also investigated and described.

Finally, the time sequence of muscle loading activity was investigated. The peak trunk loading was similar under static and 30 deg/sec exertions but varied markedly under other conditions.

Significance

These analyses have demonstrated that the trunk loadings which occur under static conditions change markedly as velocity is introduced into a lifting analysis. Thus, lifting models which are based solely upon static exertions may misinterpret the true loading which occurs at the trunk during

a lift. This research has demonstrated how such models may be adjusted to reflect the spine loading effects of motion during a sagittally symmetric task.

Publications

Marras WS, Rangarajulu SL, Wongsam PE: "Trunk Force Development During Static and Dynamic Lifts", Human Factors, 1986 (in press).

Marras WS, Wongsam PE, Rangarajulu SL: "Trunk Motion During Lifting: The Relative Cost", International Journal of Industrial Ergonomics, 1986 (in press).

Marras WS: "Trunk Motion During Lifting: Temporal Relations Among Loading Factors", International Journal of Industrial Ergonomics, 1986 (in press).

REFLEXIVE AND TACTILE EFFECTS OF HAND TOOLS

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Musculoskeletal Injuries
5 R03 OH01852-02
05/01/84 - 04/30/86
\$6,000 (\$23,536 Cum)

Objectives

Many assembly operations in industry require power tools. This investigation studied the thesis that hand tool vibration can introduce disturbances in muscular control and sensory feedback. The study found that operating vibrating hand tools can result in excessive grip exertions, which may increase the risk of cumulative trauma disorders in the upper extremities. In addition, vibration diminishes hand tactility, increases muscle tremor and localized and general discomfort, which could impair performance especially in jobs requiring tactile sensitivity, or when performing manipulative tasks.

Methodology

A survey of selected tools indicated the vibration produced by rotary power hand tools can be approximated using sinusoidal vibration with frequencies between 35 Hz to 150 Hz. Eight tools were studied. They included a heavy duty right angle sander, light duty right angle sander, handgrip orbital sander, palm orbital sander, vertical polisher, impact wrench, and trimming shear.

A simulator was developed and used in the laboratory to study the neuromuscular effects of hand tool vibration on the hand and arm. The apparatus was capable of controlling load weight, posture, vibration frequency, magnitude, and direction, at typical hand tool levels. Based on the survey findings, the apparatus was used to conduct three experiments.

In the first experiment fourteen subjects operated a simulated hand tool vibrating at 9.8 m/s² and 49 m/s² acceleration magnitudes, 40 Hz and 160 Hz frequencies, three orthogonal directions, and with 15 N and 30 N tool weights. The second experiment studied hand flexor and extensor muscle EMG using five subjects holding a handle vibrating at 8 m/s² ISO weighted acceleration, frequencies of 20 Hz, 40 Hz, 80 Hz, and 160 Hz, and grip forces of 5%, 10%, and 15% MVC. In the third experiment, five subjects operated a simulated hand tool for 30 min vibrating at 8 m/s² ISO weighted acceleration, frequencies of 20 Hz, 80 Hz, and 160 Hz, in three orthogonal directions using a 30 s: 30 s work/rest duty cycle.

Progress & Accomplishments

The study found that grip exertions were affected by tool vibration. Average grip force increased from 25.3 N without vibration, to 32.1 N (27%) for vibration at 40 Hz, and increased to 27.1 N (7%) for 160 Hz vibration. Average grip force also increased from 27.4 N at 9.8 m/s² RMS acceleration to 31.8 N (16%) at 49 m/s². Significant interactions between Acceleration x Frequency, and Frequency x Direction were also found. The largest average grip force increase was from 25.3 N without vibration to 35.8 N (42%) for 40 Hz and 49 m/s² vibration. The magnitude of this increase was on the same

order as a two-fold increase in load weight, where average grip force increased from 22.5 N to 35.0 N (56%).

Muscle responses were greatest at frequencies where grip force was affected, indicating the tonic vibration reflex was likely the cause of increased muscle contraction. Extensor activity was greater when the handle was vibrating than without vibration. These effects were greatest at 40 Hz vibration and 8 m/s² weighted acceleration using 15% MVC grip in vertical and longitudinal directions with respect to the long axis of the forearm.

Average falling ridge thresholds shifts, measured using a ridge aesthesiometer, increased 0.01 mm (50%) at 20 Hz and 80 Hz, and increased 0.03 mm (150%) at 160 Hz. Since tactility decreased with increasing frequency between 20 Hz and 160 Hz, it was concluded that loss of tactility was not likely the cause of increased grip force since grip force effects were greatest at 40 Hz vibration.

No EMG indication of muscle fatigue was observed using RMS amplitude and mean power frequency shifts after the 30 min exposure. Average muscle force tremor amplitude did not change after vibration exposure at 20 Hz, but increased 17% at 80 Hz, and 25% at 160 Hz.

Average general and right palmar discomfort was small but significantly greater after holding a vibrating handle than holding one without vibration, and was slightly greater for vibration parallel to the long forearm axis than other directions.

Significance

The following recommendations were made on the results of this research: (1) Minimize tool weight or use tool balancers to reduce grip force necessary to hold and operate hand tools. (2) Avoid using tools vibrating in the frequency range of 40 Hz and acceleration magnitudes greater than or equal to 40 m/s² to minimize effects of vibration on grip force, especially when there is a potential problem with CTDs. (3) When tactility is required to perform a task, such as inspection for smoothness, avoid using tools vibrating in the frequency range of 160 Hz and acceleration magnitudes greater than or equal to 80 m/s². (4) When performing precision motor tasks, such as manipulating small pieces, avoid operating tools vibrating in the frequency range of 160 Hz and acceleration magnitudes greater than or equal to 80 m/s².

Publications

Radwin RG: Neuromaster Effects of Vibrating Hand Tools on Grip Exertions, Tactility, Discomfort & Fatigue, Doctoral Dissertation, The University of Michigan, Ann Arbor.

MAXIMUM ACCEPTABLE LIFTS IN A SITTING POSITION

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Musculoskeletal Injuries
1 R03 OH02229-01
05/01/86 - 04/30/87
\$20,693 (\$20,693 Cum)

Objectives

Despite the recent automation and mechanization of work, it is still necessary in many industrial settings to perform some form of manual materials handling. Musculoskeletal disorders (such as low back pain, muscle soreness, tendonitis, bursitis, wrist and joint injuries, etc.) associated with repetitive exertions represent a major cause of lost work time and compensation costs. Over the past twenty to thirty years most of the research in this area has concentrated on injuries of the low back. This knowledge led to the publication of a NIOSH Technical Report on manual lifting in 1981. Curiously, the guide deals only with lifts in the sagittal plane from a standing position. In a modern industrial setting, many jobs such as production assembly or packing jobs, require lifts from a seated position. People who sit for extended periods often experience more back pain than those not so constrained. It has been shown that the stresses on the lumbar vertebra in a sitting position exceed those generated from a standing position. Despite this knowledge, little information is available concerning lifting limits from a sitting position. Knowledge of these results would be invaluable in the design of the workplace and the evaluation of the strain imposed by work tasks. The purpose of this project is to determine the maximum acceptable load that can be safely lifted with two hands, within the normal reach limits from a sitting position and to compare those values with the maximum acceptable weight in a standing position.

Methodology

The psychophysical methodology is being used to determine the maximum acceptable weight that college age students will lift in each of four positions: (1) seated, two-handed, symmetrical lift from a table, forward 38 cm, (2) seated lift from 30.5 cm below table height to table height (85 cm) with a 90 degree twist, (3) standing, two-handed, symmetrical lift from the table, forward 38 cm, and (4) standing, vertical lift from 86 cm to 134.5 cm. Subsequent to a training period, subjects lift a tray with slotted handles at the rate of 1 or 4 lifts/minute. Each subject adjusts the weight of the tray to their own preference by adding or removing flat pieces of lead over a 45 minute period. The weight of the tray, heart rate and perceived exertion are measured at 15, 30 and 45 minutes. Oxygen consumption is measured during the last 5 minutes of the 45 minute period. An indication of muscle strain will be determined from electromyographic analysis (EMG) of the back, shoulder, and abdominal muscles. The analysis of the data will determine if the amount of the weight chosen by the subjects is influenced by the lifting position. In addition, the physical strain will be assessed by the heart rate response, oxygen consumption and EMG. The perceived exertion will indicate the overall assessment of the job by the subject.

Progress & Accomplishments

A study using 8 male, college-aged, subjects has been completed. This study was completed prior to receiving the EMG equipment which was purchased by the grant, therefore no EMG data is available. As was expected the amount of weight lifted 4 times/minute was significantly less than that lifted 1 time/minute (approximately 2 kg). No significant differences in the amount of weight lifted existed between the sitting-forward lift and the standing-vertical lift. However, the loads lifted during both standing positions were significantly greater than during either sitting position. The acceptable weight for the standing-vertical lift was approximately 22% and 14% greater than the sitting-twist and sitting-forward lifts, respectively. When averaged across positions and frequencies, the maximal acceptable weight for standing lifts was 16% greater than for sitting lifts. This study also provided data relative to the oxygen consumption, heart rate, and perceived exertion of the subjects during the lifting tasks.

With the arrival of the EMG equipment, a second group of subjects will be tested to determine which muscles and the degree of their involvement during sitting lifting as compared to standing tasks. The study will be designed to investigate the muscles of the back, shoulder and abdominal region.

Significance

This project will provide data concerning the maximum acceptable weight that can be comfortably lifted from a sitting position using the psychophysical methodology. This data is not currently available. More importantly, by comparing the acceptable weight in both sitting and standing positions in the same group of subjects, we can arrive at a percentage change which can be applied to the volumes of standing data that is already available. This will greatly decrease the number of studies which need to be performed in this area in order to provide acceptable guidelines for design engineers.

Publications - Abstract(s) available

MIXTURE MODEL FOR LOW BACK PAIN REHABILITATION

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Musculoskeletal Injuries
1 R03 OH02287-01
09/01/86 - 08/31/87
\$23,464 (\$23,464 Cum)

Summary

Because of the lack of knowledge of true causes of low back pain (LBP), a great variety of non-operative treatment methods have been recommended and tried. Recently a new regime, called "back school" was introduced for the purpose of rapidly and economically returning a patient to full functional fitness and normal occupational activities. Assuming that this functional and vocational restoration is a goal of a rehabilitation program, it is apparent that early recovery and higher success rates are major concerns. Hence it is important to consider these two parameters in evaluating any individual treatment modality or the rehabilitation program as a whole.

It is proposed to construct a statistical model (mixture model) that can be used for the investigation of the recovery process of LBP patients in a rehabilitation program. The model provides (i) the percentage of patients who will be recovered, and (ii) the rate of recovery of function. The model will then be used to identify the factors that influence the process of LBP rehabilitation significantly. Approximately 400 LBP patients who participated in the program offered by one of the large urban rehabilitation centers will be studied. In this study "recovery" is defined as a return to work at least on a half time basis.

Publications - None to date

A CARCINOGEN/MUTAGEN DETECTOR FOR HPLC

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Occupational Cancers
2 R01 OH01331-04A1
08/01/86 - 06/30/87
\$160,935 (\$482,560 Cum)

Objectives

The primary objective of this project is to complete the fabrication of an electronic carcinogen/mutagen (C/M) detector and to determine the accuracy of this detector in identifying carcinogenic chemicals that are frequently found in the workplace.

Secondary objectives include 1) interfacing the C/M detector with high-performance liquid chromatography (HPLC) which serves to separate complex mixtures into component chemicals and thereby permits the response of the C/M detector to be obtained for each component, 2) modifying currently used workplace-sampling protocols to compatibility with the C/M detector/HPLC system, and 3) determining the theoretical basis of the C/M detector, which involves developing a better understanding of electron-attachment processes in biomimetic media.

Methodology

The two methodologies used in this project are pulse radiolysis and HPLC. In the former, excess electrons are generated in a non-polar medium by a pulse of ionizing radiation and the rate at which these electrons attach to solutes are observed by conductometric techniques. A diffusion-controlled electron attachment rate constant, k_e , is considered a positive response and an indication that the solute under study is an electrophile or a carcinogen, whereas a less-than diffusion-controlled k_e indicates that the solute is a non-carcinogen.

The second methodology used is HPLC which separates complex mixtures into component chemicals before the chemicals are eluted through the C/M detector (vide supra). Since the solvent flowing through the detector is restricted to non-polar media, only normal-phase HPLC can be used for this purpose. However, reverse-phase HPLC can be used for pre-separation of components not separable by normal-phase techniques, and the reverse-phase separated components can then be eluted through the C/M detector in a non-polar medium.

Progress & Accomplishments

The C/M detector is based upon k_e being an accurate measure of the carcinogenic properties of a chemical. We have recently completed a validation study of the k_e -carcinogenicity correlation that included 182 chemicals and the results of which have been accepted for publication in Carcinogenesis. Protocols in which normal-phase HPLC are used to sample occupational biohazards are being developed, and a manuscript in which a protocol to separate workplace-associated polyaromatic hydrocarbons is described is in preparation. The effect of the conduction-band energy of the medium on the measured k_e is being studied in an attempt to obtain a better understanding of the theoretical basis of the C/M detector. All aspects of these studies will be described in a manuscript being prepared for inclusion in J. Saxena's series entitled Hazard Assessment of Chemicals.

In addition, we have obtained new components for our pulse-conductivity system that should improve the efficacy of subsequent measurements of k_e , and we have enlisted consultants to provide us with additional expertise in HPLC and industrial hygiene.

Significance

Short-term in vitro tests complement more expensive and time-consuming animal tests and epidemiological studies in screening the potentially carcinogenic properties of chemicals that account for approximately 16,000 occupationally related cancer deaths in the United States each year. The physico-chemical short-term test that we have developed, the k_e test, which is based upon the liquid-phase electron attachment properties of test chemicals, is more accurate and faster than conventional short-term bioassays such as the widely used Ames Salmonella/microsome assay. Further, the electron-related basis of our test is amenable for use in conjunction with HPLC, the most rapidly growing analytical technology of the past two decades. Obtaining an understanding of the theoretical basis of the k_e test, however, could be the most significant result of this research.

Publications - Abstract(s) available

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Isildar M, Bakale G: Comparative Lethal Effects of UV and Ionizing Radiation in Ames Tester Strains of Salmonella. Radiat Res 103:461-465, 1985.

Bakale G: A Physico-Chemical Technique for Identifying Chemical Carcinogens, Meeting Report of the Eighth Interdisciplinary Cancer Research Workshop by P. Politzer and C. Parkanyi, Cancer Res. 46, 1566-1568 (1986).

Bakale G, McCreary RD: A Physico-Chemical Screening Test for Chemical Carcinogens: The k_e Test, accepted for publication in Carcinogenesis (1987).

Bakale G: Detection of Mutagens and Carcinogens by Physical Chemical Techniques in Hazard Assessment of Chemicals - Current Developments, Vol. 6, J. Saxena, ed., in preparation (Hemisphere, Washington, D.C., 1987).

Isildar M, Bakale G: Radiation-Induced Mutagenicity and Lethality in Salmonella Typhimurium. Proceeding of the Seventh International Congress of Radiation Research. JJ Broerse, GW Barendson, HB Kal, AJ van der Kogel, Eds., p. B4-19 M Nijhoff, Amsterdam, 1983.

RISK OF PATHOLOGISTS EXPOSED TO FORMALDEHYDE

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Occupational Cancers
5 R01 OH01511-02
09/30/83 - 05/30/86
\$144,728 (\$267,067 Cum)

Objectives

Recent studies by the Chemical Industry Institute of Toxicology (CIIT) suggest that exposure to formaldehyde vapor may be carcinogenic in rats. These findings have raised concern for the health of 1.75 million workers with occupational exposures to formaldehyde as well as persons with exposure from home insulation.

Methodology

This proposal is to examine the mortality experience of pathologists, a medical specialty group with formaldehyde exposure, using a non-concurrent prospective study design. Comparison populations include other physician specialists who have only limited exposure to formaldehyde. All causes of death listed on death certificates will be examined, and selected causes, notably cancers, will be verified.

Progress & Accomplishments

The study population has been constructed from lists of members in pathology associations. The vital status of the exposed and control populations has been ascertained, and death certificates to validate vital status and cause of death have been obtained. Data are being analyzed.

Significance

Results from this study will help establish specific risks, if any, and levels of risk for humans with formaldehyde exposure.

Publications - None to date

OCCUPATIONAL EXPOSURE IN ADULT GLIOMA PATIENTS

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Occupational Cancers
5 R01 OH01557-04
09/29/82 - 08/31/86
\$72,582 (\$318,304 Cum)

Objectives

A large amount of suggestive evidence on the etiology of brain tumors has come from recent studies of the rubber, plastics and petrochemical industries. These studies suggest associations between chemical exposures and tumors of the glioma series. The objective of the present research is to pursue the findings of these occupational studies by conducting a case-control study of adult glioma patients. The proposed study will collect extensive occupational and residential histories for male and female glioma patients by interview and will compare the occupational distributions to matched neighborhood controls. Chemical exposures of interest will be followed up to identify possible agents.

Methodology

Eligible cases for the current study are adults with glial tumors of the central nervous system who are residents of Northern California or Northwestern Nevada at the time of diagnosis. An attempt to population-base a subset of cases in the study through the San Francisco-Oakland SMSA SEER Registry was judged not feasible because of the Registry's lag time in assembling incidence cases in a usable form and the limitation imposed by the mean survival time of 12 months for glioma patients. We proposed an alternative of searching the California State Mortality Tapes for brain tumor deaths, identifying adult glioma cases for the Bay Area counties, and locating a next-of-kin for proxy interview. One neighborhood control, matched to the case for sex, age, and race, is interviewed for each completed case interview. Occupational histories taken from completed interviews are coded by two measures of exposure. One measure is the industry code from the Standard Industrial Classification Manual. Each industry is coded independently by two coders and discrepancies are resolved by a metacoder. In the second method, two independent coders read job histories in questionnaire and assign 9-digit Dictionary of Occupational Titles (DOT) codes to each job. A reviewer reads DOT descriptions for all codes assigned and groups them into exposure categories. A judge blind to subject reported exposures and case-control status resolves exposure-category discrepancies.

Progress & Accomplishments

As of January 1, 1986, 512 cases have been entered into the study, of which 395 had been interviewed, 20 had consented and been assigned for interview, 50 were pending consent, 16 were ineligible or unlocatable since initial study entry, and 21 individuals had refused to participate. Proxy interviews were conducted for 162 of the 395 cases. There are 263 controls. Completion of coding for this data set is currently underway in preparation for analysis. A preliminary analysis has been performed on a subset of this data.

Preliminary results based on 124 pairs of case interviews and their matched control interviews show some differences between cases and controls in the categories of interest to the study hypothesis. Only two petrochemical workers appear in these 248 subjects but both are cases. When we group exposure categories to create a broader category of work with exposure to petroleum products combining the categories of petrochemical workers, mechanics, gas station employees, and professional drivers we find 26 cases and 15 controls with a history of one or more jobs in those categories. The one-sided p value for these numbers is 0.04. Exposure to cleaning solvents shows 8 cases and 4 controls. Metal fabrication, including welding, cutting, and assembly had 7 cases and 2 controls. Rubber working has one case and no controls. Job-related exposure to radiation shows 9 cases and 5 controls. Photographic chemicals, 3 cases and 1 control. No plastics or vinyl chloride workers have appeared yet in the study. There is no difference between cases and controls on exposure to paints and dyes, construction-related exposures, farm work, machine and lubrication oils, and the category of miscellaneous exposures. The category of no exposure was quite evenly distributed among cases and controls (46 cases and 52 controls).

Significance

The results outlined above are preliminary and the numbers are quite small (most of them are not statistically significant); nevertheless, some of them are suggestive and tending in the direction of the study hypothesis. Larger numbers will resolve some of the marginal findings in this ever versus never worked analysis and also permit us to look at duration of employment with a given exposure category.

Publications

Moss AR: Occupational exposure and brain tumors. J of Toxicology & Environ Health 16:703-711, 1985.

PRODUCTION AND FATE OF HYDROXAMIC ACIDS IN HEPATOCYTES

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Occupational Cancers
5 R01 OH02027-03
08/01/84 - 07/31/87
\$73,722 (\$201,902 Cum)

Objectives

The objective of this program is to determine the extent to which thiamine-dependent enzymes participate in the toxification or latention of C-nitroso aromatic chemicals in primary cultures of rat liver hepatocytes. Specific aims relevant to the elucidation of this objective are to 1) identify the hydroxamic acids produced from selected nitroso substrates by hepatocyte cultures, 2) determine the metabolic fate of such hydroxamic acids in hepatocytes and 3) determine the relative genotoxicity of the various types of hydroxamic acids of interest, particularly with respect to covalent binding to nucleic acids and protein.

Methodology

Rat hepatocytes in primary culture are prepared under aseptic conditions and used within four days following the initiation of such monolayer cultures. Chemical substrates containing the C-nitroso group on an aromatic ring are added to such cultures, and their metabolic fates are followed by use of high pressure liquid chromatography, and in certain cases by radiation dosimetry. Particular effort is made to determine the metabolic production of certain hydroxamic acids, which are indicative of the action of particular thiamine-dependent enzymes on nitroso substrates. Authentic chemical references of such metabolites of interest have been or will be prepared by common synthetic organic methods. These reference standards are employed to confirm the production of the metabolites of greatest interest. The biochemical fates of the hydroxamic acid metabolites are also examined by such techniques. Particular effort is made to determine the extent of metabolic activation of the hydroxamic acids, and subsequent covalent binding of them to cellular molecules such as DNA, RNA and protein.

Progress & Accomplishments

Studies with five selected C-nitroso aromatic compounds in rat hepatocyte suspension cultures demonstrated that the nitroso functional group is highly reactive in a metabolic sense. Within one hour all nitroso substrates had been consumed, with concomitant appearance of expected major metabolites such as the corresponding amines, acetanilides and arylhydroxylamines; however, the latter initial metabolites were short-lived in the conversion of arylhydroxylamines to other products was a relatively rapid reaction.

More detailed studies were conducted with plated hepatocytes (primary hepatocyte cultures) on 4-nitrosobiphenyl and particularly on 4-chloronitrosobenzene. With this more refined metabolic system, the nitroso functional group still remained quite reactive. Most importantly, the conversion of 4-chloronitrosobenzene to its N-glycolyl type of hydroxamic acid primary hepatocyte cultures was positively confirmed by use of a diode array rapid-scanning UV detector with high pressure liquid chromatography.

The percent conversion of this nitroso substrate to the glycolyl-hydroxamic acid was quite low, which was not surprising in view of the

numerous pathways of metabolic conversion to which to nitroso functional group is susceptible. Furthermore it was possible to enhance the percent production of the glycolyl-hydroxamic acid through the use of certain modulators of metabolic reactions. Pentachlorophenol is an inhibitor of sulfation and was found to increase the production of the glycolyl-hydroxamic acid from 2-5 times depending upon the concentration of nitroso substrate that was employed. This suggests that the glycolyl-hydroxamic acid metabolite is further metabolized by O-sulfation. Similarly, depletion of glutathione (diethyl maleate, buthionine sulfoximine) and inhibition of glucuronidation (galactosamine) also resulted in increased amounts of the glycolyl-hydroxamic acid, although the enhancement was less than observed for pentachlorophenol.

A very notable observation was that the percent conversion of the nitroso substrate to the glycolyl-hydroxamic acid increased markedly rather than linearly as the concentration of the substrate was increased. Thus at an initial substrate concentration of 0.1 mM the percent conversion to the glycolyl-hydroxamic acid was 0.02 - 0.05%, while a concentration of 0.2 mM was converted to the this hydroxamic acid in a yield of about 0.2%. In the presence of 0.1 mM PCP the percent yield was further increased to 0.3%. The major fate of the 4-chloronitrosobenzene substrate in primary hepatocyte cultures was found to be loss by volatilization from the aqueous media. Thus, in a closed system, such as *in vivo*, it is to be expected that the percent conversions of this substrate to the glycolyl-hydroxamic acid would probably be several times higher.

Significance

This study has confirmed the original hypothesis that certain thiamine-dependent enzymes are involved in the production of structurally unique metabolites of nitroso compounds. In turn, the nitroso compounds are known intermediary metabolites of arylamines and related chemicals; therefore, it is expected that the unique hydroxamic acids derived from the action of transketolase on nitroso compounds will also be metabolites of the arylamine class of chemicals. Even though the amounts of such hydroxamic acids that are produced are very small, it is quite possible that these minor metabolites play an important role in the overall toxicological properties of many industrial and agricultural chemicals. Studies to elucidate the genotoxicity of such metabolites have not been completed.

Publications

Corbett MD, Corbett BR: Enzymatic N-oxidation of 4-nitroaniline. *Biochem Arch*, 1:115-120, 1985.

Corbett MD, Corbett BR: The reactions of C-nitroso aromatics with α -oxo acids in *Biological Oxidation of Nitrogen in Organic Molecules* (J.W. Gorrod and L.A. Damani, eds.), Ellis Horwood Ltd., Chichester, p 400-408, 1985.

Corbett MD, Corbett BR: Enzymatic N-oxidation of 4-nitroaniline. *Biochem Arch*, 1:15-120, 1985.

Corbett MD, Corbett BR: Effect of ring substituents on the transketolase-catalyzed conversion of nitroso aromatics to hydroxamic acids. *Biochem Pharmacol*, 35:3613-3621, 1986.

OCCUPATIONAL CANCER SURVEILLANCE: NEW APPROACHES

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Occupational Cancers
5 R01 OH02067-03
09/28/84 - 08/31/87
\$258,679 (\$636,463 Cum)

Objectives

There are six specific scientific aims for this project:

1. A method of monitoring occupational cancer risks is being developed.
2. The utility and feasibility of routine occupational surveillance among patients diagnosed with rare forms of malignancy, as well as with the more frequently occurring malignant tumors, is being assessed.
3. Occupational risks of blacks and females are being assessed, in addition to those of white males.
4. Brief smoking histories are obtained as an essential complement to the occupational history.
5. Various statistical methods are being tested for their ability to produce results useful for generating hypotheses about potential occupational risks of cancer occurrence.
6. An ongoing, routine system of occupational monitoring is being intergrated with the Metropolitan Detroit Cancer Surveillance System (MDCSS), a participant in the NCI SEER program.

Methodology

This is a population-based study of all newly diagnosed cancer cases for 9 selected sites or types: bladder, colon, rectum, lung and bronchus, esophagus, liver, salivary gland, eye, and mesothelioma. Cases are identified through the rapid reporting system of the population-based MDCSS. Cases fitting study criteria are interviewed by telephone within 2 to 6 months after diagnosis. This brief interview takes an average of 11 minutes to complete and includes a health history; smoking history; complete occupational history including occupation, industry, and job description; residential history, and demographic characteristics. Analytical methods will include both PCIR and SIR.

Progress & Accomplishments

Through November, 1986, 10,561 cases have been identified and entered into the study; 608 of these are in process. Of the 9,678 completed cases, 9,158 were interviewed and 520 were either refusals or non-interviews for other reasons. Thus, our current response rate for this study is 94.6% of those interviewed, 78.8% were white and 20.2% were black; 61.1% were male and 38.9% were female. To date, there have been 2234 or 24.4% of cases who never smoked cigarettes. Specific analyses of these cases will be important in elucidating occupational risks, particularly for bladder and lung, and esophageal cancers and for mesotheliomas.

In addition to the interviews completed, all data have been entered into the computer and preliminary data analysis has been initiated. A specific computer process has been written for periodic screening and editing of the data. Significant effort has been invested in the development of an analytical scheme for grouping occupations and industries in terms of shared

exposures that are potentially important for understanding occupational cancer etiology. An occupational physician consultant has assisted with this task. The Principal Investigator also has developed a socioeconomic status scale for utilization in study analyses. This scale combines data regarding education and occupation. The Principal Investigator presented a paper describing OCISS (Occupational Cancer Incidence Surveillance System) and its methodology to the Michigan Industrial Hygiene Society. The OCISS methodology is under review by the California statewide cancer registry and by the Rhode Island Occupational Health program.

Significance

This project is developing the first population-based cancer incidence surveillance system in the United States which provides monitoring across a wide variety of occupations. Insights will be gained about occupational cancer risks among blacks and women and new leads are expected to be developed regarding the occupational etiology of cancer.

Publications

Swanson GM, Schwartz AG, Brown KL: Population-Based Occupational Cancer Incidence Surveillance--Utilization of the Telephone Interview. J of Occup Med 27:439-444, 1985.

A SOLVENT AND EXPOSURE MORTALITY STUDY OF DRY CLEANERS

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Occupational Cancers
1 R01 OH02104-01A1
01/01/86 - 12/31/87
\$123,205 (\$123,205 Cum)

Objectives

This is the second year of a two year petroleum solvent mortality study of dry cleaners in Oklahoma. The objective of this study is to examine the association between workers exposure to the primary solvents used in commercial dry cleaning plants and the distribution of causes of death and mortality rates among those exposed.

Methodology

The methodology calls for the completion of the following tasks:

1. Categorize each commercial dry cleaning plant in Oklahoma, between 1941-1985, by specific solvent use over time. This task was completed during the first year.
2. Identify a cohort of Oklahoma dry cleaning plants owner/operators with known solvent exposures and duration of exposure between 1941-1985. This task was completed during the first year.
3. Among this cohort, determine the Standardized Mortality Ratios (SMR) for all deaths and for selected causes of death occurring between 1941-1985 to include cancer, kidney cancer, lung cancer and liver cancer.
4. Estimate the latency period in cases of renal cell carcinoma.
5. Identify high risk subgroups for future measurement of actual exposure levels, non-fatal acute and chronic effect on the human kidney, and effects on reproductive outcome related to exposure to petroleum or synthetic solvents among Oklahoma dry cleaners.

This study employs a non-concurrent cohort approach. About 4000 owners/operators who have ever applied for a license in the state of Oklahoma to own/operate a dry cleaning plant between 1941 and 1985 are being followed-up to ascertain living status and cause of death among those who are known to be dead. Each individual in the cohort has been categorized by solvent and by duration of exposure from employment history. Since no information is available on the cohort members social security number or date of birth, non-standard follow-up procedures are being done to follow-up this cohort. These include review of telephone directories, senior key official of the Oklahoma Association of Dry Cleaners and Oklahoma Board of Dry Cleaners, senior dry cleaners in smaller communities, next of kin, neighbors, friends, funeral homes, local chamber of commerce, state driver's license and motor vehicle registration and a state death certificate tape search to name a few. Telephone contacts are being established to verify addresses, living status and date of death. Among those who have died a copy of the certificate of death is purchased from the appropriate state health department. Among

living members of the cohort, a questionnaire is being mailed to obtain information about lifestyle factors such as smoking and to verify employment histories.

Overall and age-specific death rates will be calculated for all deaths, and for those deaths due to selected causes. These rates will be expressed as standardized mortality ratios (SMRs). Age-sex-race and cause specific distributions of deaths in the white male U.S. population from 1941-1985, and in the state of Oklahoma from 1950 to 1985 will be used as the standards of comparison. The Oklahoma specific rates for the period 1941-1949 will be estimated using projections from the 1950-1960 Oklahoma rates, and by comparison of U.S. Oklahoma trends between 1950-1960. Machine readable age-sex-race-cause specific death data are unavailable for Oklahoma prior to 1950. SMRs will be subanalyzed by solvent category, specific solvent, and grouped durations of exposure.

Progress & Accomplishments

All commercial dry cleaning plants in Oklahoma, between 1941-1985 have been categorized by specific solvent use over time. A cohort of 4000 individuals mostly white males has been identified with solvent exposure and duration of exposure. Follow-up is progressing very well. Over 2,400 dry cleaning plants were identified, with specific solvent(s) in use known in 99.5% of the cases. Of the 400 cohort members with known durations of exposure, 60% were white males exposed to Stoddard solvent without confounding exposure to perchloroethylene or carbon tetrachloride. We have ascertained living status on more than half the cohort. Of those with known status, half are known dead with a verified cause of death, the other half are living with a verified address.

Significance

Previous mortality studies of laundry and dry cleaning workers have not been solvent exposure specific, and have shown inconsistent elevation of many site-specific cancers. Perchloroethylene (PCE) has been the major solvent of interest, but non-chlorinated petroleum solvents, such as Stoddard solvent, may be related to consistent elevations in bladder and kidney cancer seen in this population.

Among the cohort, age-time-solvent and duration of exposure specific SMR's for selected causes of death will be tabulated, latency period estimated and high risk sub-groups identified. This research design will provide data to address the following questions:

1. Is there any difference in the distribution of causes of death or mortality rates among owners/operators or operators of dry cleaning plants when compared to the U.S. white males, or white male Oklahoma between 1941-1985?

2. What influence does type and duration of exposure have on age and time specific deaths?

Publications - None to date

URINARY EXCRETION OF MODIFIED NUCLEOSIDES

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Occupational Cancers
5 R01 OH02122-02
08/01/85 - 07/31/88
\$101,605 (\$196,109 Cum)

Objectives

Transfer RNA (tRNA) is a complex biomacromolecule containing a large number of modified bases. The modifications include methylation by several enzymes which are specific for bases, positions in bases, and specific for positions in tRNA. The most abundant of these enzymes are those which insert methyl groups at various positions in the macromolecule. The methylating enzymes have been found to be hyperactive in malignant tumor tissues, which therefore contain tRNAs that are different in structure from those present in normal tissues. These modified tRNAs also have a high turnover rate. It is known that patients with certain types of cancer excrete in their urine elevated levels of modified nucleosides (tumor markers). We have demonstrated abnormal levels of modified nucleosides in patients with asbestos related malignant mesothelioma. We have also shown, in a feasibility study, altered nucleoside excretion among individuals without clinical evidence of cancer but with no long-term history of occupational exposure to airborne asbestos. Measurement of modified nucleosides is therefore considered a possible approach to detection of a precancerous state or early diagnosis of cancer among individuals who are at high risk for developing a malignant disease. We are measuring levels of modified nucleosides in the urine of 1,000 asbestos insulation workers with a history of 30 years or more since first onset of exposure. The investigation focuses on whether the urinary excretion profile of nucleosides is related to the effects of asbestos exposure and whether the levels of urinary nucleosides may be predictive of subsequent malignant disease.

Methodology

We have obtained extensive clinical and laboratory information on 2,900 asbestos insulation workers from clinical field examinations performed between 1981-1983. A prospective mortality study has been designed, and the study population is currently kept under surveillance in terms of mortality experience. The nucleoside pattern will be characterized for the entire population, and a matrix of intercorrelations between nucleosides and pertinent medical and laboratory information will be generated. The association between the initial nucleoside excretion pattern observed at the clinical field examination and the mortality experience will be studied.

Progress & Accomplishments

The 1,000 individuals who constitute the study population have been selected from the 2,900 examined in the clinical field studies. All were without history of cancer or clinical signs of malignant disease at the time of the examination. We are being notified about all deaths which have occurred in the study population. They are currently in process of being ascertained, but earlier projections made in studies of this group of asbestos insulation workers seem to receive further confirmation. Approximately 50 percent of the deaths are due to cancer, and the original observation of an

extraordinarily high cancer mortality in this population will thus occur. Measurement of modified nucleosides in the first 500 urine samples is in progress. In preparation for the mortality analysis, expected mortality rates for the study population are being calculated by collaborating scientists at the American Cancer Society.

Statistical analysis is also proceeding with regard to characterization of the study population in terms of the baseline demographic, clinical and laboratory information which was obtained at the onset of the study.

Significance

The initial results of this project relate to the characterization of the study population and acquisition of mortality data. Our initial observations have shown that the study population experiences a high cancer mortality rate, similar to what has been observed in the large cohort of asbestos insulation workers from which the study population was derived. This provides us with a unique opportunity to obtain information about the predictive value of modified nucleosides in terms of the development of asbestos related neoplastic disease. The extensive amount of laboratory and clinical information available on this high risk population will also enable us to perform analyses with regard to multiple factor interactions.

Publications - None to date

FACTORS MODIFYING DIESEL PARTICULATE COMPOSITION

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Occupational Cancers
7 R01 OH02351-01*
09/27/85 - 11/30/88
\$69,031 (\$69,031 Cum)

* Previously supported under Grant Number R01 OH02119

Objectives

Ceramic particle traps are being developed for use as diesel exhaust emission controls for over-the-road as well as heavy-duty diesel engines used in mining. These devices collect entrained diesel aerosols, and on reaching the regeneration temperature, catalyze combustion of trapped materials. While they reduce the opacity of tailpipe emissions and thus are desirable from an aesthetic point of view, little is known about the physical and chemical properties and biological activity of the particles they transmit. Therefore, the possible impact of this new technology on human health needs evaluation. This is particularly true for occupational settings such as underground mines where workers may be exposed to diesel fumes at twenty-five times the levels found in congested urban canyons, with proportionately greater risk.

The long-term project objective is to assess the relative hazard to human health of particulate emissions from diesel engines with and without ceramic particle traps. The study will focus on cancer risk as opposed to non-malignant respiratory effects.

The comparative risk assessment will be accomplished through the following specific aims: 1) collection of particulate samples from a commercial mining diesel under simulated mining conditions; 2) determination of the mutagenic potency of the diesel particulate soluble organic fraction (SOF) with the Ames Salmonella/mammalian microsome test; 3) definition of the operating modes which contribute most to the production of chemical carcinogens under steady-state sampling conditions; 4) further examination of the chemical composition and biological activity of particulate emissions under conditions of transient diesel engine operation.

Methodology

Diesel particle samples (provided by the U.S. Bureau of Mines) were collected from a diesel test cell consisting of a commercial diesel mining engine, a dynamometer, an exhaust dilution tunnel and a particle collection system. The engines tested were operated in a series of 6 modes (a combination of engine load and speed), typical of mining machinery operating in a load-haul-dump cycle. The particles collected in this system accurately simulate tailpipe emissions. The variables studied in this budget period were the Corning ceramic particle trap and a manganese fuel additive (Lubrizol 3220). Two engine designs were examined including a water-cooled, Caterpillar 3304 4-cylinder and an air-cooled Deutz engine.

Progress & Accomplishments

Carcinogen Analysis. An analytical procedure for the quantification of nitrated polycyclic aromatic hydrocarbons (nitro-PAH) in diesel exhaust particulate matter was developed and validated.

Ceramic Particle Trap. Various trends were established in these studies: 1) the ceramic particle trap greatly reduces the mass of emitted particulate matter and 2) the ceramic trap transmits particles with elevated mutagenic activity. Under baseline conditions (i.e., with no trap) particles emitted have a mutagenic activity of 64-233 revertant colonies (rev)/mg — with the trap in place the mutagenic activity ranges from 46-1, 400 rev/mg. The net effect of the properly operating trap, however, is to reduce the total output of mutagenic substances. Mathematical modeling techniques that extrapolate steady-state emissions to underground air quality indicate that the ceramic particle trap can be expected to reduce particulate matter (-93%), unspecified mutagenic substances (-83%) and 1-nitropyrene (-97%) in the breathing air of miners.

Manganese Fuel Additive. A commercial, manganese fuel additive is being considered for use on trap-equipped engines because it lowers the temperature at which trapped particles are combusted in so-called trap "regeneration". The temperature reduction is an important safety consideration because it reduces the risk of mine explosions.

In these studies we find that the Mn fuel additive greatly increases the output of mutagenic substances, an important observation not previously reported in the literature. Engines operated with recommended levels of Mn generate soot with over six times the mutagenic activity (rev/mg) of baseline emissions (average of six operating modes). The output of 1-nitropyrene also is markedly increased by the fuel additive.

Significance

The development and thorough evaluation of emission control devices for diesel-powered, underground mining machinery is critical in protecting the health of miners. While existing epidemiological studies are negative or inconclusive regarding the chronic health hazard posed by exposure to diesel exhaust, it cannot be concluded that such exposures are without health consequences. The existing animal studies of diesel exhaust particle exposure use doses similar to those which miners may experience in poorly ventilated mines (P. Carey, "Summary of EPA and Other Programs on the Potential Carcinogenicity of Diesel Exhaust," PB82-128018, 1981), and are of short duration relative to the working lifetime of a miner. It has been demonstrated in recently published in vivo animal studies that carcinogens like 1-nitropyrene are "readily released" from diesel particulate matter in the lung and become bioavailable in the gastrointestinal tract (Lewtas et al., U.S. EPA Symposium, 1986).

Each of the parameters examined (particulate levels, mutagen density and nitropyrene concentration) lends support to the conclusion that the ceramic particle trap will provide a sizable reduction in cancer risk experienced by miners. The Mn additive will have a negative effect on air quality if used on machinery that is not fitted with properly functioning ceramic particle traps. The additive should not, for example, be used uniformly in partially retrofitted fleets of mining machinery or where traps have failed mechanically.

Publications

Draper WM: "Quantitation of Nitro- and Dinitropolycyclic Aromatic Hydrocarbons in Diesel Exhaust Particulate Matter", *Chemosphere* 15:437-447, 1986.

LEUKEMIA RISK IN LINEMEN

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Occupational Cancers
1 K01 OH00031-01
09/28/84 - 12/31/85
\$32,400 (\$32,400 Cum)

Objectives

An epidemiologic case-control study is being conducted to assess the risk of leukemia in telephone linemen. The study tests the hypothesis that electric power lines mounted on poles shared between the electrical utilities and the telephone companies may represent a health risk to linemen servicing the telephone cables on these poles.

Methodology

Through an arrangement with the Medical Research Department at American Telephone and Telegraph (AT&T), all deaths from leukemia that occurred among active or pensioned operating telephone company employees between 1975 and 1980 have been identified. Study cases include all active or pensioned employees who worked at least two years for AT&T, were identified through the AT&T mortality data base, died of leukemia in the six-year period 1975-1980, and did not have another underlying hematologic cancer. Specific cell types of leukemia have been identified from death certificates and company medical records. A matched control group has been selected from the Bell Systems Personnel Data Base. The controls are comparable to the cases in age, sex, race and calendar time of employment. Complete work histories for the study subjects have been obtained from telephone company personnel service record cards. Visits to telephone companies have identified specific job titles and activities associated with line work and potential exposure to electric and magnetic fields, and individual exposures will be estimated from jobs held and duration in the job. In the analysis, the proportions of cases and controls who were ever in jobs associated with field exposure will be compared.

Progress & Accomplishments

Copies of articles published since 1960 relevant to human health effects of 10-100 Hz electromagnetic field exposure (excluding direct electrical stimulation) have been obtained and reviewed. Abstracts are being written and added to the data retrieval system to create a complete, annotated bibliography. Articles on the human health effects of substances to which linemen may be exposed (such as asbestos, benzene, herbicides, insecticides, petroleum products, polychlorinated biphenyls, and solders) have been reviewed and selectively collected.

Four hundred and twenty-nine operating telephone company employees who had died of leukemia between 1975 and 1980 were identified. The names of these potential cases were submitted (prior to any exclusions) to the appropriate operating telephone company medical offices for confirmation of the death certificate diagnosis of those cases with medical records on file, additional information on cell type of leukemia, and identification of study subjects with another hematologic cancer that predated their leukemia. One hundred and thirteen individuals were excluded from the study because they

had worked for less than two years (4), were diagnosed with a hematologic cancer that predated their leukemia (14), were nonwhite (7), had chronic lymphoid leukemia (87), or both another hematologic cancer and chronic lymphoid leukemia (1). Individuals with chronic lymphoid leukemia were excluded because this form of leukemia is usually not rapidly lethal, and will be incompletely ascertained from death records such as the AT&T mortality data base. Photocopies of the death certificates for all cases but one have been obtained from the files at AT&T. A trained coder has coded these using the 1975 International Classification of Diseases. Three controls have been selected for the 316 cases by probability sampling, and are matched to cases in year of birth, year of hire, race, sex and company. The control sampling frame is the tape copy of the complete Bell System Personnel Data Base (1.2 million employees each year) for the years 1975-1980. The controls are individuals who have not died of leukemia and who were active or retired at the same time as the leukemia case.

The medical directors of each telephone company have identified a supervisor from personnel or payroll as a contact who would provide the work histories of the study subjects. We have requested work histories for the study subjects. We have also requested information on race, level of education, and jobs held prior to employment with the Bell System, when available. By December 31, 1985, we had received photocopies of work histories for 30% of the study subjects. Companies 23 and 40-42 have stated that they would require employee authorization to release work history information. Using information from personnel offices and from the actual work histories received, an alphabetical listing of Bell System occupations has been compiled. Jobs will be classified by appropriate categories and a coding scheme will be developed. Available records do not permit quantification of actual electromagnetic field exposures for each individual study subject. The most that is achievable is collection of work history data on months or years in jobs with potential exposures, and characterization of these jobs by current exposure levels. Site visits have been conducted at Chesapeake and Potomac Telephone and at Mountain Bell to obtain job descriptions for linemen and observe linemen at work rural and urban sites. Linemen can be categorized by the following job titles: installer-repairer, outside plant technician, cable splicer, and helpers who work with each category of lineman. A description of the common activities and work locations for these job classifications has been developed based on the interviews with supervisors and the review of job descriptions and daily work logs obtained from the telephone companies. A questionnaire has been developed to obtain an estimate of exposures other than electromagnetic fields by subgroups of linemen.

Significance

Recent reports have suggested that persons who are exposed to power lines are at increased risk of leukemia; this study will test that hypothesis. To date, it is our conclusion that an epidemiologic case-control study of leukemia in telephone company employees is feasible; however, since collection of work histories depends upon the cooperation of so many personnel departments, it is a source of more delay than originally anticipated.

Publications - None to date

PROSTAGLANDIN SYNTHETASE AND LUNG TOXICITY WITH BHT

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Occupational Cancers
5 R03 OH01833-02
07/01/83 - 12/31/85
\$19,600 (\$36,409 Cum)

Objectives

The major objectives of this study were to determine (1) whether butylated hydroxytoluene (BHT) is metabolized to a reactive intermediate by prostaglandin H synthase and (2) whether peroxidase activation of BHT is a significant pathway in the in vivo toxicity of BHT to mouse lung.

Methodology

Two model peroxidase enzymes, horseradish peroxidase and prostaglandin H synthase, were used for the studies of BHT metabolism. Prostaglandin H synthase was obtained from ram seminal vesicles. The in vitro formation of reactive metabolites was monitored by (1) the covalent binding of ¹⁴C-radiolabeled BHT to protein, (2) the formation of BHT-quinone methide, the purported ultimate toxic metabolite of BHT, by spectrophotometric methods, and (3) the formation of free radicals from BHT using electron spin resonance techniques. Stable metabolites of BHT were identified by conventional methods. The in vivo toxicity of BHT to mouse lung was assessed by measuring lung weights and by histopathology four days after exposure.

Progress & Accomplishments

The major findings from the past year on this project can be summarized as follows:

A. BHT was converted by peroxidase enzymes to a reactive metabolite which covalently bound to microsomal protein. This reaction was concentration dependent and was inhibited by glutathione and certain peroxidase substrates. BHT also formed two polar metabolites (one aqueous, one organic) as detected by thin layer chromatography. The formation of these metabolites increased when glutathione was present suggesting they might be glutathione or protein conjugates.

B. In the presence of butylated hydroxyanisole (BHA), the covalent binding of BHT was enhanced (+400%). In addition, two additional products were detected: BHT-quinone methide and stilbenequinone. The formation of the highly reactive quinone methide might account for the increase in covalent binding.

C. In addition to BHA, several other compounds were capable of enhancing BHT covalent binding and quinone methide formation. These included compounds which were able to form phenoxy radicals or nitrogen-centered cation radicals. Alternatively, compounds which formed peroxy radicals or semiquinone radicals inhibited BHT metabolism. A direct chemical-chemical interaction between the BHA radical and BHT resulting in the generation of quinone methide was proposed to explain the enhanced metabolism and covalent binding of BHT.

D. A free radical resulting from the metabolism of BHT by horseradish peroxidase was detected using electron spin resonance and is currently being analyzed to determine the chemical structure of the radical.

E. Microsomes from various animal tissues (mouse lung, rat lung, human lung, dog lung, guinea pig lung, sheep lung, rabbit kidney medulla, and rat small intestine) were capable of catalyzing the covalent binding of BHT to protein using either arachidonic acid or hydrogen peroxide as substrates. BHA was able to enhance BHT binding in each case.

F. In the mouse lung, subcutaneous administration of BHA (50 or 250 mg/kg body weight) was observed to significantly enhance the toxicity of subthreshold doses of BHT (0-250 mg/kg) as measured by increased lung/body weight ratio. This enhancement of lung/body ratio was dependent on the time of administration of BHA and was much more effective when BHA was injected subcutaneously than intraperitoneally.

Significance

The results presented here demonstrate an interesting post-enzymic interaction between BHA and BHT resulting in the formation of a toxic metabolite, quinone methide. This method of activation of BHT by a chemical-chemical interaction represents a method of activation of a xenobiotic compound for which there are few examples in the literature. This interaction between BHA and BHT can take place in a variety of animal tissues which contain peroxidase enzymes, including human lung. In the mouse lung, BHA was also able to enhance BHT-induced lung toxicity. These results suggest that tissues which contain peroxidase enzymes might catalyze the formation of the toxic quinone methide. These results also point the importance of considering toxic effects from combinations of chemicals that are commonly used together in addition to testing them singly.

Publications

Thompson, D.C., Chan Y.N., and Trush M.A.: the peroxidative activation to butylated hydroxytoluene to BHT - quinone methide and stilbenequinone. In: Biological Reactive Intermediates, III (Jollow, D.J., Kocsis, J.J., Witmer, C.M., Nelson, J.O., and Snyder, R., eds.). Plenum Press, New York, pp. 301-309 (1986).

Thompson, D.C., and Trush M.A. The toxicologic implications of the interaction of butylated hydroxytoluene with other of interaction of butylated hydroxytoluene with other antioxidants and phenolic chemicals. *Fd. Chem. Toxicol.* (in press, 1986).

DEVELOPMENT OF A HUMAN DNA ADDUCT MONITORING METHOD

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Occupational Cancer
1 R03 OH02238-01
05/01/86 - 04/30/87
\$21,206 (\$21,206 Cum)

Objectives

DNA adducts resulted from covalent binding by agents to the bases of DNA. Monitoring adduct formation in carcinogen exposed persons may be a good dosimeter of the risk of genetic disease because individual differences in absorption, distribution, excretion and DNA repair are effectively integrated. DNA binding is likely to be a relevant end point for genotoxicity.

The post-labelling method of DNA adduct analysis has potential to be used as a tool to monitor adduct formation in humans. No radiolabelled carcinogen is required, only microgram quantities of DNA are needed, and the method can detect adducts to a few femtomoles/microgram DNA. The method can also be used to analyze different but related compounds, so it is adaptable to various exposure situations.

To be accepted as a human monitoring tool, the post-labelling method should first be validated with other tests of genotoxicity in animal models. The quantitation of post-labelling and standard scintillation counting will be compared in mouse liver using labelled benzidine. The kinetics of benzidine adduct formation and removal (repair) will be studied. Then, the relationship between DNA adduct levels and subsequent chromosome aberrations will be determined in the liver of partially hepatectomized mice. The liver was chosen for those studies because it is the target organ of benzidine carcinogenicity in mice.

Methodology

DNA isolated from mouse liver is degraded to the 3'-phosphonucleotides, which are then labelled with ^{32}P from (Y- ^{32}P) ATP. Normal nucleotides are resolved from the adducts by thin layer, ion exchange chromatography. Because of post-labelling's requirement of only microgram quantities of DNA, adduct and chromosome analyses could be performed from the tissue of each animal.

Progress & Accomplishments

The correlation between DNA adduct levels and chromosome aberrations has been established. On the basis of individual animal livers the correlation between the two parameters is 0.624. When dose group analysis is performed, the correlation is 0.94. The shapes of the dose response curves for adducts and aberrations implies that a third process underlies both. The study of the kinetics of adduct formation indicates that metabolic activation of the procarcinogen is the most likely explanation. The time at which adduct levels peak is dose-dependent. Adduct levels as low as 1 adduct in 37 million normal nucleotides could be detected reproducibly.

Significance

These data indicate post-labelling has real potential to be used to monitor DNA adduct formation in exposed humans. Adduct levels are casually associated with chromosome aberrations and predict this significant genetic effect. Adducts could be detected at levels approaching what might be expected to result from human exposure. These studies also indicate that metabolic differences between individuals may affect adduct formation.

Publications - Abstract(s) available

CYTOGENETIC ASSESSMENT OF OCCUPATIONAL EXPOSURE
TO ANTINEOPLASTIC AGENTS

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Occupational Cancers
1 R03 OH02243-01
05/01/86 - 04/30/88
\$21,092 (\$21,092 Cum)

Summary

This study proposes to evaluate the utility of measuring the effects of low level, long term occupational exposure of hospital personnel to antineoplastic agents through conventional cytogenetics (chromosomal breakage) and to attempt to correlate those measurements with micronuclei frequency.

The study population includes nursing personnel evaluated for actual occupational exposure to antineoplastic agents through admixing and administering chemotherapy drugs. Based on documentation of exposure, nursing personnel will be placed in one of three exposure categories: no known exposure, low exposure or high exposure.

Patients diagnosed, but not yet treated for breast cancer will serve as the second study population group. Patients will be both no exposure - negative controls (pre-treatment) and very high exposure - positive controls (post treatment).

All study subjects will be interviewed to identify possible confounding factors of exposure - age, smoking and occupational or environmental exposure to genotoxic agents. Blood samples will be collected from all subjects (single sample from nursing personnel, pre-treatment and post treatment sample from patients).

Peripheral blood lymphocytes will be analyzed for determination of frequencies of chromosomal breakage and micronuclei.

Frequencies will be compared between exposure groups to attempt to identify the absence or presence of a dose response relationship between exposure to antineoplastic agents and chromosomal damage. Correlation will also be assessed between the frequency of chromosomal breakage and the frequency of micronuclei. Significant positive correlation coefficients would indicate the appropriateness of estimating chromosomal damage through the more efficient and cost effective technique of micronucleus methods.

Publications - None to date

EXPLOSION HAZARDS RELATED TO GRAIN AND FEED DUSTS

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Traumatic Injuries
5 R01 OH01122-06
01/01/81 - 07/31/87
\$99,184 (\$580,069 Cum)

Objectives

The purpose of this research project is to quantitatively characterize the explosion hazard represented by suspended and layered combustible dusts. Although much explosion testing with combustible dusts has been done for many years, almost all of these results are apparatus dependent. The results fail to characterize a fundamental physical quantity, and none of it directly pertains to layered dust hazards. Under this project the burning velocity for a suspended dust cloud and for a layer of dust is being measured. Appropriate analytical efforts are also required in order to understand the effect of several physical variables -- composition, concentration, size, moisture content, shape, volatility, etc.

Methodology

It was necessary to develop two new pieces of equipment in order to avoid criticisms associated with previous dust explosion testing. The suspended dust combustion studies are conducted in the Premixed Turbulent Combustion Bomb (PTCB), which is a spherical, one cubic meter jet stirred reactor. The combustion process is initiated at the center of the well characterized, uniform, turbulent dust cloud and during its propagation toward the vessel wall appropriate measurements are made to characterize the burning process. The layered dust testing is done in the Flame Acceleration Tube (FAT), which consists of three continuous segments of 3,000 psi working pressure steel tubing each having a length of forty feet and an inside diameter of one foot, closed at one end and open at the other. A controlled thickness and width dust layer may be placed along the bottom of the tube which is then ignited by the combustion of a presuspended dust cloud in the first twelve feet at the closed end. The history of the subsequent combustion process as it accelerates toward the open end is monitored using appropriate instrumentation. Recently a Laser Doppler Anemometer (LDA) has been acquired through an industrial gift and with this instrument it is possible to measure the velocities of dust particles in either apparatus. This allows accurate determination of both the hydrodynamic velocity and the turbulence intensity. In both facilities, for different dust conditions, the flame propagation and properties are characterized.

Progress & Accomplishments

Using the PTCB the laminar and turbulent burning velocities have been measured for methane/air mixtures and these are in excellent agreement with values obtained in a different fashion thus verifying the concept of using a jet stirred reactor for dust combustion studies. Using the LDA, turbulence intensities within the bomb have been measured with and without the presence of combustible dust. This data is in close agreement with the previously employed hot wire data. The laminar and turbulent burning velocities have been measured for many additional dusts-polyethylene, graphite, coal, Pure

Food Powder, Mira-Gel, and ground corn cobs. The laminar data for coal is in agreement with data reported elsewhere and obtained using a different technique. The data trends make good physical sense with small, dry, and reactive dusts giving the highest burning velocities. For the ignition of a turbulent cornstarch cloud the energy has been shown to be a function of both the power of the ignition source and the turbulence intensity.

Using the FAT both the burning and the flame velocities have been measured as well as the accompanying overpressures for several new dusts including-Mira-Gel, Pure Food Powder, actual floor sweepings, and Navy bean dust. For the flow enhanced dust (Mira-Gel) large overpressures (6 atm) were easily produced and flame acceleration continued to occur even after the end of the effective entrainment of the dust. For mixed grain dust the introduction of turbulence inducing grids into the tube was shown to result in flame acceleration through an enhancement of the burning velocity. Ground corn cobs may be obtained accurately sized, and through blending an bimodal size distribution for the dust may be obtained. The most severe explosions are produced by a mixture containing both sizes as the more easily entrained larger particles apparently propel some of the smaller particles into the airstream. A similar effect may be produced through the addition of a lwo enhancing agent to the smaller corn cob particles.

Significance

For a suspended dust/air mixture the turbulent burning velocity is a fundamental physical parameter. Knowledge of the quantity allows analytical prediction of flame propagation as it would occur during the course of an accidental explosion, as it is not possible to test all possible dust explosion configurations. Through the use of many different kinds of dust having different physical and chemical properties - surface area, drag coefficients, vapor pressure, etc. - it will soon be possible to develop a model of a turbulent dust flame and to determine the mechanisms on which it depends for propagation. For layered dust the combustion process depends upon successful entrainment which is surprisingly snesitive to several parameters. This dust then reacts as if it were a premixed turbulent dust cloud. The developing understanding of the entrainment mechanism will allow a significant improvement of the existing layered dust model so that the behavior of a secondary explosion will perhaps be predictable. This information would be expected in industrial operations in the United States where only in the grain industry through 17 December 86 there have been 20 explosions, 14 injuries, and 2 deaths so far in the calendar year.

Publications

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Srinath SR, Kauffman CW: Secondary Dust Explosions, Symposium on Industrial Dust Explosions, Pittsburgh, PA, June 1986.

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Tai CS, Kauffman CW, Nahatis HM, Sichel M, Nicholls JA: The Use of a Jet Stirred Reactor to Determine the Combustion Characteristics for Methane/Air, International Workshop on the Gas Flame Structure, USSR Academy of Sciences, Siberian Division, Novosibirsk, USSR, July 1986.

FEEDBACK AND SAFETY: INVOLVING WORKERS

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Traumatic Injuries
5 R01 OH01928-02
12/01/83 - 11/30/85
\$30,347 (\$92,718 Cum)

Objectives

The purpose of the study was to determine to what extent involving the direct participation of workers as a source of delivery of feedback would influence safe performance on the job.

The specific objectives were accomplished as follows:

1. To refine a system already developed for assessing job safety performance.
2. To use the refined measurement system to observe performance in order to establish pre-intervention baselines.
3. To train a group of volunteer workers to supply performance feedback using a simplified form.
4. To compare the effect of involving workers in providing performance feedback vs. currently standard practice by examining accidents and injuries, consumer satisfaction and other dependent variables.

Methodology

At a paper mill, 21 work areas were divided into functional units of from 2 to 15 people. Safety targets were identified by reviewing accident records, conducting multiple interviews with a wide spectrum of managers, hourly workers, union leaders and safety personnel; by observing workers performing on the job; and then setting priorities. For each unit, the set of targets was defined and revised until safety personnel, managers and worker representatives agreed as to the importance of each item and its definition. Items were then placed, with their definitions, on observational checklists.

Baselines data, consisting of 5 to 67 data points, were reliably collected for 17 of the 21 units. The 4 units for which sufficient baseline data could not be collected were the irregularly operating midnight to 8 AM shifts. After baseline, workers were familiarized with the system by supplying them with feedback on first one, then two, and in some cases three target behaviors that they themselves had selected from the pool of items identified as being relevant to their units.

Each of the 11 units whose performance still needed improvement and who expressed a willingness to participate had members who volunteered to serve as peer auditors. Nine of the units had sufficient baseline data to include in a statistical analysis.

Safety scores were selected as the main dependent variables. These were collected repeatedly, at least weekly, throughout the study, immediately afterward for several weeks and five additional times during the nine-month period thereafter. Consumer satisfaction data were collected from a small sample of respondents in the form of a generic Safety Climate Questionnaire.

Progress & Accomplishments

1. Improvements in safety performance from baseline to the third feedback

phase were statistically significant.

2. Of the nine units involved in the peer feedback phase, one peer auditor stopped after the first time, while the eight other posted between 5 and at least 14 (total = 63) feedback forms over a period spanning several months.

3. In terms of the safety scores, although performance diminished somewhat under peer auditing, it was, in general (5 of the 8 units), still higher than during baseline. Analyses of variance showed a significant interaction between targeted and non-targeted items and peer vs. no peer feedback conditions for the long-term follow-up. For targeted items, units that had involved peers showed a 25% performance improvement over baseline (changing from approximately 30% to 40%) nine months after all interventions had been discontinued, while safety scores for units in which peers were not involved and non-targeted items all dropped to below their baseline averages (peer feedback group, non-targeted items: -5; non-peer feedback group, targeted items: -27; non-peer feedback groups with non-targeted items: -5).

4. Accident data have been collected and currently are being analyzed, as are costs. No significant differences were found for pre-post scores on the Safety Climate Questionnaire although several factors were endorsed by groups of workers in both plants as being especially relevant to safety in their workplaces. For example, women indicated that they felt that social factors were very important to safety.

Significance

Once again, this study has shown how safety performance on the job can be improved via the delivery of specific performance feedback. By demonstrating that peers can fulfill the function of safety auditors, the research has demonstrated that this activity can be undertaken by people other than supervisors, safety or research personnel. Consequently, the feasibility for utilizing feedback systems has been expanded. Most promising is the potential that involving peers might have for supporting long term durability of change, as suggested by the data. The next step is to find ways to perfect such peer involvement, for example by identifying variables that relate to optimal peer selection and methods of implementation of the system.

Publications

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WORK AND RISK OF CARDIOVASCULAR DISEASE IN THE FRAMINGHAM STUDY

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Cardiovascular Effects
1 R01 OH02057-01
01/01/85 - 12/31/85
\$121,342 (\$121,342 Cum)

Objectives

To carry out a prospective examination of the incidence of cardiovascular disease in relation to long-term work patterns based on 30 years of surveillance of the Framingham Study cohort. To classify subjects according to employment status, work environment, employers, and to merge these work history data with other cardiovascular risk factor data, demographic and social variables and cardiovascular disease status. From this data to determine the incidence of cardiovascular disease in relation to the work history variables and to assess the net and joint effect of the work variables on cardiovascular disease incidence taking into account the major associated cardiovascular risk factors. Interactions between work history variables and other demographic and psychosocial variables are to be explored in relation to impact on cardiovascular morbidity and mortality.

Methodology

The effects of labor force participation, occupations and employers on cardiovascular outcomes such as coronary events, strokes, cardiac failure and peripheral arterial diseases is examined taking into account medical, psychosocial and demographic variables using multivariate multiple logistic regression analysis. This is done prospectively using DOT work classification codes at each biennial exam in relation to occurrence of new cardiovascular events over the 2-year interval. The relation of work variables to cardiovascular risk factors is examined including: blood lipids, blood pressure, blood sugar, cigarette smoking, relative weight and ECG abnormalities. Using multivariate analysis the effects of work variables on cardiovascular morbidity and mortality is examined taking into account the major associated cardiovascular risk factors.

Progress & Accomplishments

The classification and coding of work variables including: unemployment history, number of jobs held, part-time work, overtime work, physical demand of job, psychological stress, DOT and census occupational categories and exposures to noxious agents has been completed for each biennial exam over 30 years of follow-up on 5209 men and women participating in the Framingham Study. Work tapes including these variables, cardiovascular risk factors and cardiovascular outcomes have been created. Preliminary analysis relating these work variables to cardiovascular outcomes has been carried out identifying a number of positive findings justifying further, more detailed analysis.

Significance

Epidemiologic investigation indicate powerful environmental influences on cardiovascular risk factors and on cardiovascular events. These data provide

information on the work environment, a major component of life-style which is modifiable. Knowledge of occupational exposures as risk factors for cardiovascular disease is of great preventive importance.

Publications - Abstract(s) available

Gordon G, Isreal I, Brehm H, Berkeley J, Kannel WB: The relationship between occupation, physical demand of job and life expectancy among Framingham Heart Study males. American Heart Association 26th Annual Conference on Cardiovascular Disease Epidemiology, March 1986 and published American Heart Journal 112:820-825, 1986.

LEAD BURDENS, EXPOSURE ESTIMATES AND HYPERTENSION

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Cardiovascular Effects
5 K01 OH00011-02
09/28/84 - 08/31/86
\$19,440 (\$32,400 Cum)

Objectives

The two major objectives of this project are to develop epidemiologically useful tools for estimating cumulative lead exposure and to conduct a pilot case-control study investigating a potential relationship between lead and hypertension.

Methodology

Standard twenty-four hour diagnostic chelation was conducted on a group of patients with and without known lead exposure. These patients then underwent x-ray fluorescent determination of skeletal stores of lead at the distal ulna (styloid process), and at the temporal bone. Correlation between XRF values and excreted lead was determined. Sensitivity and specificity of XRF vis a vis standard diagnostic chelation was determined using 600 mcg Pb/24 hour as the cut-off point.

Fifteen patients with and without known lead exposure received standard 24 hour diagnostic chelation for lead. Volumes and lead concentrations were additionally measured at three hours and at six hours lead excretion at three and six hours was correlated with total 24 hour results. Five additional patients have been recruited to bring the total to 20.

An exposure questionnaire has been developed and pretested. Composite lead estimates and individual questions will be validated for patients selected without knowledge of exposure status by using results of lead excretion.

Twenty randomly selected hypertensive patients are being matched with twenty controls on the basis of age, sex, race and economic status. A pilot case-control study will compare lead excretion using six-hour chelation results and questionnaire results.

Progress & Accomplishments

The x-ray fluorescent determination of skeletal stores at the wrist was found to have a correlation coefficient of .52 with 24 hour urine lead excretion following standard chelation ($.05 > p > .02$).

Specificity was 100% but sensitivity was only 56% using 600 mcg Pb/24 hour as the cut-off, and false-negatives occurred in individuals with lead excretion as high as 2500 mcg/24 hour. Results from XRF determinations at the temporal bone showed even less epidemiologic screening.

Three hour lead excretion following chelation has a correlation coefficient of .87 compared with standard 24 hour results. Six hour lead excretion has a correlation coefficient of .97 with 24 hour results. Both relationships are statistically significant and appear to be linear. We are collecting a total of 20 subjects and plan to submit this method for publication in the near future (an abstract has already been submitted to SREPCIM).

Both questionnaire validation and the pilot case-control study are proceeding using the six-hour urinary lead collection.

Significance

The study to date has validated a shortened form of diagnostic chelation which is feasible for use as an outpatient procedure. This can be used as an epidemiologic tool for case-control studies evaluating the chronic effects of cumulative lead exposure, one example of which is the pilot study of hypertensives and non-hypertensives being conducted. Similar studies of patients with dementia, gout, or cerebrovascular accidents could use this tool.

Publications - Abstract(s) available

OCCUPATIONAL RISKS OF PESTICIDE EXPOSURE FOR FEMALES

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Reproductive Disorders
5 R01 OH00835-08
09/29/82 - 08/31/88
\$37,852 (\$317,185 Cum)

Objectives

The present project is designed to characterize some of the reproductive hazards that confront females engaged in occupations which subject them to potential exposure to pesticides. It will evaluate the effects of a known reproductive toxin on both the ovary and ovulatory ability of a non-pregnant female and on the embryological development of the ovaries of females exposed prenatally to the toxic agent. This study intends to determine how inhibition of normal reproductive activities is induced by such agents by ascertaining the effects of such chemicals on steroid hormone levels, specific follicle populations and cell surface characteristics of the different cellular components of the ovary.

Methodology

Virgin female CD-1 mice were employed in this study. Adults were randomly distributed into three treatment groups: a Kepone-treated group, a group treated with estradiol-17 β (E-17 β), and sesame oil vehicle control group. Mice were exposed to these agents for two, four, six or eight weeks. Weekly procedures consisted of five consecutive daily exposures followed by two days of no treatment. This time-table was established to mimic an ordinary five day work week which would represent the maximum weekly exposure to which a female working with such a compound, might be subjected. Kepone (0.25 mg), estradiol-17 β (0.1 mg) and sesame oil were administered in a 0.2 ml volume by oral gavage. During the final week of exposure exogenous gonadotropins were administered to determine whether the ovaries could elicit an ovulatory response to these gonadotropins. At the end of the prescribed time period, animals were sacrificed. Livers were removed from the mice and prepared for gas chromatographic analysis. Oviducts were flushed to tabulate the number of eggs ovulated in response to the gonadotropins. Ovaries were also removed to determine the number and ratio of the different follicle populations and to note any alterations in cell surface characteristics through the use of lectin conjugated probes.

Prenatal studies consisted of exposing a pregnant female mouse to either Kepone, E-17 β or sesame oil between Day 6 and Day 15 of pregnancy. Animals were sacrificed at Day 18. Fetuses were weighed and sex was determined. Fetal livers were removed for gas chromatographic analysis of pesticide incorporation. Fetal ovaries were subjected to similar procedures as described above.

Progress & Accomplishments

Results obtained, thus far, in the adult mouse study reveal that Kepone causes a significant decrease in the ovulatory response of the ovaries following four week exposures when compared to both E-17 β and vehicle controls. After eight weeks of Kepone exposure there was no further decrease in this response but rather a tendency for a greater percentage of these mice

to ovulate larger numbers of oocytes. Liver weights of Kepone-exposed mice increased significantly and linearly from two to six weeks of exposure with a plateauing of values between 12-19% of body weight at eight weeks. Similarly, incorporation of Kepone by the liver rose dramatically at first and then leveled off at the longer exposure times.

Preliminary evidence obtained in the prenatal exposure to Kepone indicates that Kepone does not induce any external malformations nor any deviation from the normal sex ratio. Fetuses are significantly smaller following Kepone exposure. The histological picture of these prenatally exposed ovaries are currently being investigated.

Significance

The decreased ovulatory response seen in adult females following Kepone exposure suggests that since there are sufficient exogenous gonadotropins available to stimulate ovulation and since ovulation is drastically impeded in Kepone-treated animals, the effect of the pesticide might be exerted at the ovarian level. Data soon to be obtained on steroid hormone levels, cell membrane alterations of different cell types in the ovary and follicle populations will be useful in determining where, within the ovary, such a toxic agent might have its target.

Publications - Abstract(s) available

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Swartz WJ, Mattison DR: Benzo(a)pyrene inhibits ovulation in C57BL/6N mice. Anat Rec 212:268-276, 1985.

EMBRYO CULTURE/TERATOGEN BIOTRANSFORMATION SYSTEM

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Reproductive Disorders
5 R01 OH01281-03
09/29/82 - 02/28/86
\$114,466 (\$309,960 Cum)

Objectives

Enzyme inducing agents, including phenobarbital, 3-methylcholanthrene, polychlorinated biphenyls, ethanol (and others, if time permits) will be employed to investigate the qualitative and quantitative effects of changes in enzyme complement on malformations elicited in the embryo culture systems. These inducing agents will be studied in Sprague-Dawley rats during the earlier stages of gestation and effects as various xenobiotic biotransforming systems will be assessed to better determine what relationships exist between metabolic activities and incidence and type of observed malformations. The relative effects of tissue homogenates, slices, various homogenate subfractions and fine tissue fragments can help us to better evaluate the contribution of the various participating enzymes.

Methodology

In cases in which bioactivation of proteratogens have been established in the embryo culture system (e.g. with 2-acetylaminofluorene), attempts will be made to identify proximate and ultimate teratogenic species. A number of approaches will be utilized in attempts to accomplish this goal. Suspect metabolites can be added directly to the embryo culture system; selective inhibitors and activators of specific metabolic pathways can be used to corroborate other evidence; free radical scavengers, antioxidants, inhibitors of lipid peroxidation, etc., may also be found useful in determining not only which metabolite or metabolites may be acting as proximate or ultimate teratogens in vitro, but also through what mechanisms the reactive intermediates may be eliciting the malformations.

Progress & Accomplishments

In an initial study, we demonstrated for the first time that an exogenous source of xenobiotic biotransforming enzymes (hepatic 9000g supernatant fraction (S9) could be cocultured successfully with whole rat embryos. We also demonstrated that an exogenous bioactivating system (rat S9) was essential for the conversion of cyclophosphamide to a teratogenic intermediate and that the bioactivating reaction was cytochrome P-450-dependent. In other published investigations, we utilized the whole embryo culture system to study the role of biotransformation on the capacity of several other teratogenic agents to elicit malformations in cultured embryos. These included cytochalasin D, salicylate, 2-acetylaminofluorene, adriamycin, niridazole, and diethylstilbestrol. A principal advantage of the embryo culture system is that it permits evaluations of chemically-elicited dysmorphogenesis in the absence of complicating maternal factors.

Early experiments in our laboratories convincingly demonstrated that cytochrome P-450-dependent systems (added as S9 fractions with cofactors) could markedly affect the capacity of chemicals to elicit malformations. For some chemicals -- e.g., cyclophosphamide and 2-acetylaminofluorene -- a P-450-dependent system was requisite to chemical dysmorphogenesis due to the

necessity for bioactivation. For cytochalasin D and niridazole, it could be demonstrated that P-450-dependent biotransformation could play a major determining role in their teratogenicity via metabolic inactivation. For salicylate and diethylstilbestrol, exogenously added biotransformation systems appeared to play only a minor or no role as a determinant of teratogenicity in vitro. An added P-450-dependent system appeared to enhance the capacity of adriamycin, fipampicin and busulfan to produce embryotoxic effects even though these agents were active without such additions. In unpublished preliminary studies, we have also found that the teratogenicity of procarbazine and aflatoxin B₁ can be markedly influenced by biotransformation/bioactivation factors.

Significance

Recent research data from our laboratories have strongly indicated that rat embryos at days 8-10 of gestation can respond significantly to certain inducers of P-450 cytochromes. When cultured with a proteratogenic agent, 2-acetylaminofluorene, the conceptuses exhibited the capacity to convert the proteratogen to sufficient quantities of reactive intermediates to elicit grossly observable morphologic abnormalities in the same cultured (target) embryos. These observations imply that control of embryotoxic responses to certain chemicals can reside within the conceptus per se and opens a host of important questions that should be answered with research.

Publications

Greenaway JC, Bark DH, Juchau MR: Embryotoxic effects of salicylates: Role of biotransformation. *Toxicology and Applied Pharmacology* 74:141-149, 1984.

Faustman-Watts EM, Yang HYL, Namkung MJ, Greenaway JC, Fantel AG, Juchau MR: Mutagenic, cytotoxic, and teratogenic effects of 2-acetylaminofluorene and reactive metabolites In Vitro. *Teratogenesis, Carcinogenesis, and Mutagenesis* 4:273-283, 1984.

OCCUPATIONAL EXPOSURE OF PARENTS AND TUMORS IN OFFSPRING

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Reproductive Disorders
5 R01 OH01413-03
02/01/82 - 01/31/86
\$189,478 (\$475,561 Cum)

Objectives

The objectives of this research are to determine the extent to which occupational exposure of parents accounts of tumors in the offspring and to determine the extent to which other environmental exposures of parents and children convey a risk for childhood tumors.

Methodology

The research projects employed a case-control approach focusing on childhood brain tumors and leukemias. Cases came from the Los Angeles County Cancer Surveillance Program (CSP), a county-wide tumor registry. After cases were identified (age 10 or less), controls were selected from friends or by random digit dialing. Parents of the cases and controls were interviewed ascertaining a complete occupational and environmental history for each. Additional information was collected from the mother about the child.

Progress & Accomplishments

Central Nervous System Tumors - Work on the original grant has provided evidence that solvent exposure to fathers is an important factor for children's CNS neoplasms. It appears that methylethylketone, freon and trichloroethylene are three specific solvents that are associated with risk. See the following table showing these risks:

Solvent Exposure in Fathers of Children with Brain Tumors

	<u>12 CNS</u>	<u>10 Controls</u>	<u>Odds ratio</u>
Solvents at all	12	7	
Daily Solvents	11	6	7.3
Carbon tetrachloride	3	1	3.0
Trichloroethylene	5	0	
Methyl ethyl ketone	7	1	12.6
Toluene	2	0	
Freon	4	0	
Paints	3	1	3.0
Petroleum Products	10	6	3.3

By examining the table it can be seen that a remarkable number of CNS fathers work with solvents. The odds ratios vary from 3 to infinity with striking findings being related to trichloroethylene (5 to 0), methylethylketone (7 to 1) and freon (4 to 0) all of which are commonly encountered industrial substances.

Leukemia - A case-control study of children age 10 and under in Los Angeles County was conducted to investigate the causes of leukemia. The mothers and fathers of acute leukemia cases and their individually matched controls were interviewed regarding specific occupational and home exposure as well as other potential risk factors associated with leukemia. Analysis of the information from the 123 matched pairs showed an increased risk of leukemia for children whose fathers had occupational exposure after the birth of the child to chlorinated solvents (odds ratio (OR)=3.5, p=0.01), spray paint (OR=2.0, p=0.02), dyes or pigments (OR=4.5, p=0.03), methylethylketone (OR=3.0, p=0.05), and cutting oil (OR=1.7, p=0.05), or whose fathers were exposed during the mother's pregnancy with the child to spray paint (OR=2.2, p=0.03). For all of these, the risk associated with frequent use was greater than for infrequent use. There was an increased risk of leukemia for the child if the father worked in the industries manufacturing transportation equipment (mostly aircraft) (OR=2.7, p=0.007) or who burned incense in the home (OR=2.7, p=0.007). The risk was greater for frequent use. Risk of leukemia was related to mothers' employment in personal service industries (OR=2.7, p=.04) but not to specified occupational exposures. Risks related to fathers exposure to chlorinated solvents, employment in the transportation equipment manufacturing industry, and parents' exposure to household or garden pesticides, and incense remain statistically significant after adjusting for the other significant findings.

Significance

Little is known about the etiology of childhood tumors. These studies have pointed to several different risk factors which offer the possibility of prevention of these devastating diseases.

Publications

Peters JM, Garabrant DH, Wright WE, Bernstein L, Mack TM: Uses of a cancer registry in the assessment of occupational cancer risks. Natl Cancer Inst Monogr 69:157-161, 1985.

Lowengart RA, Peters JM, Cicioni C, Buckley J, Bernstein L, Preston-Martin S, Rappaport E: Childhood leukemia and parents' occupational and home exposures. J Natl Cancer Inst (accepted).

REPRODUCTION HAZARDS OF DINITROTOLUENE TOLUENEDIAMINE

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Reproductive Disorders
5 R01 OH01520-03
12/01/83 - 11/30/86
\$190,839 (\$499,650 Cum)

Objectives

The general goals of this study have been the same as defined in the original application, i.e. to define the anti-fertility effects of 2, 4-DNT (DNT) and the reproductive toxicity of 2, 4-TDA (TDA) in male rats.

Methodology

Groups of adult male rats were fed varying amounts of compound in their diet for 10 weeks or were placed on normal chow for an additional 11 weeks. Fertility was tested by mating males with receptive females and determining breeding ability. The reproductive performance of male rats were evaluated by determining mating, fertility, and viability indices. (i.e. # of sperm-positive females/# of exposed females per group, # of pregnant females/# of sperm-positive females per group, and # of live fetuses/# of implantation sites). The effects of exposure to the agent were assessed further, after sacrifice of males, at 10 weeks and at 11 weeks post TDA treatment by measuring serum LH, FSH, testosterone, epididymal sperm counts, reproductive organ weights, and by examination of the cellular architecture of the seminiferous tubules.

Progress & Accomplishments

We have established that diets containing 0.03% TDA fed ad libitum to male rats for 10 weeks results in decreased mating frequency and an increase in infertile matings. Light microscopic examination of the testes from these animals revealed reduced numbers of sperm in the seminiferous tubules and cauda epididymides. No effect on the number of viable implants or resorptions was found among pregnancies from males exposed to this dose or to lower dose levels of TDA.

Treatment with 0.03% of TDA for 10 weeks reduced the weight of the seminal vesicles and epididymides, reduced serum testosterone levels, and decreased cauda epididymal sperm counts. The reduced epididymal sperm reserves persisted in the TDA-treated animals placed on normal diet for 11 weeks. Furthermore, serum luteinizing hormone concentrations were increased and weights of epididymides and testes were reduced in these 11 week post TDA-fed animals.

Significance

These studies demonstrate that TDA exerts a toxic effect on spermatogenesis in the male rat. The results indicate that TDA is capable of reducing fertility and of exerting an inhibitory effect on sperm production at nontoxic doses. It appears that TDA has deleterious effects on androgen production and action, but may not be capable of inducing mutagenic changes in the germinal cell of the male rat. The diminished cauda epididymal sperm counts 11 weeks after TDA treatment, suggests that the agent induced damage to

the spermatogonial stem cells. The precise mechanism by which TDA exerts its adverse reproductive effects remains to be established.

Publications - Abstract(s) available

Thysen B, Varma SK, Bloch E: Reproductive Toxicity of 2,4-Toluenediamine in the Rat. 1. Effect on Male Fertility. J. Toxicology and Environmental Health, 16:763-769, 1985.

Thysen B, Bloch E., Varma SK: Reproductive Toxicity of 2,4-Toluenediamine in the Rat. 2. Spermatogenic and Hormonal Effects. J. Toxicology and Environmental Health, 16:763-769, 1985.

REPRODUCTIVE EFFECTS OF LEADS OR SOLVENT EXPOSURE AT WORK

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Reproductive Disorders
5 R01 OH01910-03
04/01/84 - 03/31/87
\$74,642 (\$187,909 Cum)

Objectives

The aim of this study is to investigate, whether exposure of men and women to organic solvents or inorganic lead at work causes spontaneous abortions or malformations in the offspring.

Methodology

The exposed group consist of women and men who, according to the biological monitoring data, have been exposed to organic solvents or lead through 1972 to 1982. The cases of spontaneous abortions will be identified from the hospital discharge register maintained at the National Board of Health. A possible high risk for congenital malformations in the children of the exposed women can be excluded or confirmed by using the Finnish Register of Congenital Malformations. The figures will be compared with controls who have born a normal child.

Progress & Accomplishments

The collection of the biological measurements of solvent exposure as a computerized data file with the personal identification codes has been completed. The most common solvents monitored were trichloroethylene and styrene.

The pregnancies of the monitored workers have been identified from the Hospital Discharge Register and the Finnish Register of Congenital Malformations. The women had 1440 pregnancies from 1973 to 1983 and the proportion of spontaneous abortions to all pregnancies was 9.7%. The wives of monitored men had 4035 pregnancies and the proportion of spontaneous abortions among them was 8.8%. The number of children with congenital malformation was 14 among monitored women and 39 among the wives of monitored men.

The investigation was continued as a case-control study. All women who had been treated at hospital for spontaneous abortion or who had a malformed child were defined as cases. Three age-matched controls were selected for spontaneous abortion-cases and five controls for malformed children from the women who had given birth to a normal child. Similarly, cases and controls were selected from the wives of solvent exposed men. A questionnaire has been sent to the women or to the men and their wives to confirm the solvent exposure during the first trimester of pregnancy.

The collection of biological monitoring data of lead exposed workers has been completed. The number of biological measurements was about 60,000. The checking and correction of the data file is in progress.

Significance

The anticipated result: the possible effects of organic solvents or lead at various exposure levels on the pregnancy outcome of the workers are in prevention of health hazards. They can also be helpful for the understanding of dose-effect relationships and for the planning of occupational hygienic standards.

Publications - None to date

EFFECTS OF 27MHZ RADIATION ON SOMATIC AND GERM CELLS

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Reproductive Disorders
5 R01 OH02148-02
09/27/85 - 08/31/88
\$125,896 (\$234,664 Cum)

Objectives

To determine thresholds and dose-response relationships for the effects of 27-MHz radiofrequency radiation (RF) on mammalian somatic and germ cells in vitro under accurately measured and precisely controlled exposure conditions. The extent to which effects are dependent upon RF frequencies will be determined by comparison with the effects of other frequencies, specifically 1-, 10-, 50-, 100-MHz, and 2.45-GHz.

Methodology

Well-characterized somatic cells (erythrocytes, neutrophils, glioma [C5 and LN-71], fibroblasts, and HeLa) and germ cells (mouse ova and sperm) are exposed under conditions of accurately measured electric and magnetic field strength in a temperature-controlled coaxial line RF cell exposure system or a temperature-controlled microwave waveguide exposure system. The dependent variables will include: 1) cell viability and morphology, 2) phagocytosis and chemotaxis, 3) plasmalemma cation permeability, 4) cell cycle regulation, 5) DNA and protein synthesis, 6) sperm viability and motility, and 7) fertilization (in vitro).

Progress & Accomplishments

Frequency dependence of cellular responses in vitro has been investigated by simultaneous exposure of cell cultures to 27-MHz or 2450-MHz CW radiation under precise temperature control ($37 \pm 0.2^\circ\text{C}$). Exposure of human glioblastoma cells (LN-71) at 80 W/kg for 2 h to 2450-MHz microwaves resulted in metabolic stimulation as indicated by increased uptake of labeled thymidine and uridine and mitochondrial activity at 3 and 5 days post exposure. Simultaneous exposure of the same cell line to 27-MHz RF radiation resulted in an apparent metabolic suppression, as indicated by decreased uptake of thymidine and uridine on days 3 and 5 post exposure, with no effect on mitochondrial activity. Exposure to either frequency did not cause morphological alterations as assayed by phase contrast light microscopy. Comparative studies of effects of these frequencies (also at a SAR on 80W/kg) on structure and function of mouse sperm have indicated a reduction in ova penetration following 1 hr exposure to 2.45 GHz microwave radiation with no effects of 27-MHz RF radiation. Exposure of human lymphocytes to microwave or RF radiation at 37°C , in the range of SAR's of 45 to 200 W/kg, resulted in a dose rate dependent decrease in the effect of PHA on mitogenic stimulation. Exposure to SAR's of less than 100 W/kg resulted in an increase in PHA stimulated mitogenicity whereas higher SAR's caused a relative decrease. It has not been determined whether microwave and RF dose response relationships differ significantly.

Significance

Data generated by this study will be used in the assessment of potential health risks associated with occupational exposure to 27-MHz RF radiation generated by RF heat sealers, diathermy equipment and other devices that are known sources of human exposure. Since such devices are generally operated intermittently, comparative studies will be conducted using continuous wave and amplitude modulated 27-MHz RF.

Publications - Abstract(s) available

Cleary SF, Liu LM, Merchant R, East J: Comparative Cellular Effects of RF and Microwave Radiation. Abstracts of the 8th Annual Meeting of the Bioelectromagnetics Society, Madison, Wisconsin, June 1-5, 1986.

ENVIRONMENTAL/INDUSTRIAL TOXICANTS AND TESTICULAR INJURY

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Reproductive Disorders
5 R01 OH02191-02
09/27/85 - 08/31/88
\$118,855 (\$232,171 Cum)

Objectives

Studies in our laboratory have resulted in a model of γ -diketone intoxication which permits dissection of biochemical and morphological events specific for testicular atrophy. In preliminary experiments, we have demonstrated alterations in microtubule assembly which occur in association with testicular atrophy induced by the γ -diketone 2,5-hexanedione. We propose the following working hypothesis: 2,5-hexanedione reacts with tubulin lysyl residues to form pyrroles which form covalent cross links between tubulin monomers; covalently cross linked tubulin multimers change the in vitro and in vivo kinetics of microtubule assembly producing shorter and more numerous microtubules; microtubule modification is manifested as testicular injury because of the unique dependence of the testis on the cytoskeletal integrity of the Sertoli cell.

The goal of this project is to test this hypothesis of the pathogenesis of toxicant induced testicular atrophy by fulfillment of the following objectives:

1. To utilize a γ -diketone treatment protocol to specifically correlate the temporal onset and development of testicular microtubule alterations with the appearance of testicular atrophy.

2. To evaluate the reversibility of 2,5-hexanedione induced testicular atrophy in conjunction with biochemical resolution of the microtubule abnormalities.

3. To investigate the consequences for cells and tissues, particularly Sertoli cells and germ cells in the testis, of microtubule dysfunction caused by intoxication.

4. To delineate the chemistry and biochemistry of the tubulin modification which results in abnormal microtubule assembly kinetics.

5. To determine the generality of the hypothesis by testing microtubule properties in testicular atrophy induced by additional γ -diketones, the acrylamides and carbon disulfide.

Methodology

The assembly kinetics of pure rat testis tubulin from control, 2,5-hexanedione and 3,4-dimethyl-2,5-hexanedione treated animals will be compared, along with additional biochemical and morphological parameters, during a time sequence study of the induction and resolution of the testicular atrophy. Separation of Sertoli cells from germ cells combined with electron microscopy and anti-tubulin antibody techniques will be used to focus attention on the specific cell type within the testis which is injured by 2,5-hexanedione. Tubulin cross linking will be explored as the molecular mechanism for 2,5-hexanedione induced changes in microtubule assembly. Other compounds will be evaluated for inclusion within the class of testicular toxicants which act via microtubule modification.

Progress & Accomplishments

During this first year, our studies have focused on: 1) demonstration of an in vivo relationship between tubulin dysfunction and testicular atrophy, and 2) elucidation of the biochemical basis of the 2,5-hexanedione-induced microtubule assembly alteration. Successful development of a method for the purification of assembly competent rat testis tubulin allowed the use of an in vivo rat hexacarbon intoxication protocol which correlated testis tubulin assembly alterations with decreased testis weight and histologic evidence of testicular injury. Studies of the biochemical basis for altered microtubule assembly have concentrated on the development of in vitro conditions capable of mimicking the in vivo action of 2,5-hexanedione upon microtubule assembly and a description of the assembly abnormalities. Incubation of purified bovine brain tubulin with 2,5-hexanedione resulted in a modified tubulin preparation containing a cross linked tubulin dimer. The modified tubulin preparation displayed markedly altered assembly. Of primary importance was a striking enhancement of microtubule nucleation which resulted in more numerous and shorter microtubules, a reduced requirement for added guanine nucleotide triphosphate, resistance to calcium inhibition of assembly and the ability to assemble at low temperatures. Both in vivo intoxication with 2,5-hexanedione and in vitro modification of pure tubulin produced qualitatively similar alterations in microtubule assembly.

Significance

The proposed studies will further the understanding of the basic mechanisms underlying toxicant induced testicular injury. The alterations in microtubule assembly kinetics induced by 2,5-hexanedione are seen as a manifestation of the cytoskeletal disruption which results in testicular atrophy. A combination of morphological and biochemical techniques will be utilized in localizing the microtubule alteration to a particular cell type within the testis and defining the chemical nature of the tubulin modification. The significance of this proposal lies in the ability to generalize from the proposed subcellular mechanism to include those agents which act in a similar fashion. Also, during the course of the project, methods will be developed which readily allow early assessment of new agents which may be testicular toxicants.

Publications

Boekelheide K: Rat microtubule assembly properties are altered following in vitro and in vivo modification by 2,5-hexanedione. (Abstract) Toxicologist 6:190, 1986.

GENOTOXIC EXPOSURE ASSESSMENT BY SIMPLIFIED DNA ANALYSIS

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Reproductive Disorders
5 K01 OH00035-03
09/28/84 - 08/31/87
\$30,563 (\$94,938 Cum)

Objectives

The objective of this project is to develop a sensitive and practical test for human exposures to genotoxic agents. The test is based on observable changes in the 340 bp substance produced by Eco-R1 restriction of DNA obtained from peripheral leukocytes.

Methodology

DNA is extracted from human leukocytes subsequent to *in vitro* genotoxic challenge by ionizing radiation or various chemical agents. The 340 bp aliphoid sequence is subjected to Eco-R1 restriction, and secondary treatment to induce breakage at damaged sites. Breakage is determined by the distribution of a labeled 340 bp probe after electrophoresis and hybridization. Alternative methods of quantifying breakage are concurrently evaluated when appropriate.

Progress & Accomplishments

1. DNA Quantification - In order that the amount of blood required for analysis be kept to a reasonably small volume, emphasis was placed on the development of reliable DNA extraction and quantification procedures suitable for the amounts of DNA available from a 5 ml blood draw. A modification of the DABA technique of Kissane and Robbins (J. Biol Chem 233, 184) was developed which produced a linear fluorometric response over the range of 50 ng to 20 ug of DNA.
2. Alkaline Lability - It was demonstrated that electrophoresis followed by cerenkov counting clearly detects the action of *in vitro* exposures of the mutagen dimethyl sulfate (DMS) on leukocytes. DNA extracted from cells exposed to 4 treatment levels was restricted, labeled with ³²P, made alkaline, and subjected to gel electrophoresis. Tracks were divided into three regions by excision of the 340 bp region and the activity of each region assayed by cerenkov counting. As DMS concentrations were raised from 0 to 10. mM, the fraction of the labeled genome heavier than 340 bp fell monotonically from 93% to 3%. Similar, but less pronounced effects were obtained with the carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

Significance

The development of a practical assay for genetic damage in the occupational setting would have a number of practical implications, the most immediate being a supplement to conventional personnel monitoring. While an assay for general genetic damage would most probably lack agent specificity, the agent is known in many routine occupational situations and in many cases of accidental exposure. Another application would be the assessment of

genetic damage in exposure conditions so application would be the assessment of genetic damage in exposure conditions so complex that conventional monitoring techniques cannot produce a meaningful interpretation of the situation.

Publications - Abstract(s) available

Furlong NB, Marien KD, Flook B, and White J: Characteristics of Site Variation Among Cones of the 340 BP Tandemly Repeated ECO-R1 Family of Human DNA. J. Biochem. Gent. pp. 24,71-78, 1986

ROLE OF GAP JUNCTIONS IN ADVERSE REPRODUCTIVE OUTCOME

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Reproductive Disorders
5 K01 OH00059-02
09/28/84 - 08/31/87
\$31,482 (\$94,608 Cum)

Summary

Direct intercellular communication between adjoining cells can occur among cells in vitro and in vivo, most likely through specialized channels called gap junctions. It has been suggested that gap junctional communication plays an important role in embryonic development, germ cell maturation, and parturition. Our proposed research will investigate inhibition of junctional communication as a possible mechanism of adverse reproductive outcome, and will test the validity of several short-term assays for the detection of reproductive toxins that may act by this mechanism. The first part of our research program will entail testing known and potential reproductive toxins for their ability to interrupt junctional communication using rapid in vitro techniques. We will employ several different cell lines in an attempt to maximize the reliability of the in vitro systems as predictors for the human, in vivo situation. These cell lines will include Chinese hamster V79, human teratocarcinoma, human uterine, and rat hepatocyte lines. The second focus of our research will be to validate inhibition of junctional communication as a mechanism of abnormal morphogenesis using hydra reaggregation as an in vivo development system. By differentially labelling and later mixing and reaggregating two populations of hydra cells, we hope to demonstrate that compounds which interfere with hydra reaggregation also interrupt junctional communication in vivo. These results will be compared to the results from cell systems to determine the reliability of the cell systems in predicting developmental toxicity. Finally, we will investigate molecular mechanisms of gap junction function. We will focus our efforts on protein phosphorylation, since this has been associated with growth factors, receptor function, and junctional communication.

Publications

Loch-Caruso R, Trosko JE: Inhibited intercellular communication as a mechanistic link between teratogenesis and carcinogenesis. CRC Critical Reviews in Toxicology 16:157-183, 1985.

Loch-Caruso R, Corcos IA, Trosko JE: Acetic acid derivatives of 2-methoxyethanol and 2-ethoxyethanol fail to inhibit metabolic cooperation. In Vitro 22(Part II):45A, 1986.

Loch-Caruso R, Corcos IA, Trosko JE: Inhibition of metabolic cooperation by metals. Toxicologist 6:267, 1986.

OCCUPATIONAL FACTORS ASSOCIATED WITH BILIARY ATRESIA

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Reproductive Disorders
5 R03 OH01827-02
09/28/84 - 04/30/87
\$5,080 (\$28,995 Cum)

Objectives

Empirical observation has raised suspicion that parental occupational factors may be associated with Congenital Biliary Atresia ("B.A.") in the offspring. The principal objective of this exploratory study is to determine whether parentally reported occupations, types of employment, and/or selected chemical exposures related to the pre-conceptual, gestational, and/or neonatal periods constitute significant factor(s) associated with the occurrence of B.A., or a subgroup thereof.

Additional aims of this study include determination of:

1. Associated, selected home or avocational exposures
2. Whether teratogenic associations are related to paternal or maternal exposures, or to specific combined exposures (interactions)
3. Increased incidence of other teratogenic outcomes (including spontaneous abortion) in case sibships (correlated with parental occupation for each pregnancy)
4. Associated familial occurrence of hepatitis (both Type B and others), and other specific conditions
5. Maternal health status and perinatal complications
6. Selected demographic and socioeconomic variables

Methodology

This case-control study has selected a consecutive series of Extra-hepatic B.A. cases born within the four-year span from 1982 through 1985, from the National B.A. Registry of the American Academy of Pediatrics. For improved ascertainment, all Institutions referring to the Registry are being asked to review their files and to also include the newer (infant) cases as well as any earlier cases not yet registered. Desired sample size for cases is 300 to 400 or more.

Controls, matched with respective cases on sex and age (D.O.B. within one year), and on race whenever feasible, are being collaboratively selected through the files of each respective case surgeon. They will have had surgery under twelve months of age, for one of several non-congenital abdominal conditions (including inguinal hernia, intussusception, others). A second, population-based ("Normal") comparison group will soon be available per the data from the contemporary retrospective study on Congenital Heart Disease through the courtesy of Dr. Charlotte Ferenz, M.D., M.P.H. of the University of Maryland, School of Medicine. Validation of case and control diagnoses, and obtainment of selected clinical data, will come through receipt and review of selected medical histories. Parental data will be obtained through mailed, self-administered written questionnaires, separate ones for mothers and for fathers. Each questionnaire covers relevant job history, selected exposure history, hobby and other environmental history, reproductive and extended family history, and parent's personal health history.

Follow-up with non-responder parents will entail initially sending a "reminder" post-card and then, if no subsequent response, direct phone

contact(s). For those families with apparent communication or literacy problems, we will offer telephone interviews. Data collected by different modes will be stratified for comparisons.

Distribution and cross-tabulations of various occupations, occupational groupings (based on similar chemical exposures), and other environmental factors will be done by case-control status, after adjustment for potential environmental, socioeconomic, and prenatal and perinatal confounding factors. Multiple regression analysis will be used to weight significant variables, identify significant interactions, and adjust for confounding variables. Maternal and paternal data will be analyzed separately as well as jointly, for suspected interaction effects.

Progress & Accomplishments

The pre-testing phase, involving a sample of families of Maryland children with congenital abdominal defects was completed in March. These children were born in the three year span from 1982 through 1984. The parent questionnaire tools were thus evaluated and minor changes subsequently made, particularly in the formats of the occupational history and reproductive history sections. The response rate after the initial family contact was approximately 66%.

Currently the data collection is underway. Since April, 1986, we have established communication and sent information and referral packets to 312 pediatric surgical institutions and private pediatric surgeons' offices across the United States, soliciting Biliary Atresia cases and time/sex matched control subjects. The response has been good. With 75% of the total provider sites reporting definitively to date (of a total of 257 sites, excluding those retired, now only in administrative roles, etc.), over 400 cases and controls have been already referred or committed to this project. Further, many of the sites which have agreed to participate by referral of subjects do not yet have any estimates of their case numbers -- thus, the actual case referral total will be considerably higher once known. In addition, some of the larger institutions, each having considerable numbers of cases, will collaborate with us, but on the contingency that our staff make site visits to review case files, select subjects, and obtain referral data. Trips to these sites, throughout the U.S., will be conducted in Winter, 1987.

Contacts with referred subject families have commenced, for collection of parental questionnaire data and signed release forms for obtaining subjects' medical histories. By the autumn of 1987, data collection and processing should be completed, with data analysis to follow.

Significance

Not yet applicable.

Publications - None to date

ROLE OF EPIDIDYMAL GLUTATHIONE IN CHEMICAL-INDUCED
GERM CELL MUTATIONS

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Reproductive Disorders
1 R03 OH02258-01
05/01/86 - 04/30/88
\$17,556 (\$17,556 Cum)

Summary

The effects of altering glutathione levels in the male reproductive tract will be studied. The proposed study will attempt to establish a link between chemical-induced reduction of glutathione in the male reproductive tract with increased susceptibility of spermatozoa to chemical-induced mutations. Glutathione is an important intracellular tripeptide that participates in a number of cellular functions, one of which has been a primary interest in toxicology: the detoxification of xenobiotics. The importance of glutathione as a protective mechanism has been clearly established, within the body (e.g. liver, kidney) but little attention has been given to the reproductive tract where chronic toxicity or chemical-induced heritable changes could have serious consequences for future generations. These studies will determine whether or not a number of environmentally important toxicants are capable of lowering the glutathione levels of the male reproductive tract, and if so, whether this change will render the animal more susceptible to the action of germ cell mutagens.

The glutathione system is comprised of glutathione and several key enzymes: glutathione-S-transferases, glutathione reductase, glutathione peroxidase, and gamma-glutamyl transpeptidase. The activity of these enzymes can affect tissue levels of glutathione and therefore will be studied to determine the effects of the selected toxicants on the glutathione status of the male reproductive tract. Glutathione and its related enzymes contain a number of reduced sulfhydryls, and the activity of the entire system is essentially linked to the nucleophilic nature and activity of the thiol group. Therefore, the toxicants that will be tested were chosen because they are chemicals that either 1) have a great affinity for sulfhydryl groups in biological systems or 2) the parent compound or one of its intermediate metabolites are reactive electrophiles that will react with GSH and the intermediate metabolites are reactive electrophiles that will react with GSH and the sulfhydryl groups on the enzymes of the GSH system. Germ cell mutations represent potentially one of the most serious consequences of exposure to environmental toxicants due to induction of heritable defects transmissible to progeny. The enzymatic and non-enzymatic mechanisms which afford protection to the male germinal cell will be determined.

Publications - None to date

MALE REPRODUCTIVE EFFECTS OF ACRYLAMIDE

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Reproductive Disorders
1 R03 OH02345-01
09/29/86 - 09/28/88
\$22,511 (\$22,511 Cum)

Summary

Acrylamide (ACR) is a widely used monomer in the polymer industry. The polymeric form is widely utilized as a flocculent in the waste and water treatment industry. Historically, concerns regarding occupational and environmental exposures have centered around its well-documented neurotoxic effects. Recent studies (Zenick, 1986; Smith *et al.*, 1986) have implicated ACR as a male reproductive toxicant in rats at doses that failed to produce peripheral neuropathies in these males. The purpose of this proposal is to further extend these findings through a number of investigations. The first investigations are aimed at determining the stages of spermatogenesis most adversely affected by ACR and establishing a no effect limit to exposure to ACR. To accomplish these aims two dominant lethal studies had to be carried out. Results from the first study are presented in the preliminary studies section. A second dominant lethal study to extend the dose range downward and the next investigation to assess the factors that contribute to apparent pre-implantation loss are in progress. The apparent pre-implantation loss investigation will evaluate copulatory dysfunction which may result in abnormal mating behavior and sperm transport. In addition, spermatotoxic effects may result from ACR exposure and these will be assessed. The third investigation will examine where ACR interferes with fertilization or subsequent zygote development (i.e. produces "true" pre-implantation loss). The fourth investigation will initiate studies into the potential mechanism underlying pre- and post-implantation loss and will evaluate the potential binding of ACR to sperm and sperm DNA. The last investigation will seek to characterize the effect of dose and rate of exposure on the expression of reproductive damage. This proposal is important for a number of reasons. First, it will give greater insight into the mechanism of ACR-induced dominant lethality. It will, also, provide important information to better understand the significance of pre- and post-implantation loss. In addition, it will establish a dose relationship associated with ACR's reproductive effects. Finally, the results determined by these studies may provide information useful in identifying other occupational and/or environmental exposures which heretofore have been unrecognized as reproductive toxicants.

Publications - None to date

OCCUPATIONAL NEUROPATHIES DUE TO INDUSTRIAL CHEMICALS

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Neurotoxic Disorders
2 R01 OH00823-07
08/01/82 - 12/31/89
\$166,870 (\$715,931 Cum)

Objectives

The overall goal of this project is to investigate the mechanism(s) of joint neurotoxic action of industrial chemicals. The specific chemicals that are being studied are n-hexane alone and when used simultaneously with methyl iso-butyl ketone, toluene, or the organophosphorus neurotoxicant EPN (O-ethyl O-4 nitrophenyl phenylphosphonothioate). These studies include investigating the joint neurotoxic action of test compounds on the nervous system as well as their action on liver microsomal xenobiotic-metabolizing enzymes.

Methodology

The adult hen was used as the test animal since it is sensitive to neurotoxicity induced by organophosphorus and aliphatic hexacarbon-type chemicals. Dermal and inhalation exposure were employed since they are the major routes of occupational exposure, to these chemicals both by industrial and agricultural workers. The organophosphorus chemical used is the delayed neurotoxic insecticide EPN and the neurotoxic aliphatic hexacarbons used are: n-hexane, 2-hexanone (MnBK), 2,5-hexanediol (2,5-HDOH), and 2,5-hexanedione (2,5-HD). The non-neurotoxic methyl iso-butylketone (MiBK), which has replaced the neurotoxic MnBK in industry, was also used. In these studies the effect of n-hexane and related chemicals on the accumulation of neurofilaments, a pathognomonic feature of n-hexane neuropathy, has been studied using sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotting using neurofilament triplet protein antibodies. Liver microsomal cytochrome P-450 isoenzymes have been studied by SDS-PAGE and western blots using antibodies for isoenzyme proteins.

Progress & Accomplishments

In the first phase of these studies, the hen was established as a model test animal for aliphatic hexacarbon-induced neurotoxicity via intraperitoneal injection, inhalation, and oral and dermal administration. The relative neurotoxic effect of aliphatic hexacarbons in hens in descending order is: 2,5-HD, 2,5-HDOH, MnBK, n-hexane. The results showed that although both aliphatic hexacarbons and organophosphorus compounds are capable of producing similar signs of neurotoxicity in the hen, the morphology and distribution of the neuropathologic lesion were distinct. Concurrent inhalation of MBK and dermal application of EPN potentiated the neurotoxic effect of either chemical alone. Also, the non-neurotoxic MiBK synergized the neurotoxic effect of the weak neurotoxicant n-hexane in hens. Simultaneous dermal exposure to EPN and n-hexane and its metabolites produced either additive or potentiating effect. The chemicals MnBK, MiBK, and EPN induce chicken hepatic microsomal cytochrome P-450. Also, dermal application of n-hexane, MnBK, and 2,5-HD enhanced the dermal absorption of EPN. Simultaneous exposure of hens by inhalation to n-hexane and various concentrations of MiBK resulted in neurologic dysfunction and cross-linking of the three neurofilament triplet proteins; 70-kDa, 160-kDa, and 220-kDa in the spinal cord. The extent of cross-linking was

proportional to the concentration of MnBK . Also, these neurofilament triplet proteins exhibited a decreased Ca^{2+} - and calmodulin-dependent kinase phosphorylation. To examine the hypothesis that MnBK enhances the n -hexane neurotoxicity by inducing liver microsomal cytochrome P-450 which metabolizes n -hexane to the more potent neurotoxicant 2,5-HD, liver microsomes from hens simultaneously exposed to n -hexane and MnBK were isolated and found to have an activity that ranged from 375-744% of the control. Treatment of rats with 2,5-HD resulted in cross-linking of the spinal cord neurofilament triplet proteins. Furthermore, in 2,5-HD treated animals, there was a diminished amount of immunoreactive breakdown products of all three neurofilament proteins.

Significance

The results of this project have demonstrated that in the test animal (the hen): a) simultaneous dermal administration of EPN and inhalation of MBK vapor potentiated their neurotoxic effects, b) persistent dermal exposure to small daily doses, without skin protection of n -hexane, MnBK , 2,5-HDOH, or 2,5-HD in combination with EPN produced a potentiating or additive action on EPN neurotoxicity in the following order: 2,5-HD > MnBK > 2,5-HDOH > n -hexane, and c) concurrent exposure to n -hexane and MnBK vapors greatly enhanced the neurotoxicity of n -hexane alone. The mechanism(s) of the synergistic action of MnBK to n -hexane neurotoxicity are being investigated. The present results indicate that: 1) MnBK induces the hepatic microsomal cytochrome P-450, 2) this results in increased metabolic biotransformation of n -hexane to 2,5-HD, and 3) 2,5-HD causes the accumulation of neurofilaments in the spinal cord as the result of: a) cross-linking of the three neurofilament triplet proteins; 70-kDa, 160-kDa, and 220-kDa, b) diminishing the breakdown of neurofilaments, and c) decreasing the Ca^{2+} - and calmodulin-dependent phosphorylation of the neurofilament triplet proteins.

Publications - Abstract(s) available

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MECHANISMS OF TOXIC OCCUPATIONAL NEUROPATHY

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Neurotoxic Disorders
5 R01 OH00851-06
05/01/83 - 04/30/86
\$176,554 (\$912,712 Cum)

Objectives

These studies seek to illuminate the role of axon transport in the induction of nerve-fiber degeneration induced by agents of industrial significance.

Methodology

Axonal transport studies utilize radiolabeled markers to determine the rate of anterograde and retrograde transport and the amount of material transported in intact and injured nerves of intoxicated animals. Biochemical assay procedures are carried out to examine enzyme activity in treated nerves and to study the relationship between enzyme inhibition and nerve-fiber degeneration. Light and electron microscope techniques are used to examine central and peripheral nervous system tissues taken from intoxicated animals. In addition, a novel organotypic tissue culture system consisting of fetal mouse spinal cord, dorsal root ganglia and muscle is utilized to monitor the effect of neurotoxic compounds on living nervous system tissues during intoxication and recovery.

Progress & Accomplishments

The occupational neurotoxin acrylamide, induces distal, retrograde degeneration of peripheral nerves in intoxicated rodents. Our studies have shown that acrylamide-induced axon degeneration is preceded by dose-dependent changes in retrograde axonal transport that are temporally related to deleterious alterations in the neuron perikaryal repair responses to injury. This has led to the suggestion that acrylamide, and perhaps other agents that precipitate toxic axonal neuropathies, impede information transfer from axon to perikaryon that is required to activate repair responses needed for the maintenance of axonal integrity.

We have also examined, for the first time, the entire sequence of pathological changes in hexacarbon neuropathy using 2,5-hexanedione (the ultimate metabolite of n-hexane) and the more potent compound, 3,4-dimethyl-2,5-hexanedione (DMHD), in living organotypic tissue cultures. Our findings demonstrate different pathological responses in cultures as a consequence of dose and potency. In the presence of DMHD, neurofilament accumulation (which causes axonal swellings) and axonal degeneration appear to be two separate pathological events, both spatially and temporally. Therefore, it is unlikely that chemical interference of neurofilament transport is the sole cause of hexacarbon-induced axonal degeneration.

These studies have been supplemented by an analysis of the toxic neurological disorders induced by other substances (e.g., 2-t-butylaxo-2-hydroxy-5-methyl hexane) of industrial/environmental significance.

Significance

Peripheral neuropathy associated with alterations in axonal transport has resulted from human overexposure to a number of industrial chemicals, including the compounds studied here (i.e., acrylamide and n-hexane derivatives). The molecular and cellular mechanisms underlying the subchronic neurotoxicity of these agents is not well understood and under intensive study. We have made a major advance by defining the pathophysiological changes in nerve fibers which precede degeneration induced by certain chemical agents.

Publications

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CHRONOBIOLOGY AND OCCUPATIONAL HEALTH HAZARDS

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Neurotoxic Disorders
5 RO1 OH00952-07
12/01/84 - 11/30/87
\$134,013 (\$726,670 Cum)

Summary

The long-term objective of this study has been to determine what role metabolic rhythms have on influencing response to toxicity from physical stimuli, industrial toxins and therapeutic agents. Among those that have been, or currently are being investigated include: irradiation, paraquat, urethane, malathion, mercuric chloride, insulin, glucagon, epidermal growth factor (EGF), somatostatin, ACTH, and gastrin. The toxicity response in mice to all has been shown to be circadian-stage dependent. Moreover, some of the above polypeptides stimulate RNA and DNA synthesis in a number of tissues including the alimentary canal (EGF, insulin and gastrin) whereas others are predominantly inhibitory (glucagon, somatostatin, and ACTH), again all responses have been found to be circadian-stage dependent. The aim of the present application is to continue such studies, but also, to determine if the chronobiological findings already gained can be used to reduce overall toxicity.

The model chosen has been cell proliferation in certain regions of the intestinal tract, which also undergoes remarkable circadian variation. Can the fact that fasting, beginning at a certain circadian stage, for a span of 36 hours or less reduce the fraction of proliferating cells and thus be used to protect the gut from damage brought about by: (1) a physical agent such as irradiation which affects the mitotic spindle or (2) a chemical agent such as cytosine arabinoside, which specifically interferes with DNA synthesis? Moreover, will any of the above mentioned peptides, when given before, simultaneously, or subsequent to either a physical or chemical induced injury, protect different regions of the gut from damage or enhance repair? Another objective is to gain insight into the mechanism of action of EGF induced cell membrane protein phosphorylation in the gut of normal adult mice and those treated with irradiation.

These studies have relevance for industrial toxicology, gastroenterology, immunology, and endocrinology.

Publications

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MOLECULAR MECHANISMS OF DIKETONE NEUROTOXICITY

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Neurotoxic Disorders
5 R01 OH01972-03
05/01/84 - 04/30/87
\$46,290 (\$133,853 Cum)

Objectives

n-Hexane and the related solvent methyl n-butyl ketone (MnBK) are neurotoxic in man and a number of experimental animal species. These compounds undergo metabolism to the γ -diketone 2,5-hexanedione (2,5-HD), which is believed to be the active agent in this neuropathy. All γ -diketones tested to date induce a similar neuropathy, while non- γ -diketones are without effect. Other characteristic observations include the appearance of neurofilament (NF)-packed swellings in the distal region of PNS and CNS fibers, covalent tissue protein binding of the compounds, and an apparent direct action upon axonal components.

Previous studies in this laboratory demonstrated the formation of 2,5-dimethylpyrrole adducts from the reaction of 2,5-HD with protein amine functions. Since only γ -diketones can form the pyrrole derivative, this reaction could account for the observed structure/activity relationships. In addition, autoxidative reactions subsequent to pyrrolylation were found to yield covalently crosslinked protein species. Based on these findings, we have proposed pyrrolylation of neurofilaments as the primary initiating event in γ -diketone neuropathy. The following specific aims are being addressed in this research program: 1) Elucidation of the degree and sites of pyrrole formation in NF proteins following in vitro and in vivo exposure to γ -diketones; 2) Characterization of the physicochemical changes in NF protein structure induced by pyrrolylation; 3) Characterization of the autoxidative reactions occurring secondary to pyrrole adduct formation.

Methodology

Following in vitro or in vivo exposure to 2,5-HD (radiolabelled or unlabelled), axonal cytoskeletal proteins are analyzed for pyrrole content by several methods, including colorimetric assay, liquid scintillation counting, and autoradiography of polyacrylamide gels. Physiocochemical alterations are examined by PAGE, reversed-phase HPLC on octyl-Sepharose, and sedimentation analysis. Sites of pyrrole formation in individual NF subunit proteins are determined by means of specific cleavage with chemical and enzymatic reagents followed by analytical peptide mapping by HPLC or PAGE. Western blotting strategies with anti-NF antisera are employed to identify the high-MW protein bands in axonal cytoskeletal protein preparations following 2,5-HD exposure. Bovine serum albumin (BSA) is used as a model protein for elucidating chemical aspects of the pyrrole-forming and secondary autoxidative reactions.

Progress & Accomplishments

Studies utilizing "Western" blotting and immunostaining were initiated to characterize the high-MW protein bands present in SDS-PAGE gels of axonal cytoskeletal proteins from 2,5-HD-treated rats. The extra protein species in the region of the gel about 240 kDa were immunoreactive with both anti-NF(H) and -NF(M) monoclonal antibodies, but not anti-NF(L) antisera. In contrast,

a prominent extra band at 170 kDa was immunoreactive only with anti-glial fibrillary acidic protein (GFAP) antisera. It should be noted that, although not present in the axon itself, GFAP is a member of the family of intermediate filament proteins which also include the NF proteins. Thus, this class of proteins may be exceptionally vulnerable to pyrrolylation and subsequent autoxidative crosslinking during γ -diketone exposure.

Although the precise mechanism of the reaction of γ -diketones with amines to form pyrroles has not been established Schiff-base intermediates (imines) have been postulated. In vitro were conducted in order to detect and quantitate imine formation in 2,5-HD-treated BSA at various diketone: amine molar ratios and as a function of pH and incubation time. Use was made of NaCNBH_3 , a mild reducing agent which specifically converts imines to stable amines. BSA was incubated with $[1,6-^{14}\text{C}]-2,5\text{-HD}$ at diketone: amine molar ratios of 2:1 or 50:1, for 2 to 144 hr. Incubated samples were then divided into two equal portions, one of which was treated with NaCNBH_3 . Covalently bound radioactivity was determined by liquid scintillation counting of dialyzed samples. Imine formation was quantitated as the difference between total covalent binding in NaCNBH_3 -treated and untreated samples. These studies provided clear evidence for an imine intermediate in this reaction mechanisms.

Significance

The findings described above represent further evidence for pyrrole adduct formation and covalent crosslinking of NF proteins in γ -diketone-treated animals. Evidence has also been provided to support the hypothesis that the neurotoxic diketones act directly upon axonal cytoskeletal components. Thus, the cytoskeleton in general and neurofilament in particular may be the critical target sites for the neurotoxic diketones. Additional progress has also been made in determining whether pyrrole formation per se is sufficient to induce γ -diketone neuropathy or if additional autoxidative reactions are required. It is hoped that findings derived from this project will ultimately lead to elucidation of the molecular mechanism of action of this important class of neurotoxic chemicals.

Publications - Abstracts available

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MECHANISMS OF OCCUPATIONAL NEUROPATHIES

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Neurotoxic Disorders
5 R01 OH02003-02
05/01/85 - 04/30/88
\$192,793 (\$368,667 Cum)

Objectives

The overall goal of this project is to study the mechanism(s) of organophosphorus compound-induced delayed neurotoxicity (OPIDN) in a sensitive mammalian species, i.e., the cat in comparison with the animal of choice to study OPIDN, i.e., the chicken. The proposed studies in this project will test the hypothesis that the initial event in the mechanism of OPIDN is the phosphorylation of a neurotoxicity target protein (e.g., neurotoxic esterase) whose normal physiological function is unknown but could be related to Ca^{2+} homeostasis. Subsequent changes in cellular Ca^{2+} content leads to either (1) an increased Ca^{2+} - and calmodulin-dependent phosphorylation of neural cytoskeletal proteins, e.g., tubulin, neurofilaments, which results in aggregated insoluble filamentous polymers or (2) an increased Ca^{2+} -activated proteolytic destruction of neurofilaments. Accumulation of such structures leads to the disruption of axoplasmic transport and accumulation of mitochondria at the distal portion of the axon. Disrupted mitochondria release their Ca^{2+} stores into the axoplasm. This overloads and disrupts any still functional axonal membrane control mechanisms for intracellular/extracellular ionic gradients, which leads to focal internodal swelling and Ca^{2+} -dependent proteolysis, followed by focal degeneration that spreads somatofugally to involve the entire distal axon.

Methodology

The present studies into the mechanisms of OPIDN are carried out in the hen, the experimental animal for OPIDN, and the cat, a mammalian species that may be a better model for extrapolation to humans. Test chemicals used in these studies are the delayed neurotoxic organophosphorus compounds TOCP, EPN, or DFP which are compared to the non-delayed neurotoxicant parathion. Characterization of neurotoxic esterase is carried out using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). In vitro phosphorylation of cytoskeletal proteins is performed using $[\gamma\text{-}^{32}\text{P}]$ followed by PAGE and autoradiography. Isolation and characterization of tubulin, MAPs, and neurofilament proteins are carried out using one-and-two-dimensional PAGE as well as peptide mapping.

Progress & Accomplishments

In order to investigate the mechanisms of OPIDN in the cat in comparison with the chicken, a study was carried out to characterize this effect in the cat first. A dose-response study of the dermal administration of a single or daily doses of TOCP was performed in the cat. Clinical signs were characterized by hindlimb weakness, ataxia, and paresis. Electromyographic abnormalities resulting from acute denervation were observed in most cats that developed a neurologic deficit. These results correlated well with the histopathologic results showing lesions in the most distal portion of the longest tracts in both central and peripheral nervous systems.

Neurotoxic esterase was characterized in the chicken and the cat and found to have the following characteristics: (1) it is a membrane bound protein, (2) it has molecular weight of 155-178 kDa, (3) its target size determined by radiation inactivation is 105 kDa, and (4) it is transported in the sciatic nerve of hens at a fast axonal transport rate of about 150 mm/day. The kinetics of substrate hydrolysis and inhibition by mipafox of NET have been determined. The results indicate that no NTE isoenzymes can be distinguished by mipafox. Oral administration of a single oral 750 mg/kg TOCP enhanced the Ca^{2+} - and calmodulin-dependent phosphorylation of α - and β -tubulin, cold-stable microtubules, and microtubules-associated protein-2 (MAP-2) in hen brain. Similarly, in spinal cord TOCP enhanced the Ca^{2+} - and calmodulin-dependent phosphorylation of α - and β -tubulin (52 and 59 dKa) and neurofilament triplet proteins (70, 160, and 210 dKa).

Significance

The present studies demonstrate that TOCP-induced delayed neurotoxicity in hens results in increased Ca^{2+} - and calmodulin-dependent phosphorylation of the following cytoskeletal proteins: α - and β -tubulin, MAP-2, and the neurofilament triplet proteins in brain and spinal cord. These studies have also eliminated the following possible causes of this increase: 1) inactivation of ATPase, thus making more ATP available for phosphorylation, 2) decrease in phosphatase activity resulting in less dephosphorylation, or 3) more phosphorylation sites are available in protein substrates. The results suggest that Cam Kinase II is affected and the possibilities that either its amount or activity is increased are being investigated. The consequences of increased cytoskeletal protein-phosphorylation by delayed neurotoxic organophosphorus compounds are: 1) increased phosphorylation of MAP-2 depolymerizes cold labile microtubules, 2) increased phosphorylation of tubulin results in aggregated polymers, and 3) increased phosphorylation of α - and β - tubulins, MAP-2, and neurofilament triplet proteins by Cam Kinase II would interfere with their interactions with themselves and with each other.

Publications

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ACRYLAMIDE NEUROTOXICITY: ROLES OF OXIDATIVE METABOLISM

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Neurotoxic Disorders
5 R01 OH02020-03
08/01/84 - 07/31/87
\$64,881 (\$174,534 Cum)

Objectives

We are testing the whether or not acrylamide (ACR) monomer, a neurotoxic chemical which produces a central-peripheral distal axonopathy, inhibits enzymes of oxidative energy pathways resulting in depletion of high energy phosphates. Experiments were designed to answer the following questions.

1. Does acrylamide selectively inhibit the activity of oxidative enzymes in neural tissue?
2. Does acrylamide inhibition of glycolytic (previously reported) and/or oxidative enzymes produce a decrease in high energy phosphate levels in peripheral nerves?
3. Is there a correlation between the reduction in axoplasmic transport rates caused by ACR and a reduction of high energy phosphate levels?

Methodology

Biochemical and quantitative enzyme histochemical enzyme assays conducted both in vivo and in vitro will be used to determine whether or not neural lipoamide dehydrogenase (LPDH) and NADH dehydrogenase (NADH-D) are selectively inhibited by doses of ACR which produce a peripheral nerve degeneration. Bioluminescence assays will determine whether or not the production of ATP by brain mitochondria and/or the concentration of ATP and CP in peripheral nerves is reduced by ACR; the changes correlated with reductions in axoplasmic transport.

Progress & Accomplishments

The efficacy of ACR to alter the NADH-TR (LPDH + NADH-D) activity in fast glycolytic (low normal activity) and slow oxidative (normally high activity) motoneurons was examined. ACR decreased the enzyme activity 17-20% in slow oxidative motoneurons after 5 and 10 days of exposure. A decrease in retrograde transport of exogenously applied horseradish peroxidase was also observed. The acute effects of ACR on NADH-TR activity of these motoneurons demonstrated a 33% inhibition in the slow oxidative motoneurons within one hour of injection; the activity slowly recovered over 3 days. The enzyme activity was decreased in vitro in a dose-dependent manner from 1-10mM; the maximum inhibition was 54% at 10mM. The non-neurotoxic analogue MbACR did not significantly decrease the oxidative enzymes both in vivo and in vitro.

Quantative enzyme histochemical assays on tissues from animals acutely exposed to ACR has demonstrated a neural selectivity of enzyme inhibition. One hour following a 50 mg/kg ACR injection, the NADH-TR activity in axons (9.5%), DRG neurons (17.8%), cerebellar Purkinje neurons (17.3%) were significantly reduced. Conversely, the activity in stomach parietal cells, hepatocytes and renal cortical tubules was not significantly inhibited. In addition, MbACR did not significantly inhibit the NADH-TR activity of any of the cells except the Purkinje neurons (23.7%).

In vivo, the LPDH and NADH-D of brain and spinal cord were decreased 8.7-16.7%. Non-neural tissues were differentially affected; NADH-D was

increased in both stomach and liver but decreased slightly in the kidney. LPDH activity was inhibited in both kidney and stomach but slightly increased in liver. In vitro incubation of tissue homogenates and toxicant produced significant NADH-D inhibition of brain and spinal cord (11-29.6%) in micromolar concentrations. Kidney and liver NADH-D activity was unaffected, stomach was inhibited 16.3% by 1mM ACR. In vitro incubation of ACR with neuron-enriched fractions of the cerebral cortex produced a 39.7-51.7% inhibition of LPDH and 16.9-24.6% inhibition of NADH-D in the 5-1000uM range.

Single injections of ACR significantly reduced both the rate and quantity of anterogradely transported protein down the rat sciatic nerve. The control rate of 395 ± 32.5 mm/day was reduced to 342.7 ± 6.1 mm/day and 312 mm/day with a 50 and 100 mg/kg dose, respectively. The quantity of transported protein was decreased by 40 and 79% from control by a 50 and 100 mg/kg dose of ACR, respectively.

Determination of high energy phosphates in sciatic nerves demonstrated an increase in the concentrations of both ATP and CP following single 50 and 100 mg/kg doses of ACR. A dose dependent increase in high energy phosphates of the sciatic nerve paralleled the dose dependent decrease in protein transport in the opposite sciatic nerve.

Significance

Our results have shown that ACR, in doses which produce the central peripheral distal axonopathy, is capable of inhibiting LPDH and NADH-D activity. The inhibition is direct and dose-dependent. We have also shown that neural tissue enzymes are selectively inhibited by ACR; other non-neural tissue enzymes are generally not affected. Inability of non-neurotoxic M₀ACR to produce significant enzyme inhibition supports the hypothesis. Since neuron specific forms of these enzymes have not been reported, we consider the mechanism of selectivity due to lower levels of glutathione in neural tissue. Our results also indicate that fast anterograde transport of proteins is significantly affected at the same doses as the enzyme inhibitions. However, this relationship is coincidental since the high energy phosphates are increased at the same dose and time point of energy and transport deficits. These data refute the hypothesis that enzyme inhibitions by ACR deplete high energy phosphates blocking transport and producing a neuropathy.

Publications - Abstract(s) available

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MECHANISMS OF OCCUPATIONAL DISTAL AXONOPATHY

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Neurotoxic Disorders
5 R01 OH02065-02
05/01/85 - 04/30/88
\$147,311 (\$279,193 Cum)

Objectives

These studies are designed to further understanding of how neurotoxic agents alter normal neuronal processes to produce axonopathy. Central-peripheral distal axonopathy is produced in laboratory animals repeatedly dosed with acrylamide and is a useful model of neuronal degenerative disease. Acrylamide neurotoxicity may be initiated by an inhibition of the retrograde axonal transport system which interrupts the flow of information from axon to perikaryon. As a consequence, the neuron fails to mount the appropriate repair responses when challenged distally and the axon undergoes distal-proximal degeneration. To test this novel hypothesis we are measuring markers of repair-associated gene expression following axotomy in the presence and absence of systemic acrylamide intoxication.

Methodology

The bipolar sensory neurons of mouse dorsal root ganglia and their projections located in the sciatic nerve constitute the in vivo model system employed to study perikaryon repair responsiveness. This model has been utilized to identify and explore several biochemical markers associated with perikaryal repair events. Experimental protocols are designed to test the ability of acrylamide and other agents to block increases in repair markers stimulated by transection of the sciatic nerve. As an index of repair response to axotomy RNA polymerase activity, ornithine decarboxylase (ODC) activity, and recently polyamine content of dorsal root ganglia have been measured.

Progress & Accomplishments

We have demonstrated that nerve transection causes a significant induction of ODC activity in corresponding neuronal perikarya. Ongoing studies have confirmed that ODC induction is a useful marker of axonal damage providing a reliable quantitative biochemical measure of perikaryal-repair response.

To test the hypothesis that acrylamide blocks retrograde axonal transport and thereby prevents signal(s) which inform the neuronal perikaryon of distal injury leading to initiation of repair responses we examined the ability of acrylamide to block ODC induction following transection of the sciatic nerve. Single (50 mg/kg) or multiple (4x50 mg/kg) doses of acrylamide significantly attenuate ODC induction following axotomy. In additional experiments, acrylamide given 24 hours after nerve transection failed to block ODC stimulation indicating that timing is critical for signal interception to the neuron perikaryon. These biochemical observations reveal an acrylamide-induced effect on neuron physiology long before neurological and pathological manifestations of intoxication are evident, providing new and exciting insights into the mechanism of action of this occupational neurotoxin.

Significance

Monomeric acrylamide is an environmental and occupational neurotoxin which causes polyneuropathy in humans. Despite many years of intensive research, the exact toxic mechanism of acrylamide remains unknown. Diminished perikaryal repair capacity, in the presence of low-level axon damage, represents a novel mechanism by which toxic agents may alter neuronal function or structure. These studies provide detailed data regarding the means by which neuronal repair responses are initiated and regulated, substantially adding to our understanding of the mechanisms by which neurotoxic agents produce axonopathy.

Publications - Abstract(s) available

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VALIDATION OF A NEUROBEHAVIORAL TEST BATTERY

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Neurotoxic Disorders
7 R01 OH02384-01*
09/27/85 - 08/31/87
\$157,787 (\$157,787 Cum)

*Previously supported under Grant Number R01 OH02157

Objectives

To validate a computer-administered neurobehavioral test system for use in field situations for detecting the intoxicating effects of solvents.

Methodology

Transient changes in central nervous system (CNS) function are assessed using a computer-administered neurobehavioral evaluation system (NES). Industrial hygiene sampling, exhaled breath analysis, and urine analysis is coupled with biomathematical dose models to estimate CNS concentrations of solvent. To reduce the complexity of exposure estimation, fiberglass boat-builders are studied. They are exposed almost exclusively to styrene. NES testing is performed before and after exposure to styrene during the work day. At the beginning of work shift, after peak-exposure activity near mid-day and at the end of the work shift.

Progress & Accomplishments

Work in the first year of this project concentrated on identification and recruitment of styrene-exposed workers, development of breath sampling and analysis methods, and data collection on one-half of the subjects.

Significance

We anticipate that this study will demonstrate the utility of our NES in assessing the intoxicating effects of solvents in the field study situation and provide information directly relevant to the setting of workplace exposure standards. It should improve our ability to monitor exposure and effect and to establish dose-effect relationships in solvent-exposed workers.

Publications - None to date

PERIPHERAL NERVOUS EFFECTS OF WORKPLACE NEUROTOXINS

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Neurotoxic Disorders
7 K01 OH00064-03*
09/28/84 - 08/31/87
\$32,400 (\$64,800 Cum)

*Previously supported under Grant Number K01 OH00043

Objectives

To develop methods that are accurate, sensitive, and efficient for field evaluation of peripheral nervous system functions in workers exposed to neurotoxic agents.

Methodology

Three aspects of peripheral nervous system function have been selected for study: tremor, temperature sensitivity and vibration sensitivity. The project consists of five phases: (1) Examination and modification of test apparatus and data collection procedures, (2) reproducibility testing (3) normative data development (4) testing of exposed populations and (5) development of protocol manual.

Progress & Accomplishments

For tremor testing, peripheral equipment has been assembled and computer software integrated to allow acquisition and storage of tremor signals as well as performing Fast Fourier Transforms of signals for frequency analysis. For temperature and vibration, instruments from Sensortek, Inc., have been selected and tested. We have implemented a procedure for estimating sensory thresholds that is more efficient than that proposed by the instrument manufacturer. We have performed computer simulations comparing our procedure, the manufacturer's suggested protocol, and a third standard procedure.

We have collected data using our new protocol on more than 110 unexposed workers for development of norms for both the temperature and vibration test devices. In addition, we have collected sensory test data during field studies of solvent exposed painters. 113 painters have been tested with the temperature device and 55 were tested with the vibration device. Exposure-effect analyses on these data has been completed. Data collection for reliability and stability of these measures has been completed. Manuscripts for reliability, norms, and exposure effects in painters are near completion.

Significance

These tests and new procedures should expand our ability to study the effects on the PNS of neurotoxic agents in the workplace.

Publications

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NEUROTOXIC ESTERASE AND NEUROTOXICITY

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Neurotoxic Disorders
5 R03 OH01761-02
09/01/83 - 08/31/86
\$28,125 (\$54,585 Cum)

Objectives

Single exposure to certain organophosphorus compounds (OP) induce delayed onset of peripheral neuropathy which is associated with distal axonal degeneration of peripheral nerves and long spinal tracts. The initial event, occurring within hours of dosing, is the phosphorylation and "aging" of a target protein (NTE: Neuropathy Target Esterase), but the relationship between this event and the delayed onset of axon degeneration is unknown. The objective of this study is to determine whether the OP-NTE reaction is followed by detectable pathophysiological changes which precede the onset of the first morphological or neurobehavioral abnormality in the test animal (hen). Specific focus has been placed on the fate on retrograde axon transport since this critical system appears to be exceptionally vulnerable in toxic neuropathies.

Methodology

Studies were designed to examine the relationship between NTE/OP interaction and the fate of retragrade transport of ^{125}I -tetanus toxin (a marker protein) in hen sciatic nerve after a single, subcutaneous injection of either neurotoxic (DBDCVP) or non-neurotoxic (PMSF or paraoxon) compounds. At selected intervals after receiving one of the these compounds, animals were administered a single intramuscular (gastrocnemius) injection of ^{125}I -tetanus toxin. Retragrade axonal transport of radiolable was subsequently assessed by determining the amount of ^{125}I transported to lumbar dorsal root ganglia (sensory axons) and ventral spinal cord (motor axons). Control animals were treated with comparable doses of PMSF or paraoxon to determine if defective transport is related to NTE inhibition/"aging."

Progress & Accomplishments

The induction of central-peripheral distal axonopathy in hens singly dosed with some organophosphorus (OP) compounds, such as DBDCVP, requires greater than 80% phosphorylation and subsequent intramolecular rearrangement ('aging') of NTE in the nerve fiber. Suprathreshold biochemical reaction a few hours after dosing was shown to cause a progressive decrement of retrograd axonal transport in sensory and motor fibers that culminates, days later, in axon degeneration. The maximum transport deficit (about 70% reduction) was reached 7 days after DBDCVP (0.75-100 mg/kg s.c.) prior to the onset of nerve fiber degeneration and clinical signs of neuropathy. By contrast, PMSF (30 mg/kg s.c.), an agent that prevents the development of OP neuropathy by inhibiting NTE without the aging reaction, had no effect on axon transport, nerve-fiber integrity and clinical status when administered either alone or prior to a neurotoxic dose of DBDCVP. Paraoxon (0.2mg/kg s.c.) did not inhibit NTE and was also unable to cause either deficit in retrograde transport or neuropathy. Taken in concert, these studies demonstrate that

retrograde transport deficit is correlated with the threshold-initiating mechanism in OP-induced nerve fiber degeneration.

Significance

Peripheral neuropathy is a remarkably common response of the human nervous system to systemic intoxication with occupational chemicals, including certain OP compounds, acrylamide, *n*-hexane, methyl *n*-butyl ketone, carbon disulfide, and dimethylaminopropionitrile. These neuropathies are all associated with distal axon degeneration of long and large-diameter nerve fibers in peripheral nerves and long tracts of the spinal cord. The mechanisms underlying axonal degeneration in OP neuropathy are not understood, but the results of the current study and other data from this laboratory suggest that alterations in retrograde axonal transport may play an important role in the pathogenesis of nerve fiber degeneration in this and related toxic neuropathies.

Publications - Abstract(s) available

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OCCUPATIONAL ETIOLOGIES OF ALZHEIMER'S DISEASE

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Neurotoxic Disorders
1 R03 OH01826-01A1
12/01/84 - 05/31/86
\$24,450 (\$24,450 Cum)

Objectives

Evaluate the hypothesis that Alzheimer's disease or a closely related clinical dementia are related to occupational exposure to organic solvents and/or lead.

Methodology

A case-control study of clinically diagnosed cases of Alzheimer's disease was conducted to evaluate occupational exposure to organic solvent and/or lead as potential risk factors. Neighborhood controls were selected from the list of registered voters for Massachusetts and were matched by sex, year of birth, and town of residence. Occupation was assessed by mailed questionnaire sent to spouses or next of kin residing in the same household. An exposure index was utilized to assess exposure to organic solvent. A conditional logistic regression analysis was performed, odds ratios and two-sided 95% confidence intervals were calculated.

Progress & Accomplishments

Detailed occupational histories were gathered from spouses and next of kin for 106 cases and 214 controls. A conditional logistic regression analysis was conducted for 98 cases and their 162 matched controls. After controlling for educational level no apparent association was observed between risk of Alzheimer's disease and occupational exposure to organic solvent and/or lead.

Significance

Prior scandinavian studies have noted an excess of presenile dementia and encephalopathy in workers exposed to organic solvents and/or lead. The current study suggests that this condition may not be identical to DSM III criteria dementia of the Alzheimer's type. Further study of dementias not specifically of the Alzheimer's type may be in order.

Publications - None to date

THE EFFECTS OF CADMIUM ON THE RAT OLFACTORY SYSTEM

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Neurotoxic Disorders
5 R03 OH02038-02
09/28/84 - 08/31/86
\$3,353 (\$20,877 Cum)

Objectives

The overall goal of the project was to determine what effects chronic low-level exposure to cadmium (via inhalation) may have on the rat olfactory system. The hypothesis tested was as follows: exposure of rats to chronic low levels of Cd via inhalation results in impairment of the olfactory system, both functionally and structurally.

Methodology

Forty-two rats were water deprived and trained on a conditioned suppression task to suppress a response in the presence of an odorant stimulus. Baseline olfactory thresholds were determined. Rats were then exposed to 0, 250, or 500 mg/m³ CdO aerosol for 20 weeks. Twice a week, rats were run on a conditioned suppression task using a suprathreshold stimulus to maintain their performance. At the end of every 5 weeks, the following parameters were examined: tissue Cd levels, olfactory thresholds, and proteinuria. Cd levels in the kidney, lung, olfactory epithelium, olfactory bulb, and brain were analyzed by atomic absorption spectrophotometry. Olfactory thresholds for an odorant, isoamyl acetate (IAA), were determined. Urinalysis to determine the amount of protein present in the urine was performed. At the end of 20 weeks, 4 rats from each treatment group were sacrificed, and the olfactory bulbs were examined by light microscopy for any histological alterations produced by Cd exposure.

Progress & Accomplishments

No Cd-induced overt toxicity was observed. Tissue Cd concentrations showed both time- and dose- dependent increase. Cd concentrations in the olfactory bulb were significantly higher than those in the brain. However, anosmia was not found in any of the rats. Furthermore, the olfactory thresholds were not altered by Cd exposure. No histological or histochemical changes in the olfactory bulb were found under light microscopy. No changes in proteinuria was observed.

Failure of Cd to produce any impairment of olfactory function may have resulted from: 1) Protection by metallothionein from the toxicity produced by unbound Cd; 2) Regeneration of the olfactory system, thereby repairing any damage to the system; or 3) The possibility that there is a critical age and/or duration for Cd exposure in determining the occurrence of olfactory dysfunction.

Significance

Although no alterations in the olfactory threshold induced by low-level chronic exposure was found, this research was the first attempt to address this question. It was also the first study to apply olfactory research to

toxicology. A significant finding of this research was that Cd uptake in the olfactory bulb when compared to other routes of exposure. It is likely that inhalation exposure is a far more effective route for Cd to gain entry into the CNS, particularly the olfactory bulb. A possible additional route of Cd transport to the CNS -- the transneuronal transport from the olfactory epithelium to the olfactory bulb might have contributed to the greater Cd uptake found in the present study.

Publications - None to date

TEST FOR NEUROTOXINS USING CAENORHABDITIS ELEGANS

*

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Neurotoxic Disorders
1 R03 OH02095-01
06/01/85 - 05/31/87
\$14,748 (\$31,689 Cum)

Objectives

The overall aim of the project is to develop a screening test for neurotoxins using the nematode Caenorhabditis elegans. The test organisms are exposed to selected chemicals and changes in behavior are evaluated using computer tracking.

Methodology

A strain of E. coli is grown on experimental plates until a lawn is well formed and then 0.4 mL of an aqueous solution of the test chemical is added to the plates. The plates are desiccated for 24-hours at 50% relative humidity to allow for the excess water to evaporate. A number of three to four day-old C. elegans are placed on each plate. After 24-hours two types of evaluations are made: lethality and computer tracking to determine behavioral changes. Control cultures are tested concurrently with the neurotoxins.

With lethality tests, death is determined by the total lack of movement and/or response to probing with a needle. For behavioral studies, the worms are removed from the plates, washed, and placed on 1% agar. The worms are positioned under a video camera that is interfaced to a microcomputer. This arrangement allows for the simultaneous tracking of several hundred nematodes. The movements of the animals are analyzed to determine both the rate of movement and the number of changes in directions (reversals). In the future, responses to sensory stimulation will also be measured.

Progress & Accomplishments

Lethality studies have been completed for seven metals: Hg, Cu, Pb, Cd, Zn, Al, and Ni. For each metal a dose-response has been determined and the LC50 has been calculated using both probits and logits.

Computer tracking experiments have been conducted on Hg and to a lesser extent on Cu. The nematodes exposed to Hg show a biphasic response - at very low Hg concentrations there is hyperactivity that appears to peak at exposure concentrations of about 7 ppm Hg and as the concentration increases the activity falls to well below the controls. This peak activity Hg concentration is approximately 10% the nematode LC50 of 60 ppm Hg. The computer tracking for Cu has only shown decreased activity as the Cu concentration increases; however, very low concentrations of Cu have not yet been studied.

Significance

The lethality data has been compared to the LD50 data from mammalian studies and, except for one metal (Cd), there is a good correlation between the results. Although much more data is needed, it is believed that this

test species shows much promise as a basic model for range-finding studies and initial lethality screening tests.

The computer tracking studies are in a preliminary state, but the hyperactivity observed with Hg supports our hypothesis that computer tracking of C. elegans can provide a rapid, inexpensive means of screening chemicals for neurotoxicity.

Publications - None to date

THE EFFECTS OF IMPULSE NOISE ON THE AUDITORY SYSTEM

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Noise-Induced Hearing Loss
2 R01 OH01152-07
07/01/80 - 09/28/87
\$179,847 (\$768,726 Cum)

Objectives

The purpose of this research is to understand the relation between the parameters of impulse noise (peak pressure, duration, number, repetition rate, exposure duration, etc.) and the effects on hearing as assessed by anatomical, physiological, and psychophysical techniques in the chinchilla.

Methodology

Several mechanical or electrical-mechanical devices are used to produce realistic noise impacts. Hearing is tested, the animal is exposed to impulse/impact and its hearing is tested for the following forty days. Routine data collection consists of measurements of hearing sensitivity, auditory discrimination and cochlear histology. More detailed studies of certain experimental groups will include scanning E.M., as well as more discriminating psychoacoustic measures of hearing.

Progress & Accomplishments

This project has led to a number of new insights into the effects of exposure to impulse noise. We have provided data to support the hypothesized relation between the duration of an impulse and its hazard to hearing; we have shown (morphologically) that impulse noise can damage the cochlea by direct mechanical destruction. This mechanical damage is different than the cochlear damage following continuous noise and is probably responsible for different audiological symptoms produced by impulse and continuous noise. Several years ago we showed that impulse and continuous noise can synergistically interact. We have documented that noise induces a number of the changes in the neural code leaving the ear and have related these changes to the symptoms of noise induced hearing loss. Recently, we have finished a large, parametric study of the validity of the Equal Energy Hypothesis.

Significance

The results have direct implications for noise standards. Heretofore, we have either neglected or underestimated the traumatic potential of impulse noise. Our research shows the peculiarities of impulse noise and suggests several situations where impulse noise increases the audiological hazard.

Publications

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FUNCTIONAL CORRELATES OF COCHLEAR INJURY

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Noise-Induced Hearing Loss
5 R01 OH02128-03
09/01/84 - 08/31/89
\$132,641 (\$349,956 Cum)

Objectives

The major goal of this project is to determine how the magnitude, pattern, and growth of hearing loss and structural damage are altered as the frequency, intensity, duration, and scheduling of noise are varied. Another goal is to determine the physiological correlates (as revealed by single-unit studies) of the behavioral changes and anatomical damage induced by exposure to noise. These behavioral, physiological, and anatomical studies share the long-term goal of identifying the relations between functional deficits and injury in the inner ear. A final goal is to evaluate hearing-loss and cochlear damage as a function of age in a group of chinchillas that have been raised in a sound-shielded environment and have never been exposed to noise.

In addition to these studies, acoustic measures of spontaneous otoacoustic emissions are being made from ear canals of noise-exposed chinchillas. These measures will provide physical indicators of cochlear integrity in the intact animal and information about nonlinear and active processes in the normal and damaged cochlea.

Methodology

Experimental animals are trained by food-reward, operant-conditioning techniques so that measures of auditory function can be obtained before, during, and after their exposure to noise. Physiological studies include thresholds and tuning curves of individual fibers, rate-level functions, and population studies in noise-exposed animals. Histological evaluation of the specimens includes detailed study of plastic-embedded flat preparations so that counts of missing sensory and supporting cells can be made and cytochrome c oxidase preparations prepared for all specimens. Acoustic measures are made with a small probe microphone assembly.

Progress & Accomplishments

1. Continuous exposures. We have continued evaluating hearing loss in chinchillas exposed to an octave band of noise (0.5 kHz, 95 dB SPL) for long durations. To date, we have found that ATS is stable for as long as 700 days; that is, the hearing loss measured during days 700-730 of exposure is no worse than the hearing loss measured in the first week of exposure. However, anatomical data indicate that the longer exposures are considerably more damaging to the cochlea than the short ones, and studies of PTS after exposure indicate a steady growth of PTS.

2. Intermittent exposures. Two groups of chinchillas were exposed on different schedules of intermittent noise (Group 1: OBN centered at 0.5 kHz, 95 dB SPL, 6 hr per day for 36 days; Group 2: same OBN at 95 dB SPL, 15 min/hr for 144 days. Both exposures produced an initial shift of thresholds of 35-45 dB; however, after a few days of exposure thresholds began to decline and eventually recovered to within 10-15 dB of original baseline values even though the exposure continued. Measures of recovery made after completion of the exposures indicated minimal permanent threshold shifts in all animals.

These intermittent exposures produced less hearing loss and cochlear damage than continuous exposures of equal energy.

3. Age-related changes in the chinchilla cochlea and presbycusis. Cytocochleograms have been made for 62 ears of chinchillas ranging in age from birth to 19.2 years which were born and raised in our sound-shielded animal colony and have never been exposed to noise. Regression lines, describing growth of outer and inner hair cell loss with age indicate the function $y = 1.06(\text{age in years}) - 0.55$, where y is percent missing OHC, describes the data adequately. The function for percent missing inner hair cells is: $y = 0.25(\text{age in years}) + 0.22$.

We have also obtained behavioral audiograms for elderly chinchillas raised in the sound shielded environment with no experimental treatment (ages 8.8-19.2 yrs). The 19.2 year-old chinchilla did have some mild hearing loss which was correlated with damage in the cochlea. With the exception of this animal, all old animals evaluated to date appear to have nearly normal hearing sensitivity compared to young control ears. There is no suggestion of progressive high frequency hearing loss seen in presbycusic humans, even though cell loss does increase with age.

3. Acoustic measures. We currently have one animal with an SOAE (2.1 kHz, about 10-15 dB SPL, developed after exposure to 0.5 kHz OBN, 95 dB SPL, 18 days). This animal has now been behaviorally trained, and a study is underway correlating the fine structure of the audiogram (10-hz steps) with the locus of the SOAE.

Significance

The behavioral and anatomical studies on long-duration noise exposure will help elucidate relations between TTS, ATS, PTS and damage to the cochlea. Our finding that the ear can recover 30 dB of sensitivity during intermittent exposure is of practical and theoretical interest. This finding suggests that workers exposed on various intermittent schedules may not exhibit significant threshold shifts even though the noise exposure is damaging the cochlea and may eventually lead to a PTS. Finally, our studies of SOAEs in noise-exposed animals provide a unique opportunity to study the relations between experimentally-induced SOAEs and cochlear histopathology, which will lead to a better understanding of active cochlear biomechanics.

Publications - Abstract(s) available

Clark WW: Spontaneous otoacoustic emissions and acoustic intermodulation distortion products as indicators of hearing loss and cochlear pathology, In: Sensorineural Hearing Loss: Mechanisms, Diagnosis, Treatment, edited by Collins MJ, Glattke TJ, Harker LA. University of Iowa Press pp 209-232, 1986.

Clark WW, Bohne BA: Cochlear damage: audiometric correlates, In: Sensorineural Hearing Loss: Mechanisms, Diagnosis, Treatment, edited by Collins MJ, Glattke TJ, Harker LA. University of Iowa Press pp 59-82, 1986.

Clark WW, Bohne BA: Temporary threshold shifts from attendance at a rock concert. J Acoust Soc Am 79, Suppl 1, S48, 1986.

Clark WW, Bohne BA: Attenuation and protection provided by ossicular removal. J Acoust Soc Amer, March, 1987.

INDUSTRIAL NOISE: POTENTIATING INTERACTIONS

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Noise-Induced Hearing Loss
5 R01 OH02317-02*
08/01/84 - 07/31/87
\$134,217 (\$332,443 Cum)

*Previously supported under Grant Number R01 OH01518

Objectives

The objective of this grant is to systematically examine the synergistic effects of noise exposure and vibration on hearing. Several different types of noise exposure [four band-limited noises of equal energy, and eight impulsive noises with different levels and types (A or B duration)] and two examples of whole-body vibration at different frequency and magnitude are being combined to assess the factors that influence the interaction between vibration and various noise exposure paradigms.

Methodology

A series of 14 control groups and 20 experimental groups is being exposed to continuous or impulsive noise, vibration or a combination of noises or vibration and noise. The exposures utilize two experimental paradigms: acute exposures; those that produce an asymptotic threshold shift. Hearing thresholds and masked thresholds are obtained before exposure and over a thirty-day post-exposure period using auditory evoked potentials. The final format of the data includes a comprehensive description of the exposure, a profile of the animals hearing ability before and after the treatment and a detailed, quantitative morphological analysis of the cochlea. The morphological analysis relies primarily upon surface preparations of the organ of Corti and the vascular tissues of the lateral wall of the cochlea and includes scanning and transmission electron microscopy of interesting or unusual pathologies.

Progress & Accomplishments

The interaction between Continuous and Impulse Noise: Frequency Effects. This set of experiments illustrates some of the conditions that must be satisfied for a synergistic interaction between impulse and continuous noise to occur. The data illustrates the effect of the energy frequency spectrum of the impulse on the production of a synergistic interaction. Chinchillas were used as the experimental animal. Experimental groups containing 5 animals were exposed to one of the following noise conditions. 1) Two different octave bands of noise for one hour; 0.5-1.0 kHz at 100 dB SPL or 2.0-4.0 kHz at 95 dB SPL. 2) Impulse noise generated by an electrical spark discharge. The duration of the first positive overpressure of the Friedlander wave thus generated was varied in four steps from approximately 30 u sec. to 80 u sec. at a peak SPL of 158 dB. A Fourier analysis indicated that the energy peak of the impulse shifted from approximately 8 kHz down to 4 kHz. The impulses were presented at the rate of 1/min for 50 min. 3) The third series of exposures consisted of combinations of the above two sets of exposures. This experimental design allowed us to study the effect of a shifting impulse spectrum on the hearing loss acquired from very brief duration blast waves and also indicated, as might be anticipated, that a synergistic interaction is most likely to occur when there is a special

overlap of energy between the impulse and the continuous noise. The effects of the above exposures on hearing were measured by obtaining either evoked potential or behavioral hearing thresholds on each animal before and after exposure. The status of the cochlear sensory epithelia was evaluated using surface preparation histology.

Using a completely different experimental protocol and a more realistic set of stimuli similar results were obtained in the experiments described below. This second set of experiments showed, in addition, that equal energy considerations can be violated when synergistic interactions are encountered.

The interaction of impulse and continuous noise: Energy and spectral considerations in the production of hearing loss. Realistic industrial noise environments containing impulsive and continuous noise were modeled using a 5-day exposure paradigm which produces an asymptotic threshold shift (ATS). Pre- and post- exposure measures of hearing thresholds were obtained on 96 chinchillas using evoked auditory responses (EAR). Six control groups were exposed to octave bands of noise at 0.5, 2.0 and 4.0 kHz at 95, 90, and 86dB SPL respectively or impacts of 113, 119 or 125 dB peak SPL presented once per 1, 4 or 16 seconds, respectively. Nine interaction groups were exposed to combinations of an impulse and continuous noise. The greatest spectral overlap of energy occurs between the impulse and the 0.5 kHz octave band of noise. Although each of the different impulse noise exposures were balanced to produce an equal energy exposure, an exacerbation of hearing loss was produced in animals exposed to the 119 and 125dB impacts in combination with the low frequency (0.5 kHz) continuous noise. The audiometric and histological data are in agreement in showing that the synergistic effect gradually disappears when the spectral overlap between noises is reduced.

Significance

Synergistic interactions among various ototraumatic agents have been shown to be of importance in determining the hearing loss in a number of industrial situations. The interactions between continuous and impulse noise and vibration and noise are being studied using an animal model. The rationale for these studies is that continuous noise rarely exists as a sole hazard to hearing in most industrial and military work environments. Thus, exposure criteria which are based upon data obtained from experiments involving only the use of continuous noise may not be protective.

Publications

Hamernik RP, Ahroon W, Henderson D, Salvi RJ: The interaction between continuous and impulse noise: Frequency Effects. Proceedings 2nd Int'l Conference on the Combined Effects of Environmental Factors. September 28 - October 1, 1986, Kanazawa, Japan.

Shaddock LC, Hamernik RP, Wright CG: A morphometric technique for analysis of cochlear vessels. Hearing Research 20:109-117, 1986.

Shaddock LC, Wright CG, Hamernik RP: A morphometric study of microvascular pathology following experimental rupture of Reissner's membrane. Hearing Research 20:119-129, 1985.

NOISE-INDUCED HEARING LOSS AND HIGH BLOOD PRESSURE

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Noise-Induced Hearing Loss
5 K01 OH00042-03
09/28/84 - 08/31/87
\$32,400 (\$96,120 Cum)

Objectives

The results of our previous study suggest that severe noise induced hearing loss and blood pressure levels are associated and both may be due to long term noise exposure among susceptible individuals. An alternate hypothesis may be that in the older worker, noise exposure in addition to the aging process may lead to poor speech discrimination, discomfort to loud sounds, etc., such that it may result in reduced social interaction and interference in lifestyle. The increased stress on and off the job may affect blood pressure levels. The goals of our present study are: 1) further validation of our previous noise and blood pressure study by its replication in a group of men 55-65 who have long histories of occupational noise exposure, 2) to carry out further more detailed characterization of the audiometric profile of these men (see protocol), and 3) administration of the revised form of the Hearing Performance Inventory as developed and tested by Lamb et. al. (Journal of Ear and Hearing, 1983). This is being used to assess the communicative difficulty of noise-exposed individuals in a variety of everyday listening situations. In addition an index of social support is being administered as that was a variable found to correlate with B/P in our previous study.

Methodology

The clinical exam consists of measures of height, weight, pulse and blood pressures, as well as indepth medical and personal habits history including alcohol consumption and smoking patterns. There is also an occupational history, military and noisy hobby questionnaire and lifestyle interference inventory. This exam will be administered at the Union Hall in the morning.

Because large variations in blood pressure have been shown to exist within a given subject, it is difficult to detect differences between groups and may lead to misclassification of individuals. Therefore, multiple blood pressure measurements using standard procedures will be implemented in this investigation. Blood pressure will be determined three times within five minutes after a ten minute rest period and then repeated by a second staff member fifteen minutes later.

Standardized audiometric testing procedures are being used and the technician is supervised by a Ph.D. audiologist who possesses a certificate of clinical competence in audiology from the American Speech and Hearing Association. Other audiometric tests to be included are: 1) pure tone air conduction (already described), 2) bone conduction, 3) speech reception threshold, 4) speech discrimination scores in quiet and in competing noise, and 5) self report inventory of Hearing Performance. The revised form of the Hearing Performance Inventory has 6 basic sections. They consist of 1) questions involving understanding speech, 2) intensity, 3) response to auditory failure, 4) personal communication, 5) social, 6) occupational.

Progress & Accomplishments

After a period of recruitment of personnel into the study, we have trained 2 nurse epidemiologists in blood pressure measurement, and an audiometric technician has been recruited to work on the study. A list of eligible retirees has been also obtained and survey instruments selected. The questionnaires and methods were piloted during the period of December, January, and February. It was during this time that we also reviewed various questionnaires and hearing assessment inventories to determine which would best suit the needs of the men in this particular study. We began screening in March 1985. To date we have attempted contact of 120 individuals via letter to request participation in the study and indicating that we would be calling them to set up a clinic date in the near future. Of these, 16 were found to be ineligible because of age or life threatening illness. An additional 19 either unpublished numbers or return-to-sender addresses which must be traced. We will be sending another letter to the individuals with unpublished numbers requesting that they call our office. There have been only 8 refusals thus far. A total of 80 individuals have completed their screening. Coding of the data for statistical compilation has not begun and therefore we do not have any pertinent analysis at this time.

Significance

The results of our previous study suggest that severe noise-induced hearing loss and blood pressure levels are associated and both may be due to long-term noise exposure among susceptible individuals. An alternate hypothesis may be that in the older worker, noise exposure in addition to the aging process may lead to poor speech discrimination, discomfort to loud sounds, etc., such that it may result in reduced social interaction and interference in lifestyle and increased stress which may affect B/P levels. This study is designed to address this question.

Publications

Talbott, E., Helmkamp, J., Matthews, K., Kuller, L., Cottington, E. and Redmond, G. "Occupational Noise-Exposure, Noise-Induced Hearing Loss, and Epidemiology of High Blood Pressure" American Journal of Epidemiology. Vol 121 No. 4, April, 1985 p 501-514.

Hodgson, M.J., Talbott, E., Helmkamp, J. and Kuller, L.H. "Diabetes, Noise Exposure and Hearing Loss", Accepted to J.O.M., August, 1986.

Matthews, K.A., Cottington, E., Talbott, E., et al. "Psychological Work Environment Noise Exposure and Blood Pressure Among Blue Collar Workers", Accepted American Journal of Epidemiology, September, 1986.

ACOUSTIC STAPEDIUS REFLEX AND TEMPORARY THRESHOLD SHIFT

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Noise-Induced Hearing Loss
5 R03 OH01718-02
07/01/83 - 12/31/85
\$22,500 (\$42,330 Cum)

Objectives

Clinical and experimental observations indicate that there are individuals uniquely susceptible to noise-induced shifts in auditory sensitivity. Experimental induced temporary hearing loss varies widely in subjects exposed to exactly the same noise in level and spectral composition. The acoustic reflex (AR) provides a protective function that also varies from individual to individual. Thus, the AR may be one of the many factors that relate to the development of temporary threshold shift (TTS). The purpose of this project was to determine if dynamic properties of the AR (threshold, latency, magnitude and decay) relate to TTS produced by two-hour exposures to broadband noise and two different octave-band noise (0.5 and 3.0 kHz). Additionally, TTS was correlated to case history data, pre-exposure behavioral thresholds and ear canal volume in linear and multiple regression models.

Methodology

Two separate, two-hour noise exposure studies were completed to determine which, if any, of the properties of the AR correlate to TTS produced by different noise stimuli. Careful measurements of the AR were obtained to different eliciting stimuli prior to each exposure. Behavioral thresholds from 0.25 through 8.0 kHz were obtained from trained subjects before and after exposure. Acoustic immittance outputs were digitized and subjected to algorithms designed to evaluate AR threshold, latency magnitude and decay. Ear canal volume and case history information (age, sex, eye color, use of tobacco, etc.) were obtained on all subjects. Behavioral hearing sensitivity (method of adjustment) was established with the aid of a computer.

The first study established correlations among these measurements and TTS produced in 37 subjects exposed to broadband noise. The second study explored correlations among AR dynamics and TTS in 30 subjects who were exposed on different days to both 0.5 and 3.0 kHz octave-band noise.

Progress & Accomplishments

To accomplish the objectives of this project, studies were designed to identify correlates between AR activity and TTS produced by exposures to broadband noise and octave band noises centered at 0.5 and 3.0 kHz. Acoustic reflex magnitude and decay elicited with broadband noise correlated to many expressions of TTS. The greater the magnitude of response ($r = -.40$) and the less the amount of decay ($r = .33$), the smaller the TTS. Test results indicated a relationship between ear canal volume and the frequency at which maximum TTS occurred. Subjects with greater TTS at 3.0 kHz had ear canal volumes that were significantly greater than subjects with greatest TTS at 6.0 kHz. Acoustic reflex threshold and latency and case history information did not correlate with TTS. Multiple regression modeling that included all significantly related variables improved TTS predictability over simple

linear regression modeling. The second series of studies examined the relation of dynamic properties of the AR to the TTS that developed in thirty subjects following two separate, two-hour octave band noise exposures. Pre-exposure behavioral thresholds and several AR parameters were found to be significantly correlated to the TTS that resulted from both exposures. Multiple regression modeling showed AR adaptation, onset latency and magnitude to be only slightly predictive of the TTS that resulted from the low-frequency exposure.

Overall, results from these studies suggest that AR is one factor in the development of TTS following two-hour exposures. Only a slightly predictive relationship exists, however. The strongest single predictive property of the AR is an expression of its sustained activity.

Results from this project will be presented in four additional articles during the next year.

Significance

The development of a test battery to identify those individuals susceptible to the hazards of noise exposure has been sought for decades. The results suggest that the AR influences the development of TTS, however is only slightly predictive. Acoustic reflex adaptation (decay) accounts for less than 30% of the variability in TTS. Thus, measurement of the AR may have predictive strength as part of a larger test battery designed to include other parameters. Additionally, ear canal volume is a factor influencing the pattern of TTS. Results suggest that persons with larger ear canal volumes will suffer greater TTS in the lower frequencies than persons with larger volumes.

Publications - Abstract(s) available

Moul MJ, Gerhardt KJ, Helper EL: Further observations on personality pattern and temporary threshold shift. Hearing Instruments, Vol. 36:22-24 1985.

HEARING PROTECTION: SELECTION AND COMMUNICATION ISSUES

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Noise-Induced Hearing Loss
5 R03 OH01829-02
09/28/84 - 08/31/86
\$19,500 (\$40,308 Cum)

Objectives

This two-year research program addressed issues which have direct bearing on the selection and utility of hearing protection devices (HPDs) for attenuation of industrial noise. This aim of the first year's research was to determine experimentally the dependence HPD attenuation on the level of insertion/donning instruction given the wearer. It was hypothesized that the achieved attenuation of over-the-ear HPDs (earmuffs) would be less influenced by donning instruction differences than that of in-the-ear canal HPDs (ear-plugs). The objective of the second year's research was to investigate the influence of occlusion, using various HPDs, on speech discrimination performance in different noise levels and under different industrial noise spectra.

Methodology

In the first year attenuation study, five universal-fit earplugs, four earmuffs, and two ear canal caps were assessed. The REAT (real-ear attenuation at threshold) method was employed to determine the attenuation afforded by each HPD after each subject had donned the HPD by following one of five levels of instruction. A free-field loudspeaker system was used to present hearing test tones at 125, 250, 500, 1000, 2000, 4000 and 8000 Hz to subjects with normal hearing seated in a near-anechoic chamber. Therefore, for each HPD, a frequency-specific profile of attenuation capability was determined for each instruction condition. In parallel with this laboratory effort, a field survey of numerous typical industrial machine noise emissions was performed, resulting in 1/3-octave noise spectrum plots for each emission. By comparing the HPD attenuation spectra obtained in the laboratory with the noise spectra obtained in the workplace, optimal matching of HPD and noise source can then be accomplished. In addition to obtaining these data, a methodology was developed and applied for assessing HPD comfort.

The second year's experiment concerning HPD effects on speech discrimination utilized the actual HPDs and recordings of industrial noise sources obtained the first year. Subjects listened to male voice recordings of isophonemic word lists presented through loudspeakers in an anechoic field. Ambient conditions included three noise spectra (low frequency, white, high frequency) presented at 60 and 83 dBA. Subjects performed the speech discrimination tests both with occluded ears (earmuff, earcap, or earplug), and with unoccluded ears to determine the effect of each HPD in each spectrum of noise. A dependent measure of correctly identified speech phonemes was obtained. Individual differences in baseline hearing levels were balanced across experimental conditions using a block design.

Progress & Accomplishments

All experimental tasks and associated analyses for both the HPD attenuation and the speech communication study were completed. From the former

experiment, frequency-specific plots of HPD attenuation as a function of level of instruction given the user were produced. HPDs were found to differ widely in the level of noise protection they provided based on the level of instruction. In general, earplug the use of attenuation was found to be much more susceptible to differences in instruction than earmuff or earcap attenuation. For some earplugs tested, detailed, systematic user instructions resulted in as much as a threefold increase in dB protection over no or minimal instructions. Earmuffs were consistently more resistant to differences in instructions than earplugs. Other differences in attenuation and user comfort are discussed in the publications resulting from this project.

The industrial noise survey resulted in 1/3-octave band spectral plots for a number of typical machinery emissions. These may be used with HPD attenuation spectra for matching HPDs to noise problems.

The speech discriminations study revealed a number of important findings. Perhaps the most important was that none of the three types of HPDs degraded speech discrimination (in comparison to an unoccluded condition) in an ambient noise level of 83 dBA. In fact, the most protective (highest NRR) HPD significantly enhanced speech discrimination in the high level ambient noise. In an ambient intensity level of 60 dBA, there was significant reduction in discrimination due to wearing some of the HPDs, but at this level, protection is not necessary. The tendency of the HPDs to improve, or at least maintain, speech communication performance in high noise intensities appeared to hold for all three broadband noise spectral types tested.

Significance

The findings of these studies shed light on the importance of proper selection of HPDs for particular noise problems. Proper user instruction is critical to obtaining optimal protection from HPDs in the workplace. Furthermore, the notion that HPDs hinder communication is not supported by these data, which indicate that properly-selected HPDs may be expected to maintain or improve speech communication (over unoccluded conditions) in even moderately high noise intensities of various spectral profiles.

Publications

Casali JG, Epps BW: Effects of user instertion/donning instructions on noise attenuation of aural hearing protectors. Human Factors, 28(2):195-210, 1986.

Casali JG, Lam ST: Effects of user instructions on earmuff/earcap sound attenuation, Sound and Vibration, 20(5):22-28, 1986.

Casali JG, Lam ST: Over-the-ear industrial hearing protectors: An assessment of comfort issues. Proceedings of the 1986 Human Factors Society 30th Annual Conference, Dayton, Ohio, September 29-October 3, 1986.

Casali JB, Epps BW: Hearing protection device comfort and user preference: An investigation and evaluation methodology. Proceedings of the 1985 Human Factors Society 29th Annual Conference, Baltimore, Maryland, September 29-October 3, 1985, 814-818.

Casali JG: In defense of industrial workers' hearing. Feature article in IIE Ergonomics Newsletter, Spring 1986, 20 (4), published by the Ergonomics Division of the Institute of Industrial Engineers, Norcross, Georgia.

AUDITORY TEMPORAL ACUITY FOLLOWING NOISE EXPOSURE

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Noise-Induced Hearing Loss
5 R03 OH02141-02
09/27/85 - 08/31/87
\$21,426 (\$40,910 Cum)

Objectives

The purpose of the study is to describe subtle, temporary changes in human temporal acuity brought about by listening to moderately intense noise for a brief period of time. The effect of signal frequency, signal level, and recovery time on gap detection threshold will be examined following the noise exposures.

Methodology

Octave-band noises with a brief temporal gap will be used as the experimental stimuli. Off-frequency spectral cues will be minimized with a broad-band masker. An adaptive two-interval forced-choice psychophysical paradigm will be used to measure gap detection threshold (GDT). The GDT's will be obtained both before and after a 5-minute, octave-band noise exposure. Stimulus generation and control of the experiments will be accomplished with a laboratory microcomputer.

Progress & Accomplishments

Pre-exposure gap-detection thresholds were obtained from five normal hearing listeners at frequencies above 1 kHz (1.7 kHz, 2.4 kHz and 3.4 kHz) and from four subjects for frequencies below 1 kHz (0.4 kHz, .56 kHz and .8 kHz). Testing levels were 35, 55, and 75 dB SPL at each frequency. After pre-exposure gap-detection thresholds were obtained, each listener was exposed to 105 dB SPL pure tone at the lowest frequency in the triad, for a time sufficient to produce approximately 10 dB of TTS₂ at the half-octave frequency. At test frequencies above 1 kHz, the dependence of pre-exposed gap-detection threshold on level was independent of frequency; at test frequencies below 1 kHz an interaction of level with frequency was noted. After exposure to the fatiguing tone, the gap-detection threshold shifts and recovery curves were predictable from a determination of the amount of TTS at each frequency. That is, the elevation in gap-detection threshold observed after exposure appears to be due to the concomitant decrease in sensitivity.

For test frequencies below 1 kHz, this "attenuation" hypothesis was more difficult to test because of the pre-exposure interaction between level and frequency. However, recovery of gap-detection threshold was longer-lasting than recovery of absolute sensitivity.

The work is continuing with a new indicator of temporal acuity, the discretion of Huffman sequences played in forward or reverse time order.

Significance

The results may provide the basis for developing a more sensitive tool for evaluating the effects of noise on hearing. Information from this project may also help us understand the temporal processing capabilities of some hearing-impaired individuals.

Publications - None to date

BIOASSAY FOR DIOXIN: WORK PLACE RELATED CUTANEOUS HAZARD

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Dermatologic Conditions
5 R01 OH01533-03
11/01/83 - 10/31/85
\$41,759 (\$85,814 Cum)

Objectives

The overall goals of this project are to evaluate two in vitro markers of dioxin induced biological activity as an assay(s) for dioxinlike chemicals and the ultimate application of such an assay(s) to the detection of these chemicals in biological samples relevant to occupational settings. The two markers are 1) the induction of an in vitro keratinization response in XB epithelial cells cocultured with irradiated fibroblast feeder cells and 2) the induction of a proliferative and morphologic alteration in XBF epithelial cells, which were derived from the XB line, also cocultured with irradiated fibroblast feeder cells. The most toxic dioxin isomer, 2,3,7,8-tetra-chlorodibenzo-p-dioxin (TCDD) was used as the dioxin prototype.

Methodology

These studies are based on in vitro alterations in epithelial cell culture by TCDD. Briefly, the semiquantitative determination of this effect was determined as follows:

Epithelial target cells were suspended by trypsinization and seeded into 24-well plates (16-mm diameter wells, 5×10^4 cells/ml per well) with irradiated 3T3 cells (5×10^5 /ml per well) in Dulbecco's modified Eagle medium (DMEM) supplemented with 20% fetal bovine serum. After overnight incubation (37C, 5% CO₂, humidified) the cultures were refed with a series of 10-fold dilutions of a stock solution of test chemical or sample extract in Me₂SO (maximum concentration 0.1%). After 14 days the culture was assessed for flat-cell induction, washed with phosphate-buffered saline (PBS), fixed with Formalin in PBS, and stained with either Giemsa or 1% rhodamine B in water.

The cultures were evaluated for the flat-cell effect by phase-microscopic assessment and confirmation that the tested cultures had grown to form a confluent monolayer of morphologically flat cells as compared to the high density, control cultures comprised of multilayered fusiform cells.

Progress & Accomplishments

Initial studies were done to determine optimal conditions and evaluate each system. These studies involved the effects of frozen storage, variation of culture conditions e.g. - serum concentration, feeder cell concentration and effect of serum lot number. Experiments were also done to establish new, more stable and responsive clones from the parent lines. The results of these studies indicated that the XB cell keratinization system, which was found earlier to lose its keratinization induction phenotype with cell passage, was unable to generate subclones in which this phenotype was stable. These studies indicated that low passage frozen XB stocks could be used for continued studies of this phenotype, but that the use of this system as an assay for dioxinlike activity could be limited. These studies also demonstrated the application of the cell keratinization system to the

analysis of soot samples contaminated with polychlorinated dibenzofurans. Based on these findings, the majority of the remaining work was performed on the XBF line and the dioxin induced morphological alterations from high density fusiform to a low density, flat epithelial morphology referred to as the flat-cell effect (FCE).

Studies were done to define the FCE which was shown to be the result of a reversible inhibition of post-confluent proliferation. Both treated and control XBF/3T3 cultures grew at equal rates until confluency was reached at which time the TCDD treated cultures became quiescent while the control cultures grew to a higher saturation density. The nondividing treated cultures were shown to be viable, and upon resuspension by routine trypsinization, were capable of further growth and of again exhibiting the FCE upon exposure to TCDD.

Validation studies were performed to determine the specificity and sensitivity of the FCE to various chemicals. Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurnas (PCDFs), polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs), and pesticides (24 compounds in total) were tested for their ability to induce the FCE. The results indicated that these changes are relatively specific for PCDDs and PCDFs. TCDD was the most potent congener tested, capable of inducing the effects at a concentration as low as 10^{-11} M. The activities of other tested PCDDs and PCDFs ranged from 10^{-1} to 10^{-3} of TCDD activity.

Ten extracts of soot containing PCDFs from an office building involved in a transformer fire were tested for FCE inducing ability and the results were compared to a high resolution gas chromatographic/mass spectrometric analysis (GC/MS) for total PCDFs in the same samples. This comparison showed a good correlation, suggesting that the XBF/3T3 system has potential use as a semiquantitative assay for dioxinlike activity.

Subsequent validation studies have indicated that this bioassay was compatible with various matrices including surface swipes, soil, sediment, fish and fat. This system has also been applied to the examination and health risk evaluation of suspect compounds such as 1,2,4,5,7,8-hexachloro-xanthene from dioxin contaminated sites in Missouri and 1,3,6,8-tetrachloro-9H-carbazole from New York State's Buffalo River. Studies based on the FCE allowed detection and subsequent identification of 2,3,7,8-tetrachlorodioxin, a sulfur analog of TCDD, found in municipal sanitary sewer.

Significance

These studies demonstrate that an in vitro assay for dioxinlike activity, based on TCDD induced alterations directly related to epithelial differentiation, has been developed. Chloracne is one of the most common effects of TCDD exposure in humans and is considered to be the result of induced differentiation. The described bioassay system therefore has potential usefulness in screening biological samples of various types (blood, urine, milk) from workers in occupational settings which carry a high risk for dioxin exposure. This will allow detection of the exposure incident and a semiquantitative evaluation of the degree of exposure. Other uses include the detection of dioxinlike activity of active dioxin analogues in the workplace and evaluation of structurally similar compounds.

Publications

Gierthy JF, Crane D, Frenkel GD: Application of an In Vitro keratinization assay to extracts of soot from a fire in a PCB-containing transformer. *Fundamental and Applied Toxicology*, 1984.

Gierthy JF, Crane D: Reversible inhibition of In Vitro epithelial cell proliferation by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicology and Applied Pharmacology* 74:91-98, 1984.

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MECHANISMS OF OCCUPATIONAL LEUKODERMA

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Dermatologic Conditions
5 R01 OH01556-03
09/29/82 - 03/31/86
\$55,233 (\$149,944 Cum)

Objectives

Occupational depigmentation from phenolic and catecholic skin depigmenters (DP) involves a direct attack on melanocytes. The susceptibility of melanocytes to DP may directly stem from physical and chemical interactions of DP with pigment melanin and/or tyrosinase. Binding of DP to melanin would "select" the pigment as a target for chemical attack. Subsequent redox reactions may involve either electron transfer from DP to melanin or "autooxidation" of DP by O_2 probably catalyzed by tyrosinase. These reactions could undermine the ability of melanin to protect the cell from cytotoxic agents and could result in diffusible reactive species capable of causing melanocyte death. To test this hypothesis we propose to (1) measure the binding and redox interactions of selected phenolic and catecholic compounds of widely varying potency with model synthetic melanins, melanin-tyrosinase complexes and with melanoproteins extracted from C-57-BL-6 black mice. The kinetics of the above-mentioned redox reactions will be characterized. Experimental conditions will be chosen which maximize a given component reaction while minimizing the others. This will allow an approximate separation of the overall interaction into binding, "autooxidation" and electron transfer components. We will use these results as a basis to dissect the total DP-melanosome interaction into simpler components, (2) induce hair depigmentation by local subcutaneous or intradermal injection of DP into black mice. The relative potencies of the DP's will be estimated visually and by a turbidimetric analysis of hair melanin in alkaline-sulfide medium, and (3) seek possible correlation between DP potency and the behavior of one or more component interactions.

Methodology

The following compounds are studied: (a) t-butylcatechol (tBC), (b) 4-methylcatechol (MCAT) (substituted catechols); (c) hydroquinone (HQ); (d) catechol (CAT); (e) 4-t-butylphenol tBP (a substituted phenol); (f) DOPA; (g) 3,4 dihydroxyphenylacetic acid (DHPA), (substituted catechol). We assess the ability of each DP to act as a substrate for tyrosinase. Early experiments were done with commercially available mushroom tyrosinase; future experiments will include results with tyrosinase preparations from B-16 melanoma cells at various stages. Melanin interaction is assessed by noting changes in the product distribution or the kinetics of the reaction.

Electron transfer kinetics from DP to test melanin are determined by measurement of the rate of reduction of potassium ferricyanide, an electron acceptor. If melanin couples electron transfer from DP to ferricyanide, then reciprocal plots of initial velocity vs [DP] will be linear. From the slope and intercept, one can obtain k' , a rate parameter and K_B , the binding constant for DP to melanin. These parameters are characteristic for given DP and melanin preparations.

Animal studies are carried out with C-57-BL mice. CB-1 mice are used as controls. Hair is removed from the dorsal surface of the black and the white mice by clipping. The clipped hair is analyzed for melanin content. Mice are

injected subcutaneously five days per week for 4 weeks with 0.01-1% of each DP in 0.1 ml physiological saline on the right side of the back. Skin and hair depigmentation is assessed by visual and histological means.

Progress & Accomplishments

We have studied the thermodynamic and kinetic interaction of 10 catechols and phenols with synthetic L-DOPA melanin. Using ferricyanide as electron acceptor, we have demonstrated that DP-melanin binding can lead to alterations in electron transfer kinetics, and that the magnitude of such alterations may be roughly correlated with depigmenting potency.

Spectroscopic analysis of reaction mixtures of DP oxidized by ferricyanide and by periodate in the presence or absence of L-DOPA melanin indicates that i) melanin does not change the product distribution or extent of reaction, and ii) the quinones of those catechols which are potent DP's are stable in solution, whereas those which are poor depigmenters are not.

Preliminary studies on oxidation of DP by molecular O_2 in the presence and absence of melanin indicate that (i) such reactions are slow compared to PC and tyro-sinase-catalyzed DP + O_2 reactions and (ii) melanin does not appear to affect the course of reactions. These results indicate that non-catalyzed DP + O_2 reactions can be neglected, in the first approximation.

We have experimentally (and reproducibly) produced depigmentation with tBC, MCAT, CAT, HQ, and MMEH at several concentrations. We have also demonstrated that L-DOPA and DHPA are non-depigmenters under the same conditions. We have documented characteristic distribution patterns, have obtained histological data from skin biopsies, and have obtained preliminary data on the kinetics of reversibility, as judged by the time course of pigment reappearance.

Preliminary results with mushroom tyrosinase indicate that DOPA, tBC, DHPA, MMEH, and tBP are substrates. DOPA melanin markedly accelerates the reactions in the case of MMEH and tBP. This appears to involve the ability of melanin to (a) act as a "cofactor", and to (b) facilitate oxidation via electron transfer.

Significance

These results show the considerable influence of melanin on reactions which may lead to cytotoxicity. These results will help in filling the gaps necessary for realizing the following long-term goals: (a) early detection and prediction of environmental depigmenters, (b) attainment of a reliable regimen for effecting controlled depigmentation, (c) effective use of the appropriate DP's as chemotherapeutic agents in malignant melanoma, and (d) determination of mechanistic similarities and differences between occupational depigmentation and mechanistic similarities and differences between occupational depigmentation and vitiligo.

Publications - Abstract(s) available

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A PERSONAL DOSIMETER FOR EXPOSURE TO ULTRAVIOLET LIGHT

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Dermatologic Conditions
5 R01 OH02021-03
02/01/84 - 01/30/87
\$87,649 (\$276,594 Cum)

Objectives

There are uniformly ignored federal standards for near (315-400 nm) and far (200-315 nm) UV radiation. The standards are written with a flat response curve in the near UV and a steep curve in the far UV. The purpose of this study is to provide simple, low-cost, easily-analyzed, and interference-free dosimeters which meet the requirements of the standard and would be acceptable to the individual wearer for occupational, recreational, or therapeutic exposure to potentially carcinogenic ultraviolet light. The major hypothesis is that photochemistry of some appropriately chosen molecules could provide the required dosimeters by measuring the ratio of product to reactant.

Methodology

For the 200-315 nm region, a tube containing 0.25% 2-Butanone (MEK) in oxygen-free water is worn in an exposed place. The ethane and ethylene produced by UV photolysis are then analyzed after the end of the shift or after suspected exposure, using a gas chromatographic technique. Dilute benzophenone in isopropanol is one candidate for the 315-400 nm range, with the product again analyzed by GC.

The far UV photolysis is carried out in quartz tubes whereas the near UV photolysis uses pyrex tubing, thus eliminating any far UV photons. The molecules chosen provide a very close match to the required spectral shape for the radiation exposure standards, particularly in terms of the sharp cutoff at long wavelengths. This eliminates any possible interference from abundant long wavelength photons.

Progress & Accomplishments

We have demonstrated that MEK photolysis and analysis of the ethane and ethylene can be used as a dosimeter for far UV radiation and have built and demonstrated a prototype dosimeter package. We encountered unexpected containment difficulties in that the original gas phase mixtures tried were not stable in their containers for a long period for useful dosimetry. The approach to eliminate this difficulty is to use a water solution of one of the relevant molecules since much larger absolute numbers of molecules can easily be placed in the same container, and GC sampling is simplified.

Significance

The original hypothesis has been proven to be correct. The unexpected containment problem has been solved for the far UV, and a very useful protective device is the outcome. The device was first demonstrated informally at the NIOSH meeting in Cincinnati, November 1986.

Publications - None to date

CUTANEOUS EXPOSURE: PREDICTIVE PATHWAYS

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Dermatologic Conditions
5 K01 OH00017-03
09/28/84 - 08/31/87
\$31,174 (\$93,049 Cum)

Objectives

The ultimate goal of this research is to anticipate the detrimental toxic effects of hazardous chemical exposure via the skin. Specifically, our objective is the quantitative estimation of the toxicokinetics of occupationally-encountered molecules absorbed across human skin in vivo.

Methodology

1. The continued building of a comprehensive percutaneous absorption data base, cross-referenced to physicochemical information on the chemicals studied.

2. The preparation of both qualitative and quantitative reviews of the assembled information.

3. The development of interpretive models for percutaneous absorption (PA); the definition and identification of structure-activity relationships are targeted for investigation.

4. Exploration of experimental approaches to transdermal absorption; optimization of new methodologies for the determination of potential cutaneous toxicity.

Progress & Accomplishments

Progress has been achieved in three areas:

1. Literature database and review: The literature database has expanded significantly over the last year. A major filing system has been established and has involved cross-referencing articles by key-words and key-categories. Abstraction of PA information from the assembled literature is in progress and details of published papers are collected for input into a file management system on the NIH Prophet Computer Resource. The number of compounds is expanding such that comparison, analysis and interpretation of blocks of results can be attempted.

The database has been used to compile four reviews: a) PA in the elderly has been contrasted with that in young adults. b) In vivo approaches for the evaluation of transdermal drug delivery (and, by extrapolation, chemical exposure via the skin) have been assessed. c) The criteria, which molecules must satisfy for sufficient transdermal delivery to elicit systemic effect. d) the impact of cutaneous metabolism on both the local and systemic availability of topically contacted chemicals has been evaluated.

2. Interpretations of percutaneous absorption: Continued development of a kinetic model for transdermal input and percutaneous absorption has been achieved. Different modes of input have been considered and the potential to use the approach for the assessment of risk following topical exposure is promising. Further, in an attempt to establish quantitative structure-transport relationships for PA, simple in vitro procedures have been developed and tested.

3. Alternative experimental approaches: Penetration has been measured under both occlusive and protected (but non-occlusive) conditions. Single-

dose observations remain predictive of penetration following chronic application. Occlusion promotes absorption, the level of enhancement increasing with increasing penetrant lipophilicity. When the site is occluded or protected with a non-occlusive device, recoveries of the applied dose equal or approach closely 100%. The ability to achieve mass balance in an in vivo measurement of PA is an important advance with respect to the quality and certainty of topical exposure information. PA has also been measured in vivo by drug disappearance from the skin surface and has been compared to evaluations from urinary excretion and skin "stripping" procedures. Agreement between the data is excellent. Hence, surface disappearance may provide a simple means to evaluate PA in man, with particular advantage in the assessment of short-term topical exposure to a variety of chemical species.

Significance

Skin disease, initiated in the work place, currently represents a major occupational health problem in the United States. Prediction of the detrimental toxic effects of hazardous chemical exposure is difficult, however, because of the complexity of the PA process and a lack of any consistently identifiable relationship(s) between transport rate and chemical properties. In addition, the very diverse approaches, which have been used to measure skin penetration, further complicate the problem since the extrapolation of results to the human in vivo situation is an exercise requiring often quite unreasonable and, hence, potentially dangerous assumptions. The ultimate goal of the research proposed here is to address this area of occupational health concern. Therefore, our specific aim is acquisition of the ability to predict accurately the toxicokinetics of occupationally-encountered molecules absorbed across human skin in vivo.

Publications - Abstract(s) available

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TRANSDERMAL KINETICS AFTER MULTIPLE CUTANEOUS EXPOSURE

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Dermatologic Conditions
5 R03 OH01830-02
07/01/83 - 12/31/85
\$19,590 (\$37,851 Cum)

Objectives

The long-term goals of the project were:

- A. To identify whether multiple cutaneous exposure can be reliably estimated from single-dose percutaneous absorption experiments.
- B. To develop a physicochemical model for skin penetration kinetics that will allow prediction of a penetrant's potential dermal toxicity on the basis of (i) its chemical properties, and (ii) the structure of skin.

Methodology

In vivo multiple dose percutaneous absorption studies have been conducted in rhesus monkeys and in humans. In rhesus monkeys, benzoic acid, parathion and salicylic acid have been studied. In man, attention has been focussed on hydrocortisone, estradiol, testosterone and progesterone. Single and multiple-dose percutaneous absorption experiments have been conducted with and without occlusion of the site of chemical application. Recovery of the applied ^{14}C -dose(s) is possible and good mass balance has been demonstrated.

The model development work centered upon extension and application of a physicochemical-pharmacokinetic simulation for transdermal delivery and percutaneous absorption. Rate parameters in the model are predicted from (a) diffusion theory and the penetrant molecular weight, and (b) the octanol/water partition coefficient (K) of the substrate. Validation of the approach has been achieved by fitting in vivo data for skin penetration of a broad range of organic compounds. Extension of the model to address (i) specific input kinetics from the topical "delivery system", (ii) the effect of surface loss processes (e.g. volatilization) on absorption, and (iii) the interpretation of in vitro penetration data, has been considered.

Progress & Accomplishments

Progress toward the two basic goals of the project and in other exploratory areas has been achieved.

1. The in vivo multidose experiments in both rhesus monkeys and man have shown that a single acute exposure and measurement of percutaneous absorption is predictive of penetration following repeated application. Experiments under occlusion have demonstrated significant promotion of absorption for lipophilic steroids. A clear inverse correlation between surface recovery of penetrant and ultimate absorption has been established. Less invasive and more direct determinations of acute and chronic dermal exposure to chemicals are therefore suggested.

2. The physicochemical-pharmacokinetic model of percutaneous absorption developed in this work has been validated. Subsequently, the model has been extended to include specific input kinetics from the delivery system. Surface disappearance through volatilization (which is relevant to dermal exposure to organic solvents, for example) has been incorporated into the simulation. Further efforts have considered the assessment of in vitro skin

penetration data, in particular to address chemical delivery in volatile solvents which may, themselves, alter the kinetics of absorption.

3. Additional progress established during this project includes:

(a) Investigation of local chemical delivery into skin in vivo using laser Doppler velocimetry. (b) Calculations of body exposure from skin absorption data. (c) Chemical delivery to local subcutaneous structures following topical contact has been reviewed.

Significance

The percutaneous penetration of chemicals accounts for a significant number of toxic observations. The ability to predict and hence minimize exposure and toxic potential is routinely based upon acute exposure and a single-dose transdermal absorption determination. However, the more environmentally and occupationally relevant situation often involves multiple cutaneous exposure for which very little experimental investigation has been performed. The project aimed to resolve this insufficiency by: (i) conducting chronic percutaneous absorption studies, and (ii) developing a pharmacokinetic model for interpretation and prediction of transdermal penetration kinetics following topical application.

Publications

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Guy RH, Hadgraft J: Kinetic analysis of transdermal nitroglycerin delivery. Pharmaceut Res 2:206-211, 1985.

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OCCUPATIONAL HEALTH HAZARDS IN SKIN INJURY BY MITOMYCIN C

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Dermatologic Conditions
R03 OH01990-02
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\$10,642 (\$31,446 Cum)

Objectives

The study of factors controlling the generation of cutaneous intradermal injuries from the antitumor agent MMC. This drug is a health hazard for occupation related injuries in health care workers, and treatment related injuries in cancer patients. Current and projected usage of MMC show a substantial potential risk of cutaneous injury and present treatment methods are unsatisfactory. A mouse model and a pig model will be used, and factors of injury generation will be studied: dose effects, injury generation time, healing time, and drug activation rate. Treatment measures that will reduce, prevent, or eliminate, cutaneous injury from the use of MMC will be studied.

MMC lesions appear to resemble adriamycin cutaneous lesions. Because adriamycin induced cutaneous lesions have been reported to respond to dimethyl sulfoxide (DMSO) treatment, it is hypothesized that DMSO will be effective against MMC induced cutaneous lesions. Furthermore, the reports of DMSO's effectiveness against adriamycin ulcers, when applied ditally to the ulcer site, will be evaluated in MMC induced ulcers.

Methodology

Skin Toxicity Studies: Dehaired mice were given topical MMC intradermal (ID) injections at various doses, and assessed for cutaneous toxicity. Topical and intradermal applications of various antidotes were made following ID exposure and evaluated for efficacy against MMC. Further antidotal studies are in progress. Because of the marked antidotal activity observed with DMSO, further studies have been pursued, and are in progress, to determine the optimal DMSO application schedule, the effects of delayed DMSO application, and the maximally tolerated MMC dose.

Pig model studies have used methods similar to those used in mice. Anesthetized and dehaired pigs were given topical MMC intradermal (ID) injections at various doses, and assessed for cutaneous toxicity. Topical and intradermal applications of various antidotes were made following ID exposure and evaluated for efficacy against MMC. Additional studies in pigs have investigated the rate of drug inactivation and disappearance from the ID injection site, and the effects of delayed DMSO application. These studies involve sacrifice of the animals, extraction of the drug, and analysis of the drug by HPLC methods.

Progress & Accomplishments

Skin Toxicity Studies: In mice, MMC produced dose-dependent toxicity. Ulcers appeared within 1-5 days following injection, with erythema greater in size than the central ulcer. Erythema increased during the first week, and then resolved over a 2-4 week period. MMC ulcers had a central necrotic appearance and resembled adriamycin induced ulcers. MMC did not produce uniformly larger ulcers with larger doses, but larger doses did require longer healing times.

In pigs, much the same results were observed as in mice. MMC also produced dose-dependent toxicity. Ulcers also appeared within 1-5 days following injection, with erythema greater in size than the central ulcer. As in mice, erythema increased during the first week, and then resolved over a 2-4 week period. MMC ulcers similarly had a central necrotic appearance and resembled adriamycin induced ulcers. MMC did not produce uniformly larger ulcers with larger doses, but larger doses did require longer healing times. In contrast to ulcers in mice, ulcers in pigs took longer to completely heal.

In mice, DMSO had a profound effect in reducing the size of the erythema and development of ulcers. Further studies are in progress to determine the maximum tolerated ID dose of MMC, with and without DMSO application. In pigs, DMSO did not appear to have as dramatic an effect as was observed in mice. There was little difference in erythema and ulceration size between MMC lesions treated with and without DMSO. Further studies are in progress to analyze the effects of DMSO on the maximally tolerated MMC dose, when the drug is given intraperitoneally. Studies measuring the MMC levels in skin of pigs and mice, using HPLC methods, are in progress. It is expected that there will be marked differences in tissue drug levels between pigs and mice. In vitro studies are in progress, attempting to compare pig and mouse skin for activation of MMC. If possible during the grant period, similar studies will be pursued using human skin. These studies will attempt to determine if human skin more closely resembles the results obtained in mice or those obtained in pigs.

Significance

Reliably produced MMC ulcers in mice and in pigs have been demonstrated, and the characteristics of healing profiled. DMSO appears to give substantial protection against MMC-induced skin toxicity in mice. DMSO does not, however, appear to offer any protection against MMC-induced skin ulceration in pigs. It is unclear as to how human skin can be predicted to behave in these conditions, and the direct application of these results to clinical setting will require further study.

Publications - None to date

THE EFFECTS OF PHENOLS ON CUTANEOUS IMMUNE REACTIVITY

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Dermatologic Conditions
5 RO3 OH02091-02
04/01/85-03/31/87
\$22,650 (\$45,037 Cum)

Objectives

Phenols have complicated effects on biological systems. For example, phenols with nucleophilic side chains in the para position (parasubstituted phenols -- PSP) applied topically to the skin induce proliferation of keratinocytes and pigment cells. PSP markedly alter the numerical density of Ia positive epidermal Langerhans cell populations, i.e., increase or decrease their number depending on the dose of PSP applied. Langerhans cells (LC) are immune "macrophages" in the epidermis. Their surface Ia markers seem essential for their interaction with T-lymphocytes and for initiation of immune reactivity. Alterations in Ia positive cells by PSP alter cutaneous immune reactivity. From other work completed, we suggest one mechanism by which PSP alter LC is by initiating the oxidation of arachidonic acid to prostaglandins and/or leukotrienes. The objective of this proposal is to study the effects of PSP and prostaglandins on the cutaneous immune response in vitro and in vivo. Commonly available compounds like 4-hydroxy anisole, monobenzyl ether of hydroquinone, and butylated hydroxytoluene will be applied to the skin of mice. Tissue will be stained by a variety of techniques, and alterations in the number and morphology of LC determined. Animals with excessive or depleted LC will be contact sensitized to common allergens/haptens to determine whether these agents alter immune reactivity. Skin of animals known to be sensitive to allergens/haptens will be depleted of Ia positive LC and challenged to the antigen in order to determine whether LC have a role in the efferent cutaneous inflammatory response. Epidermis with augmented or depleted numbers of LC will be made into single cell suspensions. The ability of LC in these suspensions to present antigen to syngeneic or allogeneic T-cells will be measured in vitro using mixed epidermal lymphocyte reaction (MELR) techniques. Further, we will perform a series of studies to determine the effects of prostaglandins, thromboxanes, and leukotrienes on the function of Langerhans cells in vitro and in vivo.

Methodology

1) Screening of Phenols: Identification of PSP which alter the population density of Langerhans cells (cells/mm²). We have tested some of the more common phenolic agents such as 4-hydroxy anisole, monobenzyl ether of hydroquinone, and butylated hydroxytoluene on the population density of Langerhans cells. The compounds in low (.001%) to high (10%) concentrations in 95% ethanol diluent were applied to the ears and back of DBA/2 or C57BL/6 mice. The epidermis from treated and control groups of mice were surgically excised and, following a two-hour incubation in .020 M Na EDTA, the resulting epidermal sheets were stained for Ia positive Langerhans cells by either indirect immunofluorescence, indirect immunoperoxidase or ATPase staining.

2) Sensitization and Elicitation of Contact Sensitivity. An ear swelling thickness technique was utilized to measure differences in cutaneous immune reactivity.

3) In Vitro Langerhans Cell Functional Assays. Single cell suspensions

of treated and control epidermal sheets were prepared using trypsin and DNase I digestion techniques. Access to a fluorescence activated cell sorter (FACS) was used to enrich for Ia positive LC. Enriched Langerhans cell suspensions (~85%) obtained from various treatment groups were observed for in vitro accessory cell function, including both MELR and mitogen stimulation studies using splenic and peripheral mononuclear blood responder cells.

4) Determination of Whether the Effects of PSP are Mediated by Prostaglandins, Leukotrienes or Other Products of Arachidonic Acid Metabolism. Briefly, to determine if some of our previous findings observed following topical application of various PSP were being mediated via arachidonic acid or its metabolites both in vivo and in vitro experiments as described above will be performed using various prostaglandins and leukotrienes.

Progress & Accomplishments

We have completed our in vivo experiments involving MBEH, 4-hydroxy anisole, and BHT. MBEH at a 20% concentration when topically applied produced a significant increase in identifiable Ia positive and ATPase positive Langerhans cells with an associated increase in intensity of the contact hypersensitivity response.

Low doses of BHT (.001%) and 4-hydroxy anisole (1.0%) when topically applied to C57BL/6 mice produced a significant increase in Ia+ Langerhans cells for 4-hydroxy anisole treated mice only. Large concentrations (5.0%) BHT and 4-hydroxy anisole (10.0%) did not produce significant changes in the density of Ia+ cells/mm². There were no significant changes in contact hypersensitivity reactivity to BHT and 4-hydroxy anisole. In vitro studies of LC enriched treated epidermal cells are planned for the upcoming year which should provide additional data on the functional aspects of these epidermal macrophages following phenolic compound exposure.

To further examine potential biochemical mechanisms involved with our original PSP hypothesis, MBEH was replaced with a biologically relevant molecules, arachidonic acid, prostaglandins and leukotrienes. Only arachidonic acid and PGE₂ when topical applied affected the CHS response by suppressing both the afferent and efferent limbs of the cutaneous immune response. Other prostaglandins and leukotrienes had no significant effect on the CHS response in vivo. In vitro experiments with these compounds are currently under way.

Since this proposal was submitted as of June 1, 1984, an additional newly identified dendritic epidermal cell has been discovered and is currently under investigation by several laboratories including our own. The cell is an Ia negative dendritic cell which expresses Thy-1, Ly-5 and asialo GM1 surface markers. The function of this cell is unknown, although it may serve as a suppressor or natural killer cell of the skin.

We have completed a series of studies in which C57BL/6, Balb/C, and DBA/2 mice have been topically treated with BHT, 4-hydroxy anisole and 20% MBEH in an attempt to identify any morphological or density changes in this cell. At low concentrations (.001%) BHT, (1%) 4-hydroxy anisole and 20% MBEH, the density of identifiable Thy-1+ cells (cells/mm²) increased by 50% vs. diluent (95% ethanol) treated animals.

Additional studies are planned using our parasubstituted phenolic compounds in an attempt to identify the functional role of this Thy-1+ dendritic epidermal cell using FACS sorting and MELR techniques.

Significance

Langerhans cells are believed to play an important role in initiating contact allergic reactions in the skin such as poison ivy or a variety of industrial related skin diseases. Our study is designed to establish whether other common phenolic compounds can enhance or suppress cutaneous immune reactivity by direct or indirect mechanisms which may modulate Langerhans cell function. Completed in vivo studies using topically applied MBEH and 4-hydroxy anisole both showed a significant increase in the density of identifiable Ia+ LC. In addition, MBEH treated mice revealed a more vigorous afferent and efferent (inflammatory) response to the allergen DNFB vs. control mice. These data suggest (potentially) that PSP exposure may actually exacerbate allergic contact dermatitis.

The recent discovery of an additional dendritic bone marrow-derived epidermal cell increases the skin's complexity with regards to normal cutaneous immune function. This cell may potentially serve as a suppressor cell and may either directly or indirectly affect Ia antigen expression on the Langerhans cells. In other words, the Ia + LC may be an "on signal" for allergic contact dermatitis, while the Thy1+ cell serves as an "off signal" for allergic contact dermatitis. Clearly, in light of our findings with regards to the function of the Langerhans cell following parasubstituted phenolic exposure, additional in vivo and in vitro studies are needed to assess the role of the Thy-1+ dendritic epidermal cell following phenol exposure. Further, via FACS analysis, we can now more precisely observe changes in Ia antigen density of epidermal cells following environmental allergen exposure. The density of Ia+ antigen expression is believed to be a major determinant for the intensity of the cutaneous immune response which we and others have reported.

It is our consensus that results of this initial study will provide new and important information on the effect of common phenolic compound exposure on the cutaneous immune response and may have useful applications for preventing or treating allergic contact dermatitis in the home and work place.

Publications

Rheins LA, Nordlund JJ: Modulation of the population density of epidermal Langerhans cells: A method to enhance or suppress cutaneous immune reactivity. J Immunol 136:867, 1986.

ZINC AND CALCIUM EFFECTS ON GUINEA PIG NICKEL DERMATITIS

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Dermatologic Conditions
1 R03 OH02182-01
09/27/85 - 08/31/86
\$21,602 (\$21,602 Cum)

Objectives

Nickel sulfate (NiSO_4) is a leading cause of ACD in the human populations of North America. Based upon its position in the first transition group of the periodic table, Ni is predicted to interact directly with Zn and indirectly with Ca (via Ca:Zn direct interaction) in biological systems. Little is known about the normal metabolic interactions of the three essential minerals Ca, Ni, and Zn; even less is known about such interactions in specific disease states. Zinc is a keratogenic element. Nickel:Zn and Ni:Ca interactions and associated skin diseases have been noted during animal feeding trials. Calcium:Zn interactions have been noted in Zn-deficiency skin disease of swine. Oral Ni toxicity in chicks has been prevented by increased dietary Zn. Although current topical, intramuscular and dietary restriction approaches to the treatment and prevention of Ni-induced ACD are not usually practical or effective, trials using dietary supplements of Zn or Ca have not been reported in the literature. By selecting one essential macro-element (Ca) and one essential trace element (Zn) that appear to interact with Ni in vivo, this research investigated whether supplemental intakes of these two minerals influence the development of ACD due to Ni exposure. The specific research aims are to: 1. to produce ACD in guinea pigs with both NiCl_2 and NiSO_4 , 2. to quantitate immunologic changes associated with nickel ACD in the guinea pig by intradermal skin test and labeled thymidine incorporation in the lymphocyte transformation test (LTT), and 3. to investigate the possibility that supplemental oral Zn and Ca may affect the degree of ACD expression in this animal model. The hypotheses tested are: increased oral Zn supplements will reduce or prevent Ni-induced ACD (via a direct Zn:Ni interaction); increased oral Ca supplements will either not affect (Ca, Zn, Ni independence) or will exacerbate Ni ACD (indirect Ca:Ni interaction, via Zn).

Methodology

Non-pregnant female Hartley strain guinea pigs, average weight 200-250 grams, are purchased from Harlan Sprague Dawley, Inc. in lots of six. They were each sensitized, using as an allergen either NiSO_4 or NiCl_2 in Freund's complete adjuvant in a protocol of intradermal and topical exposure. After 21 days they were each tested for delayed hypersensitivity by intradermal injection of 0.1 ml of the allergen (e.g. NiSO_4 or NiCl_2) at concentrations of 0.5%, 0.25%, 0.125%, 0.0625%, and 0.03125% and 0.1 ml of isotonic saline was used as a control. These tests were read at 48 hours by measuring the diameter (mm) of any observed erythema and/or induration at the injection site. The following day each guinea pig was anesthetized and bled via cardiac puncture. Lymphocytes are separated on a discontinuous Ficoll-Hypaque Gradient (0.085 sp. gr.), washed, placed in microtiter culture plate wells, and cultured in RPMI 1640 medium for 5 days at 37C with 5% CO_2 .

Mucophytohemagglutinin (PHA-M), a nonspecific mitogen, and three or more dilutions of the allergen (e.g. NiSO_4) were used in the lymphocyte transformation test. During the last 18 hours of incubation, 0.5 Ci of tritiated thymidine ($^3\text{HTdR}$) is added to each well. The cells were harvested onto glass-fiber filters with a semi-automatic harvester and measured for incorporation of $^3\text{HTdR}$ in scintillation vials containing a toluene-based cocktail in a scintillation counter. The stimulation indices (SI) was computed by dividing the arithmetic mean of quadruplicate determinations of counts per minute for stimulated wells, by the arithmetic mean of quadruplicate determinations for unstimulated control wells.

The effect of Zn and calcium of Ni ACD was measured in NiSO_4 exposed guinea pigs randomly assigned to the following groups: 1) 250 g ZnSO_4 /ml deionized distilled water (DDW), 2) 500 g ZnSO_4 DDW, 3) DDW, 4) 500 g CaSO_4 /ml DDW, 5) 1,000 g CaSO_4 /ml DDW, and 6) tap water.

Progress & Accomplishments

An experiment to compare NiSO_4 and NiCl_2 as allergens by exposing guinea pigs revealed that the lymphocytes of both the NiSO_4 exposed and the NiCl_2 exposed guinea pigs yielded higher SIs than the corresponding controls, but the SIs for the NiSO_4 and NiCl_2 exposed guinea pigs were almost the same, 1.9 and 2.0 respectively. Therefore only one allergen (NiSO_4) was used in the experiment to test for Zn and Ca effects. In this second experiment, the SIs of both ZnSO_4 treatment groups were lower than the SIs of the guinea pigs drinking only DDW; those receiving 250 g ZnSO_4 /ml DDW were significantly lower. Both the CaSO_4 treated groups had SIs very close to those of the Ni sensitized controls. The two highest concentrations of NiSO_4 used in the LTT assays (2.70×10^{-15} and $1.08 \times 10^{-5} \text{ M}$) suppressed blastogenesis. There was a wide range in the size of intradermal skin test responses. There were no associations between the size of the reaction and either the treatment group or the SI for individual guinea pigs exposed to NiSO_4 . Mean skin or whole blood Zn concentrations were not statistically different between the NiSO_4 exposed control and treatment groups, or between sensitive ($\text{SI} \geq 2.0$) and non-sensitive animals within groups.

Significance

These preliminary results help to refine the LTT for use in the ACD guinea pig model. The high proportion of sensitized control animals with stimulation indices greater than 2 indicates that this is a desirable model and test system. The poor NiSO_4 stimulation of NiSO_4 sensitized lymphocytes when the LTT concentrations of NiSO_4 were high (10.80 and $27.00 \times 10^{-5} \text{ M}$) is consistent with other observations of cell toxicity attributed to nickel.

The protective effect of ZnSO_4 should be further tested in other animal species and man. Increasing intake of zinc by low-level ingestion is a practical approach and could greatly reduce the incidence of nickel ACD, which is now the most frequent form of ACD in the United States.

Publications - None to date

OCCUPATIONAL STRESS AND HEALTH OF WOMEN LPN'S AND LSW'S

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Psychological Disorders
5 R01 OH01968-02
12/01/84 - 11/30/88
\$195,134 (\$364,893 Cum)

Objectives

The short-term longitudinal interview study of women who are licensed practical nurses ($n = 156$) and licensed social workers ($n = 248$) focuses on the relationship between occupational stress and health-related outcomes in women. Building on the investigators' prior research, the study extends our understanding of occupational based psychosocial stress to include not only job conditions and personality traits but also: (a) occupancy of non-workplace roles; (b) the quality of experience in occupational and in non-workplace roles. Quality of experience refers to the balance between rewarding and distressing attributes of a role and is measured by scales developed previously by the investigators.

Two categories of outcomes are examined: (a) physical health indices, including both self-reports and blood pressure measurements; and (b) mental health indices, including role strain, anger, and anxiety.

Major hypotheses concern the effects on physical health and psychological distress of job conditions, occupancy of non-workplace roles, and quality of roles. In addition, possible moderators of the relationship between stressors and health outcomes are examined, e.g., the interactions of work and family roles, and personality variables such as trait anger and anxiety.

Methodology

A stratified sample (race, parental status, partnership status) of LPN's and LSW's, ages 25 to 55, who live within specified zip codes (an approximately 25-mile radius of Boston) was drawn randomly from the professional registries of the two occupations. By enlisting the cooperation of relevant groups and publicizing the study widely, we have obtained an excellent response rate of 96.6%. In individual interviews a variety of measures -- both standard scales and scales developed for this study -- assess role quality (the balance between rewarding and distressing attributes of a role), psychological distress, and physical health, (including blood pressure). During the four years of the project, data will be gathered at three points in time, one year apart. A 10% random sample of subjects was reinterviewed to establish the test-retest reliability of certain scales, e.g., job conditions, rewards, and concerns.

Causal analytic techniques, including LISREL, will be used to evaluate specific hypotheses concerning the effects on health and psychological distress of job conditions, occupancy of non-workplace roles, and quality of experience in roles. In addition, we will examine the interactions of occupational and family role variables as these may moderate the relationship between occupational stress and health outcomes.

Progress & Accomplishments

(a) Sampling: As part of the process of identifying and drawing a sample, demographic studies were conducted of women LPN's and LSW's that resulted in a decision to stratify the sample by race, parental status, and

partnership status. In this way, the potential confounding of such variables as race and physical health will be avoided. Moreover, because of the low proportion of black women among LSW's, we have developed a census of all black social workers in our geographical area; we need to identify and include all such women to insure adequate numbers for analyses.

Because of unforeseen difficulties with the LPN registry, we were unable to locate the targetted number of women. The major problems were: (1) LPN's move frequently and the registry is out of date, making it extremely difficult to locate subjects; (2) the "partnered with no children" cell is very rare for LPN's since by age 25 most married LPN's have children; and (3) the actual number of existing LPN's was much lower than the estimates provided to us.

(b) Development of Measures: Major investment was made in developing measures of job conditions, social ties, physical health, rewards and concerns of various roles, and costs and benefits of multiple roles. For example, based on research currently in progress by other investigators on women's physical health, prior measures of physical health were modified to include previously neglected stress-related symptoms of women such as urinary tract problems and menstrual difficulties. Further analyses have been carried out of the rewards and concerns measures previously developed by the principal investigators in order to refine them.

In exploring procedures for determining blood pressure and accessing health records, the decision was made to collect data on blood pressure from all S's and to omit accessing of health records. Because subjects are not being identified by worksite, and because they vary greatly in their life situation, preliminary studies showed that too much uncertainty and diversity existed with respect to obtaining adequate health records. Other problems arose because of the variety of health professionals consulted, faulty memory for details, etc. Interviewers were certified by Red Cross instructors in obtaining blood pressure data; due attention is being paid to issues of reliability and validity.

Several new measures were developed and pilot tested for the second wave. Most importantly we included measures of role change concerning each major social role, i.e., paid worker, partner, mother and daughter. For each change, we ask subjects to indicate: (1) the degree to which the change was expected; (2) how much control they felt they had over it; and (3) how positive or negative it was.

We also included a measure of sex-role attitudes, items about leisure activities and friendships and about the rewards and concerns associated with aging, and open-ended questions about the role of grandmother and volunteer. Several of these areas were suggested to us by subjects, who have taken an unusual interest in the progress of the study.

Significance

Results of the first wave are being analyzed.

Publications - Abstract(s) available

IMPACT OF AUTOMATION ON INDUSTRIAL INSPECTOR STRESS

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Psychological Disorders
5 R01 OH02024-03
09/28/84 - 08/31/87
\$108,627 (\$287,927 Cum)

Objectives

The objective of this research is to determine how both human stress and human performance vary with the level of automation in an industrial inspection job. Industry is demanding higher quality of its workers and of its inspectors who are charged with maintaining and monitoring that quality. At the same time, sophisticated automation is becoming available to extend the human inspector's capabilities on some tasks within the inspection job. Inspection is known to be a stressful job and, with these two trends, a job which is likely to increase in stressfulness.

Methodology

An industrial inspection device is to be used to provide varying levels of automation. The Color Video Comparator (CVC) superimposes television images of the inspected item and a standard item in rapid succession onto a single color monitor. Any defects appear as changes in visual pattern between the successive images. In cooperation with AT&T Technologies, Inc., a stimulus set of life-sized photographs of a printed circuit board has been obtained. These photographs are of either a perfect board or one containing up to two of four different defects. Unemployed industrial workers are trained on this task, both using unaided visual inspection (the control condition) and at one of six levels of automation on the CVC:

1. Freely moving CVC to search board.
2. CVC moves in steps across board.
3. CVC moves in pre-determined step sequence.
4. Paced predetermined sequence, equal times for all areas.
5. Pattern recognition algorithm to automate search.

Performance measures are search time, stopping time and error rate.

Stress measures are visual stress (Critical Flicker Fusion Frequency), postural stress (self-report scales and measures of ancillary behaviors) and information processing load (heart rate variability, GSR). In the final year, AT&T Technologies is cooperating in replicating parts of the experiment using actual circuit board inspectors to give measures of job stress as well as task stress.

Progress & Accomplishments

The printed circuit boards, CVC and subject pool were obtained in Year 1 and the experiment at the first level of automation was run and analyzed. It was found that automation level 1 was slower than free inspection for stopping time ($p < 0.01$), but slightly better for defect detection ($0.05 < p < 0.07$ for three defects). Stress measures were not significantly different for this level of automation.

As a result of this experiment and analysis, the data collection has been expanded (GSR, Blood pressure) and shortened (2 vs 3 days). All levels of automation are being run again in year 2 with the new measures for consistency. Liaison is being maintained with AT&T Technologies for the final in-plant studies.

Significance

The results of the project will be of value to designers of automated and semi-automated systems as they will help to specify the level of automation which gives the best balance system performance and operator stress.

Publications

Drury CG, Kleiner BM: A comparison of blink aided and manual inspection using laboratory and plant subjects, Proceedings of 29th Human Factors Society Annual Meeting, San Antonio, p 670-674, 1984.

NEUROPSYCHOLOGICAL EFFECTS OF CHRONIC SOLVENT EXPOSURE

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Psychological Disorders
5 K01 OH00028-02
09/27/85 - 09/26/88
\$32,400 (\$64,800 Cum)

Objectives

Discrimination of the specific neuropsychological deficits occurring with chronic low-level exposure to different neurotoxic substances and development of a conceptual framework to explicate the mechanisms of these effects are of primary importance in the field of behavioral neurotoxicology. These issues are particularly germane to individual health and welfare as knowledge about them will allow development of screening techniques and preventive health measures aimed at minimizing the neurological, behavioral, social, and safety hazards which may accompany neurotoxic exposures.

This present study will explore these areas by longitudinally examining a group of workers experiencing chronic exposure to mixed solvents in the silk screening industry. It is predicted that exposed workers will show significant deficits on neuropsychological testing in the functional areas of visuospatial reasoning and short-term memory and that they will have affective complaints. In addition, solvent exposed workers are predicted to have difficulties on tests of cognitive flexibility which are associated with frontal lobe functioning.

Methodology

This three-year study will test a group of workers exposed to a little-investigated group of mixed solvents to be compared with non-exposed workers. Following medical, neurological, and demographic screening, each worker will undergo a short (60-75 minute) battery of neuropsychological tests, including a standard screening battery and additional tests of cognitive flexibility. Retesting will be completed at 12-month intervals.

Research design is modeled after that used in a study on lead-exposed workers in order to maximize comparability of data collected on solvents to existing data on lead.

The present investigation will utilize traditional neuropsychological methodology to examine the specific cognitive deficits which may accompany chronic exposure to this group of mixed solvents, assess relationship of duration of exposure to degree and nature of neuropsychological deficits, examine changes in test performance within subjects over time, and explore the nature of cognitive flexibility in workers with this exposure. Comparisons will be made between the neuropsychological data on this population and those derived from the parallel completed study of lead workers in order to discriminate specific cognitive and affective characteristics of lead and solvent encephalopathies.

Progress & Accomplishments

To date the subject groups to be evaluated for solvent exposure have been identified and agreement to complete the study has been made with management. Preparations have been made to begin testing in 3/86. The data on a lead comparison group have been collected and analyzed.

Significance

Explication of the specific neuropsychologic impairments occurring in workers chronically exposed to a particular group of mixed solvents and comparisons of solvent and lead induced neuropsychologic dysfunction may provide clues to explicating the mechanisms (psychologic, neuropathologic, metabolic) underlying specific neurotoxic encephalopathies. In addition, tasks assessing cognitive flexibility will help determine whether cognitive rigidity may be contributing to the functional intellectual deficits accompanying exposure to these solvents and to gain further information about brain-behavior relationships by examining effects of this exposure on tasks known to be sensitive to cerebral frontal lobe dysfunction.

Publications - None to date

A BEHAVIORAL EVALUATION OF TOLUENE AND ETHANOL

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Psychological Disorders
5 R03 OH02071-02
09/28/84 - 08/31/86
\$23,360 (\$47,043 Cum)

Objectives

The contribution of the study is to examine effects of low concentrations of toluene on human abilities which could result in sub-clinical CNS disorders affecting work, performance, or health. Toluene was chosen since it is a common solvent whose air concentrations in industry usually range from 25 to 100 ppm. Acute neurobehavioral effects of toluene vapor are measured by a microcomputer test battery and expressed in "ethanol equivalent units". Forty-two subjects were exposed for seven hours to 0, 75, or 150 ppm over three days. The behavioral evaluation assessed symptoms, visuomotor skills, visual perception, manual dexterity, and short-term memory at 8:00 am, 12:00 noon, and 4:00 pm. The criterion for choosing behavioral tests required prior validation, ease in scoring and interpretation, comparability to tests used in epidemiological studies, and recognized by the National Institute for Occupational Safety and Health and World Health Organization. The acute effects of ingested ethanol were evaluated in an identical manner. Forty-two different subjects were administered 0, 0.326, and 0.648 g/kg body weight, approximating 0.03 and 0.06 blood alcohol concentration. The rationale for including the ethanol component is: 1) it serves as a positive control for the toluene experiment, and 2) it permits a comparison of behavioral effects between solvents.

Chronic effects of solvent exposure have been equated to premature aging. Similarly, it would be useful to express an acute solvent effect by an index of "ethanol equivalent units" since acute neurobehavioral ethanol toxicity is well described.

Methodology

The study was completed in three experiments. The pilot study established the required number of subjects to detect a 5 to 10 percent difference in performance ($\alpha=.05$; $\beta=.10$) in the toluene and ethanol experiments. It is also permitted an assessment of menthol, the odor masking agent, and an assessment of statistical methods. Subjects were then randomly assigned to one of three orders of exposure presentation in both experiments (0, 75, 150 ppm toluene or 0, 0.03, 0.06 blood alcohol concentration). The results of the pilot study ($n=20$) suggested 41 subjects provided sufficient statistical power. Paid volunteers with at least one year of college, aged 18-35 years old, with no history of solvent exposure, and who consume less than 30 grams ethanol per day will be chosen. Inter-subject variation in solvent absorbance was monitored in breath at each test point, and accompanied by urinary hippuric spot sample. The data analysis will use adjusted scores computed by a multivariate regression analysis. The adjusted scores will then be used in a repeated measures of analyses study design.

Progress & Accomplishments

The three goals of the pilot study were accomplished within the first six months of the study. First, inter-subject variability over the test period suggests that forty new subjects should be chosen for experiments two and three. The actual number of subjects ranged from 20 to 59 subjects on important tests ($\alpha=.05$; $\beta=.10$). Second, the behavioral effects of 0.195 ppm menthol was not detectable on any test results which permits its use as a masking agent. Third, learning trends or improvement over 10 repeated sessions was particularly apparent in tests with a fixed stimulus. The digit span test, Sternberg Test, and Symbol-digit test had significant learning effects. We have since eliminated memorization of repeated digits by randomly generating those numbers. Finally we have identified and signed up a voluntary pool of qualified subjects.

The toluene and ethanol experiments were completed by December, 1985. Forty-one different subjects were used in each experiment. The data is currently being reduced and analyzed. In addition to the performance test scores, each subject's motivation, fatigue, and ethanol tolerance was rated subjectively and objectively. Self-reported symptoms were collected in response to the question "Over the last four hours, what reactions did you have while being tested on the computer?". Observations on sleep and other narcotic effects were also recorded by test administrators.

Accompanying performance scores and symptoms, toluene and ethanol concentrations in the breath were recorded at each test period. The urinary concentration of hippuric acid, a toluene metabolite, was also recorded as a spot sample for each test point.

The data reduction and analysis should be complete by May, 1986.

Significance

Successful completion of these studies should represent an improvement in research methodology by: 1) using a standardized test battery, 2) by introducing an index of solvent potency in ethanol equivalent units, and 3) determining if an acute solvent effect exists at relevant industrial exposures which will aid in interpretation of epidemiological investigations of chronic solvent effects. Most of the test battery has been recommended for use in future epidemiological studies by NIOSH and WHO, thus the results will be useful in the design and interpretation of future studies.

Publications - None to date

A MODEL FOR PREDICTING GLOVE POLYMER PERMEATION

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Engineering Control Systems
5 R01 OH01932-03
12/01/83 - 11/30/86
\$72,733 (\$199,089 Cum)

Objectives

The objective of this work is to utilize physical-chemical parameters for solvent polymer systems in models to predict pertinent permeation parameters; most notably the three-dimensional solubility parameter (3-DSP) of the solvent and polymer and the molecular size and shape of the solvent. Using a multiple regression model, these parameters will be regressed against permeation parameters such as steady-state permeation rate and breakthrough time for known polymer-solvent combinations. Then, from the model permeation parameters can be predicted for unknown combinations.

Methodology

Solubility parameters for polymers are determined using a method developed by Hansen in which polymer samples are submerged in approximately 55 different solvents. Those solvents which affect the polymer most will have solubility parameters similar to the polymer. For molecular size and shape of the permeant molecule, it is realized that some combination of the two concepts will have to be used. All permeation tests have been completed using ASTM D-739 test methods. Both infrared and gas chromatographic detection techniques have been utilized.

Progress & Accomplishments

Progress to date has resulted in the construction of a model for Viton^R using the difference between the solubility parameters for Viton and 19 solvents regressed against steady-state permeation rate and breakthrough time. For steady-state permeation rate, the correlation coefficient was 0.69 while for breakthrough time the correlation coefficient was 0.65. The results from this model were used to predict breakthrough times and permeation rates for unknown solvents. In addition, upper and lower 95% confidence limits were calculated for permeation rate and breakthrough time respectively. Several other experiments have been completed which relate to methods for relativizing permeation test data with respect to several variables. Both the thickness and temperature have been varied in order to determine the relationship between those two variables and permeation parameters. The relationships for Viton have, for the most, part, indicated relationships previously shown in literature. A third experiment explored variables regarding breakthrough time. Breakthrough time is a function of both the sensitivity of the analytical instrument and the permeant polymer system. Consequently, it can be somewhat arbitrary. The error caused by differences in analytical sensitivity has been determined. This error can be quite significant. In addition, we have utilized a new concept, accumulation time, to characterize the time necessary for a certain small amount of mass to permeate through the membrane. This parameter will be more consistent from one analytical device to another and should improve modeling techniques used in this grant.

Significance

The results for Viton which will be published soon, indicate clearly the significance of the work. Minimally, from these results, industrial hygienists can predict if Viton will be suitable for their particular application. Until the molecular size variable is added to the model, inaccuracies will remain so that once the industrial hygienist has predicted that a certain glove is appropriate, that glove should be tested. However, since there are at least ten glove polymers on the market, elimination of eight or nine of these by use of this model will be very useful to the industrial hygienist. Work is now underway to apply similar models to butyl and nitrile rubber.

Publications

Perkins JL: Use of the Three Dimensional Solubility Parameter to Predict Glove Permeation. Am Ind Hyg J 46:455, 1985.

Perkins JL, Ridge MC: Use of Infrared Spectroscopy in Permeation Test, in Performance of Protective Clothing, RL Barker, GC Coletta (eds), American Society for Testing and Materials, STP 900, 1986.

Perkins JL, Holcomb AB, Ridge MC, Wang WK: Skin Protection, Viton, and Solubility Parameters, Am. Ind. Hyg. J., December, 1986.

FUNDAMENTAL INVESTIGATION OF EXHAUST HOODS

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Engineering Control Systems
5 R01 OH02132-02
05/01/85 - 04/30/88
\$84,540 (\$180,599 Cum)

Objectives

The long-term objective of this grant is the optimization of exhaust hoods used in industrial ventilation, maximizing the capture ability and minimizing the air flow through the hood. Such an approach will reduce worker exposure as well as the cost of operating the exhaust system.

Methodology

The theoretical portion of the study utilizes superimposition and conformal mapping techniques to solve the flow field equations for oblong, circular and triangular hood openings. The experimental verification of the theoretical results is carried out by the measurement of airflow using a hot wire anemometer. The flow measurements are carried out in front of specifically designed hoods to represent a wide variety of hoods. The ultimate test of the hoods will be carried out using a tracer gas.

Progress & Accomplishments

To date we have carried out the theoretical investigations leading to closed form solutions for single or multiple hood openings with flanges and with or without flanking planes. Experimental verification of the theoretical equations for hoods without flanking planes is complete and hoods with flanking planes is underway. The results are reported in a number of papers which are in various stages of publication.

Significance

In the results obtained to date, we were able to show that with minimal effort it is possible to reduce required flow rate for a hood by about 15 percent while increasing the hood capture efficiency to 100 percent under ideal conditions. It is also shown that under non-ideal conditions (cross drafts) the capture efficiency of the hood can be kept at very near to 100 percent with minimal penalty in flow rate. The complete research results should enable designers to apply the principles developed in increasing the economic and capture efficiencies of hoods.

Publications

Esmen NA: Numerical Expressions for Ventilation Parameters Ventilation 85: Proc. 1st International Symposium on Ventilation for Contaminant Control, HE Goodfellow (Edit) Elsevier Amsterdam (1986), p 623-632 also p 735-742.

Durr DE, Esment NA, Stanley C, Weyel DA: Pressure Drop in Elbows. Applied Ind. Hyg. Scheduled for publication 5/87

Durr DE, Esment NA, Stanley C, Weyel DA: Pressure Drop in Flexible Ducts. Applied Ind. Hyg. Scheduled for publication 5/87.

Esment NA, Weyel DA: Aerodynamics of Airflow in front of Arbitrarily Shaped Symmetric Hoods. Amer. Ind. Hyg. Assoc. J. (in press).

CAPTURE EFFICIENCY OF LOCAL EXHAUST VENTILATION SYSTEMS

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Engineering Control Systems
5 R03 OH02101-02
04/01/85 - 03/31/87
\$14,175 (\$32,545 Cum)

Objectives

1. Development of a Theoretical Model of the three dimensional velocity field into a flanged circular hood, both with and without a crossdraft blowing perpendicular to the hood centerline.
2. Empirical validation of the above model.
3. Development of a theoretical model of capture efficiency based on the validated flowfield model using computer aided design.
4. Validation of the capture efficiency model.

Methodology

A modified potential flow solution was developed for the flow field into the flanged circular hood. An empirical validation was conducted using hot film anemometry for hoods operating with and without crossdraft. BASIC programs have been written for the IBM XT and AT personal computers that enable graphic plots of the flowfield to be displayed in two dimensions. These plots were used to construct models of capture efficiency, which were evaluated using a Sulfur Hexafluoride tracer gas system with portable Gas Chromatograph.

Progress & Accomplishments

All objectives are complete except a field validation of the model.

Significance

The work presents a method for assessing the effects of crossdrafts on hood performance and quantitatively evaluating a hood's performance. The potential for determining the adjustments needed to a given system in order to reduce the employee's exposure from one level to another are discussed.

Publications - Abstract(s) available

Flynn MR, Ellenbecker MJ: The Potential Flow Solution for Air Flow into a Flanged Circular Hood. Am Ind Hyg Assoc J 46(6):318-322, 1985.

NOISE CONTROL FOR HONING AND OTHER MACHINES - PHASE II

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Engineering Control Systems
5 R44 OH01951-03
09/15/85 - 08/31/87
\$ 86,514 (\$221,314 Cum)

Objectives

The major long-term objective of this project is to provide source noise control for machine tools such that operators will be protected against permanent hearing loss due to noise exposure. The primary aims of this research are:

Develop a noise control treatment for the honing machine, identify machines with similar noise generating mechanisms, and develop specific noise control treatments for the selected machines.

Methodology

In our Phase I study, we determined the method by which tonal sounds are generated by honing machines. We also identified a number of potential noise control treatments including: Lanchester dampers, dynamic absorbers, increased structural damping, and increased structural stiffness.

In Phase II, these noise control treatments are being developed in consultation with machine design engineers, damping consultants, and being analyzed and tested both in our laboratory and in the field. Only the most successful will be marketed in Phase III.

In addition to the honing machine, we are developing noise control treatments for a number of other machines with a similar type of sound generating mechanism.

Progress & Accomplishments

During the first year of the Phase II project, we have nearly completed the design of the noise control treatments for the honing machine. We are currently refining these treatment. Then we will fabricate the holders and test them in the production facility.

We are also identifying other machines with similar noise generation mechanisms and have located possible candidates for measurement and possible application of noise control technology. Among the potential candidates are friction saws (on the order of 65 inches in diameter), lathes, grinders, inertial welders, inertial presses, and disc brakes. We shall extend our initial literature search, perform measurements on untreated machines, and determine which machines may be treated. The sound pressure levels generated from candidate machines and the vibrational modes of the radiators will be measured. We will discuss previous attempts of control with the manufacturers, users, and our consultants. Then, we will select a number of machines to be treated.

Proposed noise controls will be designed and evaluated. Possible variations of the noise control mechanisms will be investigated and prototype treatments will be designed. Then, we will fabricate and test the prototype treatments on the chosen machines.

Significance

There are about 18,000 operators exposed to honing noise; this noise exposure can be reduced as a result of the research in Phase II. If the analysis techniques used in analyzing the honing machines are applied to other machine tools, additional employees will benefit from noise control -- about 500,000 employees in total.

Publications

Kuntz, H.L. and R.D. Bruce, "Generation of Squeal Tones in Vertical Honing Machines," Proceedings, 1985 International Conference on Noise Control Engineering (Inter-Noise 85), Munich, September 18-20, 1985, p 207-210.

NEW METHODS FOR QUANTITATIVE RESPIRATOR FIT TESTING

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Respirator Research
5 RO1 OH01301-03
09/29/82 - 08/31/86
\$71,790 (\$205,901 Cum)

Objectives

Quantitative respirator fit testing is required or recommended before workers may enter work environments in which hazardous air contaminants are present. A fit test is performed by measuring the concentration of a test aerosol leaking into the breathing space between the respirator and the wearer's face. At the time this grant proposal was written, di-2 ethyl-hexyl phthalate (DOP) was used as a test aerosol in the conventional fit testing method utilizing a photometer as the sensing unit. DOP, however, was at that time suspected to be a human carcinogen. New methods were, therefore, proposed to ensure safe and reliable fit testing. At the time of grant initiation, field tests by several investigators showed that the actual protection workers achieved through respirator wear was in many situations quite different from the protection predicted through conventional fit testing in the laboratory with no correlation apparent between field and laboratory testing. The P.I. postulated that the difference is due to particle-size dependent leakage through the faceseal of the respirator. It was further postulated, in the presence of fractional leakage, exposure to different aerosol size distributions would yield different protection factors not recognizable by the conventional fit testing technique or any other technique that measures in a narrow particle size range. Before any new respirator fit testing method can be recommended utilizing a specific instrumental measurement technique, basic questions on particle-size dependent leakage into the respirator cavity need to be resolved.

Methodology

Aerosol leakage into half-mask negative respirators was measured for particle sizes from 0.05 μm to 5 μm . Since this covers a span of three decades, several aerosol generation and measurement systems were developed. A fine aerosol test agent composed of a nebulized corn oil and the smoke from burning incense was used to measure small particle sizes from 0.05 μm to 0.3 μm . For the larger particle sizes from 0.1 μm to 5 μm , corn oil aerosol was used when testing with liquid aerosols, and limestone aerosol was used when testing with solid aerosols. The liquid aerosols were generated by different compressed air nebulizers, and the dry aerosols were generated through use of a fluidized bed. Humans wearing respirators were exposed in a specially designed fit testing chamber. The fine aerosol was measured by a system composed of a charging device, an electrostatic aerosol classifier (EAC) and a condensation nucleus counter (CNC). The larger aerosols were measured by an active scattering aerosol spectrometer (ASAS) and an aerodynamic particle sizer (APS). Much development work had to be performed to make the instruments compatible with each other and with aerosols generated. All experimentation was approved by the University's Committee on Human Research.

Progress & Accomplishments

Testing with humans turned out to be considerably more complex and difficult than with lifeless models. The aerosols that have leaked into a given respirator cavity are modified by deposition in the wearer's respiratory system so that the exhaled aerosol is different from the inhaled one, both being measured by the same fit testing instruments which probe the respirator cavity during inhalation and exhalation. Furthermore, the particles grow in the humid environment of the respiratory system and are exhaled at a larger size, decreased in number, and at higher temperature and humidity levels. Nevertheless, reliable and repeatable data were eventually obtained.

Our data show that there is essentially no particle size dependent leakage in the size range 0.2 μm to 1 μm . Below 0.1 μm , diffusion to the leak surfaces removes some particles and a moderate decline in relative particle concentration inside the respirator cavity is noted. Above 1 μm , the decline in relative particle concentration is more pronounced.

When holes of known size were made into well-fitting masks, the percent aerosol measured inside the mask for each particle size range decreased as the hole size became smaller. The shape of the leak hole affects the particle size distribution inside the mask. A long slit leaks less aerosols than a circular hole having the same cross-sectional area. Both types of leaks occur in actual respirator wear. For a given probe location leak holes in different locations resulted in measured concentrations that differed by a factor as high as four. For a given leak hole two different probe locations resulted in measured concentrations that also differed by a factor of four. The hole location, hole shape and the probe location did not affect equally the measured percent aerosol inside the mask for all particle sizes.

Significance

Based on our findings, conventional quantitative fit testing has become questionable in its ability to reliably predict the worker's protection to hazardous air contaminants.

Publications

Tackett DL: Quantitative Respirator Fit Testing Using Ultra-fine Particles, M.S. Thesis, University of Cincinnati, 1984.

Holton PM: Particle Size-Dependent Leakage Through the Faceseal of Negative-Pressure Half-Mask Respirators, Ph.D. Thesis, University of Cincinnati, 1986.

A FUNDAMENTAL STUDY OF RESPIRATOR AIR FILTRATION

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Respirator Research
5 R01 OH01485-03
09/28/83 - 03/31/87
\$98,842 (\$269,788 Cum)

Objectives

The principal aim of this research is to study the characteristics of particulate air filters of the type used in respiratory protection devices. Commercially available respirator air filters will be studied to determine their efficiency as a function of particle size in the size range of interest in respiratory protection, namely 0.01 to 10 μm . In addition, the resistance of the filter media and their dust holding capacities will also be measured, and the role of electrical charge in the conventional mechanical and electrostatic filters will also be determined. A further aim of this research is to make a comparative study of several filter testing methods based on modern aerosol instrumentation and to develop one or more of these methods to the point where they can be routinely used for filter evaluation.

Methodology

Two approaches have been used to measure the filter efficiency as a function of particle size. In one approach, a monodisperse aerosol is generated by means of a vibrating-orifice aerosol generator or an electrostatic classifier aerosol generator. The aerosol concentration upstream and downstream of the filter is then measured with an optical particle counter or condensation nucleus counter. In a second approach, a polydisperse aerosol is generated by an atomizer and the concentration and size distribution of the aerosol upstream and downstream of the filter measured with a single particle optical counter. From this, the efficiency vs. particle size curve is determined. The flow rate through the filter is also varied to study the effect of flow rate on filter efficiency.

The filter loading study is made by generating a concentration aerosol by atomization and loading the filter with this concentrated aerosol. Upon reaching a certain loading level, the filter with the loaded dust particles is analyzed to determine their efficiency as a function of particle size. In addition, the filter pressure drop is also measured. From this, the effect of dust loading on filter pressure drop and efficiency is determined.

The theoretical study consists of solving the Navier-Stokes equations on the computer to determine the filter pressure drop as a function several filters parameters. The filter efficiency and the influence of electrical charge on filter efficiency will also be determined numerically. The result will then be compared with the experimental data generated in this program.

Progress & Accomplishments

An experimental system for respiratory filter performance evaluation has been successfully developed during the first year and the system has been applied in the second year to the study of the efficiency and pressure drop characteristics of several commercially available respirator filters. The data are being analyzed for comparison with filtration theory. In addition, filter pressure drop calculation was initiated during the second year for the

case of rectangular fibers found in some commercial respirators using permanently charged filter fibers.

During the third year, the focus is on completing the measurement of filter efficiency and pressure drop, and the development of the filter pressure drop and efficiency models. Comparison of the theoretical and experimental results will also be undertaken during the third year. The result of the research will also be documented for publication in journals.

Due to the occurrence of a fire in the laboratory, much of the experimental apparatus was destroyed or damaged. Effort was made in rebuilding the test facility. This rebuilding work is now complete and additional data are being generated.

Significance

The end result of this research will be an improved understanding of the characteristics of respirator air filters in general and the availability of improved filter testing methods with potential industrial and practical applications. The research should benefit further respiratory protection device development and encourage the correct use of these devices for health protection in the work place.

Publications

BYH Liu: Performance of High Efficiency Respirator Air Filters. Proc International Symposium-Workshop on Particulate and Multi-Phase Processes and 16th Annual Meeting of the Fine Particle Society. p 45-1, Miami Beach, April 22-26, 1985.

B. Fardi: A Fundamental Study of Respiratory Air Filtration. Ph.D. thesis, Mechanical Engineering Department, University of Minnesota, Minneapolis, Minnesota, June, 1987.

RESPIRATOR PERFORMANCE MODEL FOR PARTICULATES

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Respirator Research
5 R01 OH01595-03
09/23/83 - 02/28/87
\$62,011 (\$164,867 Cum)

Objectives

The overall objective of this grant is to provide a better understanding of the effect of particle size on the performance of air-purifying respirators for protection against particulate exposures. This is accomplished through experimental measurement of filter and leak performance as a function of particle size and flow rate and the use of these data in a computer model to predict overall performance for a respirator with a given size and type of leak as a function of airborne particle size distribution and work rate of the wearer.

Methodology

The first phase involves measurement, in the laboratory, of the performance of respirator filters and facial seal leaks as a function of particle size using a mineral oil test aerosol and a high resolution optical particle counter. Filter measurements are made at 7 steady flow rates from 2 to 150 L/min and 14 particle sizes from 0.1 to 11.0 μm . Leakage measurements are made at these conditions for three types of leaks and four leakage rates. The second phase uses these data to develop a computer model that gives total penetration (filter penetration plus leakage) as a function of particle size for any inhalation flow rate, leak type and size. The model is integrated over the breathing cycle associated with five work rates and over log normal size distributions commonly found in industry. The result gives theoretical protection factors for any particle size distribution and for specific jobs where data on work rate, particle size distribution, and leakage rates are available. The model will be applied in conjunction with existing respiratory deposition models to give protection factors based on total and regional deposition as a function of work rate and particle size distribution.

Progress & Accomplishments

The experimental phase of this project has been completed. The model development phase is now complete. The model consists of programs, spread sheets, and regression equations that allow one to predict overall respirator performance as a function of work rate and aerosol size distribution based on QNFT measurement. We are currently evaluating applications of the model to various industrial situations and to the distribution of fit factors found in industry. A paper on the experimental chamber is in press and two, one on the experimental measurements and one on the model, have been submitted for publication. A paper on applications of the model is in preparation.

Significance

The ability of airborne particles to penetrate the filters and facial seal leaks of air-purifying respirators depends strongly on particle size as does the respiratory hazard associated with these particles. Thus, the

actual protection factor obtained for airborne particles will depend on the particle sizes present in the work place environment. The respiratory hazard depends on the efficiency of deposition at various sites in the lung and is affected by both the reduction in concentration and the change in size distribution of inhaled aerosol caused by the respirator. With a few exceptions current respiratory protection factors do not distinguish between exposures to aerosols of different particle size. This study characterizes the effect of particle size distribution on the performance of representative single-use and half mask respirators. No general data exist to describe the performance of respirators as a function of particle size or particle size distribution.

Publications

Hinds WC, Kraske G: Performance of PMS Model LAS-X Optical Particle Counter (extended abstract) in Aerosols: Science, Technology and Industrial Application of Airborne Particles. Liu, Pui, Fissan, Eds, p 31-34, Elsevier, New York. Presented at First International Aerosol Conference Sept. 17-21, 1984, Minneapolis, MN.

Hinds WC, Kraske G: Performance of PMS Model LAS-X Optical Particle Counter in J of Aerosol Science, 17(1) 1986.

Hinds WC, Kraske G: A Bench-Scale Aerosol Test Chamber, App. Ind. Hyg. (2) 1987, in press

IN-MASK MONITORS AND RESPIRATOR BREAKTHROUGH

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Respirator Research
5 R01 OH01603-03
09/23/83 - 11/30/86
\$131,948 (\$405,539 Cum)

Objectives

The objective of this program is to demonstrate that small, chemically sensitive colorimetric films being developed by Moleculon can be combined with miniature electro-optic sensing technology to provide a compact, convenient and reliable method for monitoring in-mask levels of hazardous gases and vapors in air purifying respirators. Such monitoring can both alert the user to early signs of respirator failure, and provide increased safety for the use of cartridge filters close to, but not beyond, their end of life.

Methodology

Initial research has been devoted to better quantifying the response of formaldehyde-sensitive films, with much emphasis on the thin-film chemistry involved, and the sensitivity and stability obtained. Further work has examined the applicability of the same concept to another hazardous gas, hydrogen sulfide. Currently under investigation is the actual use of the formaldehyde sensing system to detect and monitor end of cartridge life for formaldehyde removing cartridges, with emphasis on the experimental test apparatus involved and accurate supporting measurements of low formaldehyde concentrations.

The chemistry underlying the formaldehyde sensing system is a highly modified, nonaqueous Schiff reaction scheme free from hazardous components. The hydrogen sulfide sensor relies on the reaction of certain bismuth salts with hydrogen sulfide in organic solution, bismuth being unusually nontoxic among the metal ions colorimetrically sensitive to sulfide vapor. Both the sulfide and formaldehyde systems are continuously monitored by a tiny dual-beam spectrometer and its associated electronics.

Progress & Accomplishments

Both the formaldehyde and sulfide systems appear to have the sensitivity required for adequate in-mask and cartridge end of life use. The stability and sensitivity of the formaldehyde sensing film have shown fluctuations requiring extensive chemical and coating research. Tests now under way with real respirators are expected to demonstrate the merit and high utility of this approach.

Significance

There are many industrial settings where the use of respirators is the only immediate option for respiratory protection, yet too little is known about the service life of air purifying cartridges toward particular challenges. This leads to uncertainty in the level of protection provided by current masks, requires the uneconomical disposal of cartridges which may

retain more than adequate protective capacity, and limits the certification of protective masks for broader use. The kind of inexpensive, in-mask monitoring under investigation here is intended to address all of these important concerns, and will in time greatly increase our understanding of the factors determining cartridge reliability.

Publications - None to date

COMFORT AND ACCEPTABILITY OF RESPIRATORY PROTECTION DEVICES

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Respirator Research
5 R01 OH01608-03
09/23/83 - 08/31/86
\$57,754 (\$152,190 Cum)

Objectives

The long-term objective is the development of criteria for the design and construction of breathing devices, such as masks and hoods that protect the wearer against occupational and environmental pollutant hazards, to minimize the unpleasantness and discomfort of such masks to make them acceptable to the wearer. The specific aim of the immediate research is to determine the human response to temperature and humidity inside face masks during rest and moderate work in order to quantify the limits of acceptable mask conditions for the wearer.

Methodology

The responses (subjective and physiological) of six subjects wearing ventilated (24 liters/min) face masks during rest and work of 15 min duration in still air were observed over a wide thermal range of ambient and mask conditions. For rest (1 met), the ambient conditions were 25, 30 and 35°C at a 10°C dew point with mask conditions of 27, 30, 33, and 36°C at medium and high water vapor levels (55 and 90% RH). For work (3 met on cycle ergometer), the ambient was 16 and 21°C and mask conditions were 21, 27 and 33°C with water vapor levels of 61 and 86% RH. The subjects wore trousers and a long-sleeved shirt (0.6 clo). For both of these conditions (rest and exercise) an additional set of experiments was run to quantify the responses to a 3% CO₂ level in the mask. The thermal conditions were limited to a 25°C ambient for rest with mask air temperatures of 27, 30, 33 and 36°C and 16°C ambient for 3 mets of exercise with mask air temperatures of 27, 30 and 33°C. Skin temperatures, heart rate and skin wettedness levels were measured continuously. The subjects' judgements of acceptability, thermal sensation, degree of discomfort, sense of skin moisture, and the difficulty of breathing were gathered 14 and 1 min before removing the mask and 5 minutes after.

Progress & Accomplishments

Temperature and comfort sensations for the whole body were affected substantially by the temperature and humidity conditions of air in the mask. A cool mask made the warm body feel cooler, whereas a warm mask made the cool body feel warmer. The thermal sensation experienced under the mask increased linearly with the measured upper lip skin temperature. This skin temperature was independent of whole body ambient conditions but directly related to the air temperature in the mask. The subjects' judgement of the surrounding environment being thermally acceptable for the whole body was strongly influenced by ambient conditions. Hot environments were less acceptable than cooler ones; however increasing the air temperature and/or humidity in the mask decreased whole body acceptability further. In the mask itself, acceptability decreased with increasing mask air temperature and humidity. Mask temperatures cooler than 33 and 27°C for 1 and mets respectively were 100% acceptable. Mask temperatures greater than these were less acceptable.

The loss of acceptability was greater for humid mask conditions. Mask acceptability decreased sharply when thermal sensations under the mask were warmer than neutral.

The subjects perceived breathing to be more difficult with increasing mask temperature and humidity. This perception at rest conditions with a humid mask was significantly higher (more difficult) than for the drier mask conditions at temperatures above 30°C. Breathing difficulty was found to be linearly related to the energy content of the inspired air (as described by the thermodynamic property of enthalpy) above enthalpy thresholds of 88 and 70 kJ/kg for the 1 and 3 met activity levels. Increasing the CO₂ content of the mask supply air from 0.03 to 3% had no affect on thermal physiological or subjective responses or on acceptability. CO₂ affected respiration and its perceived difficulty during exercise but had no affect during rest.

Significance

The significance of this research is that if protective breathing devices can be made that minimize the discomfort or unpleasantness to the wearer, they will be worn more often or continuously and thereby improve the protection of exposed individuals. This research seeks to define the design criteria for such comfortable breathing protection.

Publications - Abstract(s) available

Gwosdow AR, Nielsen R, Berglund LG, DuBois AB: Comfort and Acceptability of Respiratory Protection Devices as Affected by Temperature and Humidity. American Industrial Hygiene Association Journal, May 1986.

RESPIRATORS: PHYSICAL AND PHYSIOLOGICAL CONSIDERATIONS

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Respirator Research
5 R01 OH01617-02
11/01/83 - 10/31/85
\$94,583 (\$182,183 Cum)

Objectives

A. To provide descriptive - psychophysiological data of the physical stress and physiological strain imposed while wearing a pressure-demand, air-supplied, full-facepiece respirator during maximal and submaximal work to exhaustion.

B. This descriptive data will be used to provide criterion guidelines for selection of worker capability for wearing a respirator.

C. The physiologically descriptive data will be used to verify a computer simulation program on the work of breathing and to propose design modifications of currently produced industrial respirators.

Methodology

Standard maximal ramp and submaximal treadmill protocols were used to provide physical stress to 38 subjects both with and without pressure-demand respirator wear. Each subject required six days of testing. One day included a complete pulmonary function assessment while the other five days were for treadmill testing during which breath-by-breath assessment of cardiopulmonary variables were made. In addition psychophysical scales of perceived exertion and breathing were used during the treadmill tests. Finally the subjects were required to provide a psychological profile of anxiety, which was used "a priori" to predict the presence or absence of psychological distress.

Progress & Accomplishments

A complete set of data was collected on 38 subjects resulting in a data base containing 560 lines of data for each individual subject. As these subjects varied in age, fitness levels and anthropometric parameters our major aim of providing descriptive psychophysiological data relevant to the working population has been achieved. Subsequent multiple regression analysis will enable us to provide guidelines for selection of worker capability for wearing a respirator. In addition design modification have been suggested and computer simulation of the work of breathing has been accomplished.

Significance

This data base will form the basis of our recommendations for pre-screening workers for respirator wear. In addition, it is apparent from the data that significant changes in current respirator performance characteristics will be required to meet the needs of 95% of the working population. Finally the successful use of computer simulation modelling provides the possibility that certification of specific respirators can be accomplished by simulation alone.

Publications - Abstract(s) available

Stojack WB, Raven PB: Prediction of distress for individuals wearing industrial respirators. Amer Ind Hyg Assoc J 13:263-272, 1985.

Wilson JR, Morgan WP, Raven PB: Psychophysiological screening for Respirator Wear. J Int Soc Respir Prot, 4:34-61, 1986.

Raven PB: Clinical pulmonary function and the physiological effects of industrial respirators. J Int Soc Respir Protection 1:1-18, 1983.

Jackson AW, Raven PB: Statistical and research design considerations concerning industrial respirator research. J Int Soc Respir Protection 1:31-40, 1983.

Raven PB: Respirator research, where it needs to go: The man-respirator interface. J Int Soc Respir Protection 1:49-66, 1983.

Stojack WB, Raven PB: Development of a quantitative fit testing program for the Clark County Fire Development. J Int Soc Respir Prot, 2:288-295, 1984.

OPTIMAL DESIGN OF RESPIRATOR CARTRIDGES

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Respirator Research
5 R01 OH01644-03
04/01/84 - 03/31/87
\$76,656 (\$211,038 Cum)

Objectives

Respirator cartridge performance is examined through the fundamental theories of mass transport, as used by chemical engineers and physical chemists, to determine optimal design parameters for respirator cartridges.

Methodology

An experimental system consisting of a vapor generator, dilution air stream, charcoal beds, and vapor detector allows the performance of respirator cartridges to be monitored continuously. Mathematical modeling of the observed performance of respirator cartridges as a function of time permits the geometry of the respirator cartridges to be systematically modified to give optimum performance.

Progress & Accomplishments

We developed, using a microcomputer, a rapid method of determining the basic factors, (i.e., the adsorption coefficient and mass transfer efficiency) which control the performance of a respirator cartridge. This computer program was then used in analyzing the performance of respirator cartridges exposed for extended periods of time to high concentrations of contaminant, in a rapidly flowing stream of air, at humidities as high as 100%.

In the process of analyzing these data, we developed the first satisfactory theory for the performance of a respirator cartridge at extremely high relative humidities. This equation is as follows: $W = W_0 \exp(kE^2)$, where W =gm uptake/gm adsorbent, p =density of liquid solvent, k =a structural constant for the adsorbent, W_0 =pore volume of adsorbent, and E =potential energy for adsorption. The potential energy for adsorption from a humid atmosphere is $E/RT = (1+k_1) \ln(P/P_s) + k_2 \ln(RH/100)$, where P is the vapor pressure of the solvent, P_s is the vapor pressure of the liquid solvent, T is to absolute temperature, R the ideal gas constant, and RH is the relative humidity, and k_1 and k_2 are constants describing the effect of the ambient humidity on the adsorption of the vapors of the solvent. By the above equation, the adsorption coefficient can now be determined at any temperature, vapor concentration, and relative humidity. A very important result of the above theory is that when the $RH = 100\%$, the term containing k_2 vanishes; the resultant equation under these conditions gives a simple correlation between the adsorption coefficient from dry air and from air totally saturated with water vapor. This equation fits experimental data for the adsorption of trichloroethylene at relative humidities ranging from 5 to 85% and concentrations ranging from 300 to 1300 ppm, to within 2% error.

An important prediction from the above equation is that even in an atmosphere saturated with water vapor, a respirator cartridge may have a useful residual efficiency. This was proved experimentally using vapors of xylene, methylethyl ketone, and ethyl acetate. For all three vapors, protection for 8 hours could be shown using a standard size respirator cartridge in a air flow of 20 lpm of air completely saturated with water vapor. The key factor learned from this work is that instead of writing off the use of respirator cartridges at high relative humidities, we should determine the circumstances under which they will be effective. Protection against benzene under the same conditions was studied detail. With the standard respirator cartridge, because of the very poor mass transfer of benzene vapors (number of theoretical plates remaining below ten), protection for 8 hours could not be demonstrated. However useful protection for 4 hours could readily be obtained. Next it was found that a change in the geometry of the respirator cartridge permits a respirator cartridge containing 40 grams of adsorbent to protect against benzene in air at 100% RH for more than 8 hours. This demonstrates the importance of understanding the kinetic factors influencing mass transfer.

Significance

By modeling and designing respirator cartridges to operate under adverse conditions, we increase our confidence in their safe use in more normal circumstances. Particularly important is the equation giving the adverse effects of a high relative humidity. As the experiments with benzene showed, ignoring relative humidity may cause unacceptably high exposure to occur without any warning.

Publications

Underhill DW: Pulse residence in short chromatographic columns. Anal Chem 57:826-9, 1985.

Underhill DW, Forbes SL: Modeling adsorption bed behavior using a microcomputer. J Air Pollut Control Assn 36:61-4, 1986.

A MODEL AND TESTS FOR PREDICTION OF RESPIRATOR ADSORBENT BEHAVIOR

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Respirator Research
5 R01 OH01646-03
09/23/83 - 02/28/87
\$119,866 (\$329,300 Cum)

Objectives

The Wheeler-Dubin model has been proposed for use in the workplace to predict breakthrough times of activated carbon-containing respiratory cartridges. The Wheeler-Dubin Model has been shown to be an accurate predictor of adsorptive behavior (within $\pm 15\%$) for small carbon masses (<10 g) with a single adsorbable vapor, low volumetric flow rates (<0.05 L/s) and high inlet concentrations ($<10,000$ ppm). The purpose of these investigations is to extend the Wheeler-Dubin Model to realistic conditions of use, i.e. multiple adsorbable vapors (binary vapor mixtures with water as the second vapor), large adsorbent masses (30-40 g), high flow rates (1-0.3 L/s), etc., so that accurate predictions of respirator adsorbent cartridge behavior can be made. Three phases have been planned for these investigations; Phase I involved the development of the large bed system, verification of the model and preliminary investigations with binary vapor mixtures; Phase II included investigations of the stability and reliability of the large bed system, and evaluation of commercial cartridge packings reproducibility and the degree of carbon activation. Phase III investigations include the extension of the binary vapor investigations with the inclusion of additional vapors (other than carbon tetrachloride) and the effects of reference vapor polarity on predictions.

Methodology

Carbon adsorbents are first characterized using a reference vapor such as carbon tetrachloride. Once the reference adsorbent has been characterized, it can be used in the application of the Wheeler Kinetic breakthrough model. The kinetic data are developed by passing vapor laden-air through an adsorbent bed. The effluent is monitored until a chosen exit-to-inlet ratio (<0.04) is achieved. These data are then plotted as the natural log of the exit-to-inlet ratio versus time. From this curve, the rate constant and the kinetic capacity are determined. These data are then used to predict the behavior of other vapors. The effect of water vapor will be determined in two modes, one in which water is pre-equilibrated with the adsorbent and a second which utilizes dry carbon. These adsorbents are then challenged with vapor-laden (a binary mixture of the test adsorbate and water vapor at the test concentration) air. Again, the effluent is monitored until a chosen exit-to-inlet ratio (<0.04) is achieved. These data are then plotted as the natural log of the exit-to-inlet ratio versus time. From this line the rate constant and the kinetic capacity are determined. The data can then be used to predict the behavior of other vapors in the presence of water vapor.

Progress & Accomplishments

Major progress has been made in completion of the first two phases of the proposed work. Initial investigations of the third phase are in progress.

In Phase I, the high flow/large adsorbent mass gas/vapor system for challenging adsorbent was constructed and tested. Initial data were developed on the generation of mixtures of vapors. Preliminary data on the effects of water vapor on adsorption of the standard reference vapor, carbon tetrachloride, have been developed. These data indicate that water vapor at low concentrations (RH's below 50%, 23⁰ C) can adversely effect the predictions of the model.

In Phase II of the investigation, the stability and reproducibility of the large bed system were established. An additional investigation was completed which focused on the reproducibility of respirator adsorbent packing-volumes and a comparison of the extent of activation of the adsorbents. This demonstrated that commercial activated carbon-containing respirator cartridges from three of the major suppliers are associated with significant differences in the degree of adsorbent activation and in the amount (as determined by the mass of adsorbent) of carbon in the cartridge.

The influence of adsorbate polarity on reference vapor selection was investigated. Polarity may be important for reference vapor selection due to the influence of electrostatic forces in the adsorption of polar vapors. Adsorbate polarity was investigated by determining the adsorption isotherms and characteristic curves for the three isomers of dichlorobenzene. In terms of molecular weight, vapor pressure, density, and electronic polarization, the three isomers are very similar; the major difference being the range of dipole moments; 1,2-dichlorobenzene, 2.50 D; 1,3-dichlorobenzene, 1.72 D; and 1,4-dichlorobenzene, 0.00 D. If adsorbate polarity influenced adsorption, the three curves should have different slopes. The slopes of the three curves were not statistically different at the 95% level. It was concluded, therefore, that electrostatic forces do not influence vapor adsorption when adsorbate polarities are between 0 and 2.50 D, and a single reference vapor can be selected for breakthrough prediction.

The Dubinin/Wheeler Model was modified to account for increased axial dispersion in large carbon beds. Increased axial dispersion resulted in a decrease in the ratio of the kinetic saturation capacity at 1% to complete breakthrough. Calculation of breakthrough time for small beds assumed this ratio was equal to 1.0. This assumption was found to be invalid for large beds. The modified Dubinin/Wheeler Model was used to predict 1% breakthrough times for activated carbon beds similar in size to organic vapor air-purifying respirator cartridges at environmental conditions consistent with respirator use. Breakthrough times were found to deviate from experimentally determined values by as much as 38%; with the average deviation equal to 16.5.

Significance

Currently, there is no reliable method for predicting the adsorptive behavior of respirator adsorbent cartridges in use. Although a number of approaches to this problem have been attempted, none have been demonstrated to be widely successful. The Wheeler-Dubinin model has been demonstrated to be an accurate predictor of adsorbent bed behavior. The continued development of this model will permit its general application in the field, thus filling this void.

Publications - Abstract(s) available

Trout D, Breysee PN, Hall TA, Corn M, Risby T: Determination of Organic Vapor Cartridge Variability in Terms of Degree of Activation of the Carbon and Cartridge Packing Density. Am Ind Hyg Assoc J 47:491-496, 1986.

ASSESSMENT OF RESPIRATORS AMONG AGRICULTURAL WORKERS

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Respirator Research
5 R01 OH01650-03
09/23/83 - 08/31/86
\$86,645 (\$254,103 Cum)

Summary

The agricultural sector is both a large (nearly 12 million) and understudied occupational environment. Exposure to complex mixtures of organic dusts and chemicals are responsible for a number of respiratory diseases among agricultural workers - including silo filler's disease, farmer's lung, occupational asthma, and most commonly bronchitis and obstructive airways disease. High rates of respiratory symptoms and airways obstruction have been documented among animal confinement workers and grain handlers. These exposures are often difficult to control, for both technical and economic reasons. Therefore, the use of a respirator in these working environments has become a common request from those adversely affected. Yet, there has been no systematic study of respirator efficacy with exposure to these organic dust and gas aerosols.

Three generic respiratory types will be tested - the single use half mask, the reusable half mask, and the powered air-purifying respirator. Four exposure groups will be tested - 50 swine confinement housing workers, 50 poultry confinement housing or processing workers, 50 grain handlers, and later 12 active farmers with farmer's lung using only the most efficient respirator. Respirator protection factors associated changes in lung function and respiratory symptoms, respirator acceptance, the association between respirator efficacy and acceptance and physical fitness, and the possible effect of smoking will all be evaluated. Ambient and in mask sampling for total dust, endotoxin, and ammonia will provide environmental indicators. Change in respiratory function over an exposure period, change in respiratory and eye symptoms and the worker's response to a questionnaire on various aspects of respiratory acceptance will be the medical indicators. Results of this study should provide the basis for development of a "model" respiratory program for these and other organic dust exposures.

Publications - None to date

RESPIRATOR TOLERANCE

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Respirator Research
5 R01 OH02005-03
08/01/84 - 07/31/87
\$81,067 (\$232,928 Cum)

Objectives

1. Identify determinants of tolerance to respirator use.
2. Characterize pattern of adaptation to respirator use.
3. Develop a measure of psychophysical sensitivity to respirator loads and assess effect of such sensitivity on respirator tolerance
4. Estimate inaccuracies induced by use of a mouthpiece in respirator research studies.
5. Compare laboratory to worksite measures of respirator effect.
6. Assess population variability of pattern of adaptation and respirator type load sensitivity.

Methodology

The study has three components: detailed exercise laboratory studies, field studies of respirator use, and limited studies of a large number of subjects to assess population distributions of load sensitivity and respiratory pattern adaptation.

Added work of breathing, respiratory pattern, respiratory volumes, oxygen consumption/CO₂ elimination, and heart rate are measured in the exercise laboratory studies. A respiratory inductive plethysmograph (RIP) is used for measurements when mouthpiece cannot be used (eg., field studies and studies of mouthpiece artefact). Psychophysical sensitivity is assessed by the technique of magnitude estimation based upon application of Stevens's Psychophysical Law. Field studies are performed using ambulatory recording techniques on which analog data are recorded on a small FM tape recorder which the subject wears; data are played back and digitized subsequently.

Progress & Accomplishments

The effects of inspiratory resistance, expiratory resistance, dead space, and exercise have been characterized alone and in combination. Loading of a phase of respiration leads to its prolongation. Peak ventilatory work rates and pressures (forces) are minimized by adaptation of the respiratory pattern. Resistances have relatively greater physiologic impact than do the dead space loads. Mouthpiece use does not appear to introduce significant artefact, particularly at exercise.

Psychophysical load sensitivity appears to be an important determinant of both the respiratory pattern of adaptation and of the subjective response to breathing with a respirator type surrogate load.

Two-thirds of the large population sample has been studied. The field studies have been initiated.

Significance

The work has characterized the normal pattern of adaptation to respirator use. This may be useful for establishing medical surveillance programs, for setting standards for respirator certification (e.g., by NIOSH), and for respirator design. The role of interpersonal differences in respiratory pattern adaptation and demonstrated relationship between psychophysical load sensitivity and both subjective and objective effects of respirators suggests that personal characteristics of the user may be important determinants of how well a respirator will be tolerated by an individual worker. Standard tests (e.g., spirometry) may not adequately reflect a worker's ability to use a respirator, and the anecdotal reports of poor tolerance by some apparently normal workers may be explained on this basis.

Publications - None to date

ASBESTOS FIBER COLLECTION BY NIOSH-APPROVED RESPIRATORS

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Respirator Research
1 R01 OH02154-01
01/01/86 - 12/31/87
\$85,453 (\$85,453 Cum)

Objectives

The goal of this research is to use an asbestos aerosol to investigate the performance of respirators certified for use with asbestos. Since asbestos is a very hazardous material and since such respirators are widely used to protect workers from asbestos exposure it is very important that certified respirators perform as expected. Unfortunately, the present NIOSH certification procedure does not require that respirators be tested against asbestos. It is the goal of this research to investigate the significance of this and other possible deficiencies in the certification procedure.

Methodology

A representative sample of certified air-purifying respirators will be tested. The performance of these respirators against asbestos will be compared to that for silica, the standard dust used in the NIOSH certification procedure. Respirator penetration will be measured under steady flow conditions, as is done in the certification test, and under cyclic flow, to simulate actual use conditions. Replicate tests will be performed for each combination of test aerosol and flow pattern. The measurement will be analyzed by standard statistical techniques, including analysis of variance and linear regression, to determine the effects on performance of aerosol type, flow pattern, and respirator model. In addition, aerosol size distributions will be determined by scanning electron microscope for asbestos, in order to determine aerodynamic diameter. This will allow for some modeling of the collection efficiency of the respirator filters for two types of aerosols—fibrous and non-fibrous.

Progress & Accomplishments

Preliminary experiments were performed to determine size penetration characteristics for ten different electrostatically-charged respirator filters, using latex spheres ranging in size from 0.1 to 2.0 micrometer. Three replications were made of each filter. Predictions developed using these data show that silica penetration through these ten filters will be between 2 to 3 percent. These data will allow us to choose 4-5 representative respirators for silica and asbestos tests to follow.

Significance

The results of the silica and asbestos tests at two different flow conditions will demonstrate the following:

- (1) Are silica and asbestos collected similarly by the respirators tested?
- (2) Is it possible to predict asbestos penetration from silica penetration results?
- (3) Are penetration results under steady flow similar to those at cyclic flow?

Publications - None to date

AN ADVANCED RESPIRATORY PROTECTIVE DEVICE

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Respirator Research
1 R43 OH02312-01
12/16/85 - 12/31/87
\$48,629 (\$48,629 Cum)

Summary

Masks and filters are worn over the head or face in order to protect individuals from exposure to toxic chemical vapors. In these devices, there is no convenient or effective way to tell whether the adsorbent in the mask is fresh or used. A sensor that could fit inside the protective sorbent bed and tell the remaining useful lifetime of the filter would be ideal.

In this work, a micropower microsensor is designed and fabricated. This sensor can fit inside a carbon adsorbent bed and tell whether the filter is operating properly or whether it is used and hazardous vapors are penetrating the protective device.

The sensor is designed into a cannister filter and the feasibility of an advanced respiratory protective device that can alert the user when it is no longer effective is assessed.

Publications - None to date

HUMAN METABOLISM OF HALOTHANE MECHANISMS OF TOXICITY

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Other Occupational Concerns
5 R01 OH00978-06
09/01/80 - 11/30/86
\$58,318 (\$1,037,346 Cum)

Objectives

Our major goal is to establish the molecular mechanism by which metabolism of the halogenated inhalation anesthetic, halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), causes hepatotoxicity in patients and operating room personnel. In that halothane causes both acute intracellular toxicity as well as delayed immunologically-mediated hepatotoxicity, understanding the mechanism of its toxicity would provide insights into the toxicity and ensuing mutagenicity/carcinogenicity of other halogenated hydrocarbons found in the work place and the environment. Our approach is four-fold: 1. Establish the pathway by which metabolism of halothane under hypoxic conditions produces free radical metabolites that lead to peroxidation of cell membrane phospholipids and how some of these peroxidized phospholipids, once released into the cell, may be converted to leukotriene B-4 (LTB₄), a potent mediator of inflammation and activator of macrophages. 2. Investigate the role of free radical halothane metabolites or phospholipid oxidation products (such as leukotrienes) in activating hepatic lysosomes or in activation of external macrophages to attach to and damage the hepatocyte producing the signal. 3. Determine whether the detachment of ribosomes from the endoplasmic reticulum that occurs following exposure to halothane (and most other halogenated hydrocarbons) is caused directly (by modification by a reactive metabolite) or indirectly (due lipid peroxidation). Measure whether there is increased degradation or decreased expression of cytosolic mRNA, which would result in a decrease in bound ribosomes, and whether there is modification of the protein anchor sites that bind ribosomes to the endoplasmic reticulum. 4. Determine whether reactive metabolites of halothane react with cellular components and are rendered immunogenic once exposed on the outer surface of the plasma membrane of the hepatocyte. Measure possible binding and cytotoxic activation of neutrophils and Kupffer cells toward hepatocytes that have metabolized halothane.

Methodology

The four goals described above are discussed in order: 1. We purify the major enzymes involved in halothane metabolism, human cytochrome P-450 and P-450 reductase, from livers of human heart transplantation donors and reconstitute these enzymes into phospholipid vesicles. In this system we isolate phospholipid hydroperoxides, which are precursors of leukotrienes, and analyze them by direct chemical ionization mass spectrometry. We study metabolism of halothane in monolayer cultures of rat liver hepatocytes in oxygen concentrations as low as 0.5% oxygen. 2. Resident liver macrophages, Kupffer cells, and human neutrophils are isolated and added to hepatocyte monolayers that have been exposed to halothane at varying oxygen concentrations. 3. Levels of mRNA following exposure to halothane are determined by isolating poly-adenylated RNA and translating it in an in vitro system. 4. Antibodies, raised against halothane metabolites in rabbits, are added to monolayers of hepatocytes that have been exposed to halothane and then cytotoxicity is measured.

Progress & Accomplishments

The four goals described above are discussed in order: 1. We have shown that the formation of LTB_4 from leukotriene A_4 in human liver microsomes is cytochrome P-450 dependent (1). Furthermore, we have shown that the metabolism of LTB_4 by rat hepatocytes is reduced severely at low oxygen concentrations (2). We mimicked the effects of halocarbon metabolism in rat hepatocyte monolayers by addition of t-butylhydroperoxide or by raising the intracellular concentration of calcium with a calcium ionophore, and demonstrated that hypoxia dramatically increases the toxicity of hydroperoxides (3) and calcium ionophores (4). 2. We have done preliminary experiments on the cytotoxicity of neutrophils and Kupffer cells toward hepatocytes pre-coated with LTB_4 . We have shown that activation of human blood neutrophils with the tumor promoter phorbol myristoyl acetate (PMA) causes dramatic cytotoxicity to hepatocytes. We have shown that injection of leukotriene D_4 in the right ventricle of the dog produces symptoms like adult respiratory distress syndrome (macrophARDS) (5). 3. We have established a technique for isolating mRNA from hepatocytes to determine if a reduction in mRNA is due to increased destruction or decreased expression. 4. We have raised antibodies in rabbits to trifluoroacetylated rabbit serum albumin (trifluoroacetate is the main metabolite of halothane) and have established that the antibodies are specific for the trifluoroacetylated adduct.

Significance

Our results demonstrate the importance of lipid peroxidation and hypoxia in the development of acute hepatic damage following exposure to halothane. Our studies with leukotrienes, neutrophils, and Kupffer cells confirm that the immune system is involved in liver damage. Formation of antibodies to products of drug metabolism, especially those exposed on the outer surface of the hepatocyte, could explain chronic hepatotoxicity. The continued presence of activated macrophages in tissues is known to be carcinogenic and mutagenic.

Publications

Gut J, Costa AK, Trudel JR: Oxygen concentration - dependent metabolism of leukotriene B_4 by hepatocyte monolayers. *Biochim. Biophys. Acta* 878: 194-199, (1986)

Costa AK, Heffel DF, Schieble TM, Trudell JR: Toxicity of t-butyl hydroperoxide in hepatocyte monolayers exposed to hypoxia and reoxygenation. *In Vitro*, in press (1986)

Costa AK, Schieble TM, Heffel DF, Trudell JR: Toxicity of calcium ionophore A23187 in monolayers of hypoxic hepatocytes. *Toxicol. and Appl. Pharmacol.*, in press (1986)

Shapiro JM, Mihm FG, Trudel JR, Stevens JH, Feeley TW: Leukotriene D_4 increases extravascular lung water in the dog. *Circulatory Shock*, in press (1986)

LEAD AND CADMIUM TOXICITY RELATED TO INDUSTRIAL EXPOSURE

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Other Occupational Concerns
5 RO1 OH01522-02
04/01/84 - 03/31/86
\$80,999 (\$148,757 Cum)

Objectives

Industrial exposure to cadmium and lead is a widespread and serious occupational problem. This proposal is directed toward evaluating the effects of these two metals, in single and combined exposure, in the human subject, and in an animal model, and with in vitro techniques. It is proposed that those workers at risk because of occupational contact with these two heavy metals be examined to determine if there are abnormalities of renal function that will serve as hallmarks of early toxic effects, prior to the development of established (and potentially irreversible) disease.

Methodology

The classical clearance technique has been utilized in both the human subject and the experimental animal. The evaluation of tubular transport function has also been assessed by employing the brush border membrane vesicle (BBMV) technique utilizing rat kidney tissue.

Progress & Accomplishments

A group of workers from a chemical plant located in Cleveland, Ohio were studied. In addition to the determination of the usual parameters of renal function, the subjects were investigated utilizing two new specialized techniques. The body burden of lead was evaluated with a standard three-day test in which lead excretion in the urine is promoted by infusion of the chelating agent, EDTA. These observations were compared to data obtained, in collaboration with investigators at the Brookhaven National Laboratory, utilizing an x-ray fluorescence technique to estimate tibial lead content. In addition, lead and cadmium content were determined employing a neutron activation technique. These findings were compared to blood and urine cadmium levels. The results are as follows: 1) There is a direct linear correlation between tibial lead content and lead body burden as measured by the determination of urinary lead excretion following EDTA chelation. 2) There were few indications of overall renal functional abnormalities in this group of workers.

In animals given 2% lead in their drinking water, the clearance technique and BBMV transport were utilized to evaluate the effects of the lead ingestion. While no animals have shown evidence of a phosphaturia or of alterations in glomerular filtration rate, BBMV studies did indicate a defect in transport at the proximal tubular level.

Significance

With regard to the human experiments, these studies have verified that a new non-invasive technique can supplant the older methodology, involving the intravenous infusion of a potentially toxic chemical, in the assessment of whole body lead burden.

The animal studies point up the fact that early transport abnormalities due to lead may occur, which do not necessarily become evident as disturbances of glomerular filtration rate or abnormalities of overall excretion rate. However, carefully performed clearance studies, based upon information gleaned from the rat studies, could be designed, which would pick up these early defects in kidney function due to lead toxicity.

Publications - None to date

EFFECT OF UNUSUAL WORKSHIFT ON DICHLOROMETHANE TOXICITY

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Other Occupational Concerns
5 R01 OH01531-02
12/01/83 - 11/30/85
\$57,753 (\$113,287 Cum)

Objectives

The major objective of this proposal was to determine if workers exposed to chemicals during an unusual workshift, 12 hours per day instead of the normal 8 hours per day, may be at greater risk than those on the usual workshift. Two major aims were investigated. One was the development of a rodent model to mimic man. The second was whether dichloromethane demonstrates a greater toxicity when administered by inhalation during the unusual workshift using carboxyhemoglobin measurements as the endpoint for toxicity. Also the influence of forced exercise on biotransformation of dichloromethane to carbon monoxide was determined. Additional aims added during the course of the investigation were aniline as a test compound with methemoglobinemia as an endpoint and trichloroethylene with indicators of hepatotoxicity being measurements of serum enzymes and microsomal mixed function oxidases.

Methodology

Rats were exposed for 8 hours/day or 12 hours/day to dichloromethane by inhalation. Carboxyhemoglobin measurements were made after single and repeated exposures. In some cases, pyrazole was used to prolong the half-life of dichloromethane by inhibiting its metabolism and in other cases, the animals were subjected to forced exercise (rotating cage). For aniline, similar methodology was used. Rats were exposed to varying concentrations of aniline and methemoglobin levels measured at appropriate time points. Aniline in fat and blood samples was analyzed by gas chromatography after repeated exposure. With trichloroethylene, serum sorbitol dehydrogenase, ethylmorphine demethylation and glucose-6-phosphatase activity were determined. Chamber concentrations were monitored continuously using an infrared gas analyzer.

Progress & Accomplishments

To compare the effects of a 12-hour exposure schedule to those of an 8-hour schedule on carboxyhemoglobin (COHb) formation resulting from dichloromethane (DCM) inhalation rats were exposed to 200, 500, or 1000 ppm DCM for 8 hr/day for 5 days or 12 hr/day for 4 days. The unusual exposure schedule had no effect on COHb levels. The metabolic pathway for the formation of CO appeared to be saturated even at the lowest concentration of DCM. To examine the possible increase in the retention of inhaled DCM in the longer exposure schedule, single exposures for 8 and 12 hr were compared. The peak DCM blood level was dependent upon the DCM exposure concentration, but the half-life was independent of the duration of exposure and the concentration of DCM. The half-life of COHb was prolonged by increasing the DCM concentration, but was not affected by the exposure period. Pyrazole decreased the COHb level and increased the blood DCM level in both groups of rats exposed to DCM.

When rats were placed in a cage which rotated at 7 rpm during the last 15 minutes of every hour of exposure, there was no difference immediately following exposure to 500 ppm dichloromethane or at one or two hours post-exposure between the COHb levels or blood levels of DCM in these animals and those similarly exposed but without exercise. At 1000 ppm and a rotation rate of 11 rpm, there was a slight decrease in the COHb and DCM levels in the exercising animals immediately after exposure and at one hour post-exposure.

Experiments examined the effects of altering the exposure schedule of rats to various concentrations of aniline from 8 hr/day for 5 days to 12 hr/day for 4 days. When compared to the first day, methemoglobin (MetHb) prior to and following exposure increased with the days of exposure at 50 ppm aniline. At 150 ppm aniline, the pre-exposure MetHb level increased with the days of exposure whereas the post-exposure MetHb plateaued after the second exposure. The pre-exposure MetHb levels were significantly different between the two groups at 50 or 150 ppm aniline whereas the post-exposure MetHb levels were not. Following a single exposure for 8 or 12 hr to 100 ppm aniline there was no difference in the maximum MetHb level, the peak aniline level in blood and fat, or the rate of aniline elimination from fat and blood. Repeated exposures to 100 ppm for 3 days did not produce a significant difference in aniline level in blood or fat between the 8 and 12 hr/day exposure groups immediately following the last exposure. However, the aniline level in blood was significantly higher in the 12 hr/day exposure group when determined on the next morning.

When groups of rats were exposed to 500 ppm trichloroethylene, ethylmorphine demethylation was increased in the group exposed for 12 hours for one day but not for 8 hours/day. After one week of exposure hepatotoxicity was greater in the 12 hour/day group than in the 8 hour/day group as determined by measurements of microsomal glucose-6-phosphatase activity.

Significance

The studies demonstrate that for chemicals with short half-lives, such as DCM, workers would not be expected to be at greater risk to their toxicity if encountered for 12 hours per day rather than the usual 8 hours per day. However, for compounds with long half-lives, such as aniline and trichloroethylene, alterations in permissible exposure limits may need to be considered.

Publications

Kim YC, Carlson GP: The effect of an unusual workshift on chemical toxicity. I. Studies on the exposure of rats and mice to dichloromethane. *Fundam. Appl. Toxicol.* 6:162-171, 1986.

Kim YC, Carlson GP: The effect of an unusual workshift of chemical toxicity. II. Studies on the exposure of rats to aniline. *Fundam. Appl. Toxicol.* 7:144-152, 1986.

Carlson GP, Kim YC: Effect of forced exercise on dichloromethane blood levels and metabolism to carbon monoxide in rats. *IRCS Med. Sci.* 14:795, 1986.

BENZENE TOXICITY IN BONE MARROW PRECURSOR CELLS

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Other Occupational Concerns
5 R01 OH01542-03
09/30/83 - 08/31/86
\$71,480 (\$208,089 Cum)

Objectives

The goal of this project is to examine the relationship between in vivo and in vitro toxic effects of benzene and its metabolites on bone marrow, hemopoietic cell proliferation and differentiation. Specifically, experiments are directed toward studying the interaction of the hemopoietic microenvironment with developing precursor B-lymphocytes and precursor granulocytes/monocytes after acute exposure to benzene or its metabolites.

Methodology

A coculture system that consists of adherent, bone marrow stromal cells and an agar overlayer of bone marrow precursor cells has been established to study how benzene or its metabolites alter stromal cell supported growth of precursor granulocytes/monocytes. A second culture system which involves the tandem use of liquid culture followed by agar culture was used to investigate how benzene or its metabolites affect maturation of bone marrow, precursor B-lymphocytes. Marrow cell suspensions were first depleted of mature surface IgM positive B cells by using antibody-coated Petri dishes. These cells were incubated in liquid culture for 48 hours after which time the number of precursor B-cells that have matured into surface IgM positive cells was assessed phenotypically with fluorescent antibody and functionally via an agar colony forming assay.

Progress & Accomplishments

Initially dose-response studies were undertaken to characterize the in vitro effect of benzene metabolites on adherent stromal cell colony formation. It was established that the TD_{50} (dose that reduced colony number by 50%) was 0.018 mM for benzoquinone, .0025 mM for hydroquinone 0.060 mM for benzenetriol, 0.125 mM for catechol and 0.190 mM for phenol. In addition, both hydroquinone and benzoquinone were shown to impair the ability of adherent stromal cells to support the hemopoiesis of granulocyte/monocyte colonies in agar. Further studies were done to determine if in vivo exposure of mice to benzene or its metabolites would result in similar damage to the stromal cell microenvironment. Both B6C3F1 and DBA male mice were given benzene (100 mg/kg) or phenol (100 mg/kg) intraperitoneally, twice a day 4 consecutive days. On day 5 bone marrow from the femurs and tibias was removed, plated in culture and assayed for the ability of stromal cells to proliferate and to support granulocyte/monocyte colony formation (hemopoiesis) in agar. Although stromal cell growth and proliferation were unaffected by the treatments, the ability of these stromal cells to support hemopoiesis was reduced to a greater degree among B6C3F1 mice than among DBA mice which paradoxically showed an increase in the ability to support hemopoiesis but no preferential effect among strains was evident. These results suggested that benzene, but not phenol, is metabolized differently between the two mouse strains and that bone marrow stromal cells of the hemopoietic microenvironment are sensitive targets for benzene or its oxidative metabolites.

To investigate the mechanism involved in stromal cell responses to in vivo benzene exposure, we have begun to test the hypothesis that benzene metabolites are affecting stromal cell secretion of regulatory factors such as colony stimulating factor or prostaglandin E2 (PGE). Male B6C3F1 mice were given benzene (100 mg/kg), indomethacin (2 mg/kg) or a combination of benzene plus indomethacin was given one hour prior to benzene. On day 5 marrow cell suspensions were prepared from the mice. Benzene had no effect on bone marrow cell number, granulocyte/monocyte precursors or stromal cell colony number, but the ability of stromal cells to support hemopoiesis in agar was significantly decreased. In contrast, benzene had no effect on stromal cells taken from mice pretreated with indomethacin. This observation was subsequently corroborated by in vitro experiments which demonstrated that indomethacin, which decreases PGE levels in stromal cell cultures, can ameliorate hydroquinone induced toxicity.

We also have demonstrated that short-term exposure to benzene metabolites alters the ability of bone marrow B-lymphocyte precursors to mature into functional B lymphocytes. Marrow cells from B6C3F1 mice were first depleted of mature surface positive IgM (B-cells) lymphocytes, exposed to 0.0001 mM hydroquinone for one hour and then plated in liquid culture. Pre-B (sIgM negative) cells were then allowed to mature in culture for 0, 24, 48, or 72 hours and production of newly formed B cells assayed both by sIgM expression and colony formation in soft agar cultures containing LPS. Acute exposure to hydroquinone reduced the number of sIgM+ cells generated whereas small pre-B cell numbers were enhanced as compared with control cultures. The number of adherent cells found in these cultures were decreased. These results suggest that hydroquinone may block the maturation of B lineage cells at the pre-B cell stage. The reduction in adherent cells in treated cultures also suggests that toxicity is associated with damage to regulatory cells for the B lineage.

Significance

Our studies have demonstrated that bone marrow stromal cells, components of the hemopoietic microenvironment, are sensitive targets of benzene or its metabolites. This toxicity apparently results form an imbalance in stromal production of regulatory molecules which are necessary for normal myeloid and lymphoid development. These observations are significant in that benzene exposure is known to lead to leukemic transformation and sustained effects on cellular differentiation in the marrow hemopoietic microenvironment may contribute to the development of carcinogenesis.

Publications

Gaido K, and Wierda D, Bone Marrow Stromal Cell Factors Involved in the Regulation of Hemopoiesis. Blood (Suppl. 1) 66: 151a, 1985.

King AG, Landreth KS, and Wierda D: Hydroquinone Inhibits Bone Marrow Pre-B Cell maturation in Vitro. Toxicologist 6: in press, 1986.

Gaido K, and Wierda D: Hydroquinone suppression of Bone Marrow Stromal Cell Supported Hemopoiesis in vitro is Associated with Prostaglandin E2 Production. Toxicologist 6, in press, 1986.

Wierda D, and Gaido KW: Indomethacin protects against in Vivo Benzene Inhibition of Stromal Cell Function. Toxicologist 6, in press, 1986.

CHROMIUM DISTRIBUTION AND TOXICITY IN MAMMALIAN CELLS

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Other Occupational Concerns
5 R01 OH01630-04
09/30/83 - 08/31/87
\$87,586 (\$332,680 Cum)

Objectives

To study the mechanisms of chromium toxicity and the intracellular distribution of chromium in vertebrate cells.

Research on chromium toxicity to the present was based on two complementary research methods, single cell (cytochemical) and HPLC analysis. These techniques were chosen because of their sensitivity and ability to detect minute changes of chromium action.

Progress & Accomplishments

CHO cells were cultured with chromate-51 (Cr(VI)) to study the site of chromium interaction with cell biomolecules. After incubation, cells were homogenized and separated into nuclear, mitochondrial, microsomal, and cytosolic fractions. Greater than 75% of the radioactivity was found in the cytosolic fraction. The cytosolic fraction was subjected to chromatographic investigation. Two HPLC columns were used, viz., anionic and ion-pair. At least three different dialysable biomolecules interacted with chromate or its reduced derivative, Cr(III). These biomolecules were the nucleotides (ATP, UTP, CTP, GTP and ADP), glutathione and a metallo- peptide/protein. Glutathione accounted for 50% of the radioactivity, the nucleotides for 30%, and the metalloprotein for the remainder.

A tissue culture study was conducted on the effects of chromium on chondrocytic differentiation. Mesenchymal cells from stage 22-24 chick limb buds were dispersed and cultured as micromasses, where they differentiated into chondrocytes. Addition of chromium (VI) to the cultures indicated that the production of proteoglycans (as detected by Alcian blue staining) was more sensitive to chromium's effects than was cell proliferation. Whereas Alcian blue nodule formation was inhibited by 1.0 μ M chromium (VI), cell proliferation (as detected by cell counts) was not. Chromium (VI) was added to cultures at daily intervals, and these studies indicated that the interval of d 1-2 was the most sensitive period. ADP-ribose transferase activity in these cultures was measured; the pattern of enzyme activity in control cultures was high 1 and 24 h after the start of culture, decreased abruptly between 24 and 48 h, and then decreased more gradually. In the presence of chromium (VI), elevated ADP-ribose transferase levels were maintained throughout the culture period. We suggest that, in the presence of 1.0 μ M chromium (VI) or higher concentrations, the balance of events favors nucleolytic action rather than repair of damage.

Significance

Our working hypothesis was that, "if chromium affects chromatin superstructure in such a manner as to alter it, or to prevent Cr-chromatin interaction, or to prevent the precise re- combination/formation after its cell cycle transit, this Cr-chromatin interaction will be deleterious to the cell's survival." We still believe that chromium-chromatin complexes could alter chromatin conformation, expressed as karyological (nuclear) changes

which normally accompany the cell cycle traverse. In assessing the utility of the two techniques which we used, viz., single cell cytochemistry and HPLC, we found that the single cell methodology was more useful in analyzing chromium's toxic action. After spending considerable time in the development of HPLC isolation and analysis of nucleotides from small amounts of tissue, we used these techniques to analyze nucleic acid digests from chromium-treated cells. We were not able to demonstrate any significant effects using HPLC analysis of nucleic acid digests except at chromium concentrations in excess of 1.0 μM . We believe that the reason for the sensitivity of the single cell technique is that chromium, at the outset, is toxic to only a small proportion of the many cells that make up the affected tissues and, so, in order to study these few affected cells, we will utilize techniques which are capable of analyzing for the "one cell among many". We will study the in vivo effects of chromium on liver, lung and kidney toxicity using an innovative CAM (computer-assisted microscopy) system to quantify cytochemical changes after the administration of chromium to mice.

Publications

Uyeki EM, Nakanishi S, Fremming L: Chromium effects on chondrocytic differentiation in vitro. J Tox Environ Health 19:137-45, 1986.

Denniston ML, Uyeki EM: Distribution and HPLC study of chromium-51 binding sites in Chinese hamster ovary cells. In Press, J Tox Environ Health, 1986.

EVALUATION OF OCCUPATION LISTED ON ROUTINE DATA SOURCES

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Other Occupational Concerns
1 R01 OH01957-01
07/01/84 - 01/31/86
\$36,833 (\$36,833 Cum)

Objectives

The aims of this project are:

1. to estimate the comparability of useful occupation and industry calculated from a complete occupational history with the subject's own designation of usual occupation,
2. to compare each of these entries (calculated usual occupation and industry and self-designated occupation) with occupational entries of the death certificate and cancer registry abstract.

Methodology

The proposed research is intended to compare, for a sample of incident New Jersey cancer cases and controls, occupational data which have already been collected from three independent sources: personal interview, cancer registry abstracts, and death certificates. The information recorded on the death certificate and cancer registry abstract will be compared with the more accurate information collected from a lifetime occupational history obtained during personal interview.

Progress & Accomplishments

All data have been coded and formatted for comparability. Data analyses are nearing completion.

Significance

The long-term objective of this research is to suggest to epidemiologists and other researchers the most effective uses of routinely collected occupational data by describing how accurately these data sources characterize the usual occupation and industry of individuals. If the death certificate or cancer registry abstract often describe last occupation/industry or current occupation/industry, then the value of these sources for occupational epidemiologic studies will need to be reassessed.

Publications - None to date

IMPROVED PERSONAL PROTECTION AT HAZARDOUS WASTE SITES

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Other Occupational Concerns
5 R01 OH02066-02
09/28/84 - 04/30/87
\$120,938 (\$238,559 Cum)

Objectives

Goal 1. Investigation of the utility of FTIR spectroscopy performed with an ATR attachment for the acquisition of spectra of hazardous waste materials having a variety of physical and chemical properties.

Goal 2. Investigation of the applicability of forward and reverse search algorithms used in combination with spectral libraries to the identification of components of the hazardous waste mixtures, and the sensitivity of these searches to the presence of minor components of mixtures.

Goal 3. The development of a compatibility testing scheme that utilizes FTIR analysis data, including guidelines on the use of such data to define personal protection strategies.

Methodology

All experiments were performed using a Nicolet 20 SX-1280 FTIR equipped with a 160 megabyte storage module, and a Harrick PLC SIM ATR system. Software development used adaptations to the programs PAIRS and LSF MIX using Fortran 77 and Macro languages.

All samples were prepared from Aldrich (Gold) solvents, or in the case of actual hazardous waste samples, obtained from the U.S. EPA (NEIC).

Progress & Accomplishments

The first step in the achievement of this objective was to define what is meant by "hazardous waste materials":

Matrices:

- A. Mixture of hydrocarbons from n-decane through n-eicosane
- B. # 2 fuel oil
- C. Authentic waste oil from remedial action site
- D. Mixture of acrylic enamel paint solvents
- E. Synthetic acrylic enamel paint sludge
- F. Authentic paint sludge from remedial action site

Compound classes (of target analytes):

- | | |
|------------------------------|--------------------|
| 1. Chlorinated, cyclic | 5. Phenolic |
| 2. Polychlorinated biphenyl | 6. Isocyanate |
| 3. Chlorinated, aliphatic | 7. Organophosphate |
| 4. Non-chlorinated, aromatic | 8. Nitrile |

Subsequent investigation found these definitions to be inadequate.

Therefore, an investigation into the frequency of occurrence at hazardous waste sites of compounds of significance to health was initiated. Several hazardous waste samples were procured from the U.S. EPA and a variety of other sources. These samples have been subjected to extensive analysis by other methods.

Goals 1-3: A Nicolet 20 SX - 1280 FTIR was acquired, installed in our laboratory, and all personnel trained in its use. Software was installed in the instrument, including forward search routines, the Aldrich medium and high resolution spectral libraries, the Boolean logic program GRAB, and the

artificial intelligence program PAIRS.

Goal 1: Progress into the investigation of the applicability of ATR for spectral acquisition is complete. Questions of ATR versus transmission cells center on the questions of shifts of absorbance peak maxima and absorbance ratios. This project is central to our present effort.

Goal 2: Investigation of forward search routines was completed. Investigation of the program PAIRS for the identification of functional groups of principal constituents of simulated hazardous waste mixtures was completed. Application of PAIRS to the identification of the principal components of simulated hazardous waste mixtures was completed. Development of the program PAWMI was completed. The programs LSF and LSF MIX, as well as a variation on these programs written in our laboratory, were investigated for spectral curve fitting and quantitation, and found to be inapplicable. However, the program DECON was found to be useful to deconvolve overlapping peaks. An automatic rule generator and optimizer for PAWMI is being developed. The application of the automated version of PAWMI to spectra that have been deconvoluted will complete this objective.

Goal 3: The goal of incorporating these findings into a hazardous waste site personal protection strategy has not been addressed.

Significance

The above progress represents a very substantial portion of the work that can be accomplished in the laboratory towards investigating the utility of FTIR for the improvement of personal protection strategies at hazardous waste sites. The advances have been accomplished in analytical chemistry that will allow the industrial hygiene advances, but not without actual field testing. Proposals have been submitted to perform field testing of the method, and application of the findings to industrial hygiene during that field trial.

Publications

FTIR spectroscopy applied to hazardous waste: Preliminary test of material analysis for improvement of personal protection strategies. Am Ind Hyg Assoc J 46:181, 1985.

Application of the hazard ranking system to the prioritization of organic compounds identified at hazardous waste remedial action sites. J. Haz. Waste Haz. Mat. 3:221, 1986.

Computerized IR spectral interpretation of compounds frequently found at hazardous waste sites. Anal. Chem. 58:1156.

An IR screening technique for automated identification of bulk organic mixtures. Anal. Chem. 58:1981, 1986.

Qualitative screening of hazardous waste drum mixtures. In press, Environ. Sci. Technol., October, 1986.

POLYIMIDE SORBENTS FOR AIRBORNE TOXIC AND CARCINOGENS

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Other Occupational Concerns
1 R01 OH02108-01
01/01/86 - 12/31/87
\$141,155 (\$141,155 Cum)

Objectives

The long-term thrust of this research have been to characterize the performance of four promising polyimide sorbents which can be used for collecting toxic, mutagenic, or carcinogenic chemicals from workplace, indoor or outdoor air, and performing chemical and biological assays on the collected samples.

The effect to which the air sampling process maintains ideality with the polyimide sorbents was studied during the past year. Laboratory experiments were conducted to gain insight to the fundamental and practical understanding of those factors producing nonideal behavior. Specifically, the aim was to study: (a) the effect of air humidity on analyte retention by the polyimide sorbent; (b) the competition between different classes of analytes in a mixture for sorption sites on polyimides and Tenax GC (included for comparative purposes); (c) the recovery of analytes using thermal and supercritical fluid CO₂ conditions.

Methodology

Four promising polyimide sorbents were evaluated and compared with Tenax GC. Experiments were conducted with six chemicals to probe four types of molecular interactions which might occur during adsorption. These were:

(1) water (Class AB, strong hydrogen bonding), (2) ethanol (Class AB, weaker hydrogen bonding, electron donor properties), (3) 2-butanone and nitromethane (Class B, no active hydrogen bonding, electron polarization properties), and (4) hexane and benzene (Class N, no active hydrogen bonding, Van der Waals and $\pi - \pi$ forces, respectively).

Using a deuterated tracer pulse technique with mass spectrometric detection, the method of Parcher (1,2) was modified. A mixture of the five chemicals in the absence and presence of 90% humidity was introduced in a continuous gas stream (frontal) into the sorbent bed, and -10, 0, 10, 20 and 30 min (at 0 min the frontal stream was begun) intervals discrete injections (~5 μ g) of the corresponding deuterated chemical were made into the frontal stream. The percent change in the breakthrough volume (BV) of the deuterated chemical was determined relative to no "polluted frontal stream" and no humidity.

Radiolabeled γ -BHC, hexachlorobiphenyl, anthracene, and parathion, representing the chemical classes of semivolatile chlorinated, aliphatic, and aromatic compounds, polynuclear aromatics, and organo-phosphate pesticides, respectively, were used to demonstrate the ability of supercritical carbon dioxide to desorb these chemicals from polyimides and Tenax GC. The identity of the desorbed compounds were verified using TLC and mass spectrometry. A comparison was also made to thermal desorption for the same chemicals.

Progress & Accomplishments

All experiments were completed which addressed the three specific aims and chemicals cited above during the first year.

The results provided information to the following questions: (a) Does air humidity alter the BVs of analytes for polyimides and Tenax GC? (b) Are BVs for individual analytes altered by competition between analyses in a mixture for the sorption sites? Are there a variety of adsorption mechanisms operating on the sorbent surface and are they chemical class-related?

The results suggest that the sorptive capacity is a function of several sorption mechanisms (analyte-sorbent) at the molecular level, as it may be predicted by the various chemical functionalities present on the sorbent surface. For example, humidity did not appreciably affect BVs on Tenax GC, whereas effects were observed on polyimides. Competition was demonstrated between analytes; however, these effects differed between sorbents. The deuterated pulse tracer technique was effective in demonstrating percent changes of (decrease or increase) >5% in BVs.

Recoveries from Tenax were >90% for the selected chemicals cited above and except for anthracene were >80% on the polyimides. These results were compared to thermal desorption from Tenax GC for the same chemicals where, even under the more favorable conditions for thermal desorption, the recovery for hexachlorobiphenyl and parathion was well below 50%.

Significance

The deuterated pulse tracer technique yields extremely rich information and is useful in probing the mechanisms of analyte-sorbent interaction and competition under practical sampling conditions. This technique could serve as a standard technique for assessing any sorbent under consideration for sampling of organic vapors from air.

Recovery of analytes using supercritical fluid CO₂ allows the range to be extended to semivolatile chemicals which are not recoverable by thermal means. Also, conditions are mild (40°C) permitting analysis of labile, polar chemicals while minimizing potential artifacts attributable to thermal desorption. A method based upon supercritical fluid CO₂ should permit more sensitive analyses than can be achieved with liquid desorption.

Publications - Abstract(s) available

Raymer JH, Pellizzari ED: Toxic Organic Chemicals from 2-6-Diphenyl-p-Phenylene Oxide Porous Polymer Using Supercritical Carbon Dioxide and Thermal Desorption Methods. Anal. Chem., in press.

PLASMA PROTEINS: MARKERS OF CHEMICAL EXPOSURE

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Other Occupational Concerns
1 R01 OH02149-01
01/01/86-12/31/88
\$101,559 (\$101,559 Cum)

Objectives

A significant number of people are exposed to a variety of chemicals at work sites which may be responsible for toxic manifestations. Such exposure(s) can bring about changes in plasma proteins in terms of their function, concentration or covalent modification. These changes, besides being responsible for toxic effects, can also be used as markers of chemical exposure. The intent of this research is to develop method(s) which can identify the changes in plasma proteins caused by chemical exposure.

Methodology

Effects of chemical exposure on plasma proteins were studied in terms of their biological activity, concentration, and covalent modification. These changes were measured using bio- and immunoassays, electrophoretic and chromatographic techniques. Covalent modification will be further characterized by peptide mapping, compositional and sequential amino acid analysis. The structure of the modified amino acid(s) will be determined by spectral techniques and further confirmed by independent synthesis.

Progress & Accomplishments

In vitro covalent binding of 1,2-dibromo- and 1,2-dichloroethanes with plasma proteins was studied. Incubation of plasma proteins with ^{14}C -dibromoethane (DBE) showed a time dependent increase which attains a maxima in two hrs. The two hour sample when subjected to gel filtration column gave two protein peaks. The second peak (mol. wt. < 60,000 daltons) accounted for > 90 of the total radioactivity applied. This pooled protein fraction was subjected to anion-exchange high-performance liquid chromatography which demonstrated that approximately 65% of the radioactivity was associated with albumin while approximately 23% with α_1 -acid glycoprotein. The ^{14}C -DBE bound albumin was further purified using affinity chromatography and is currently being digested in order to identify the amino acid by which ^{14}C -DBE is bound. Similar results of incubations were obtained with 1,2-dichloroethane (DCE) but the extent of binding is less than expected because chlorine is a poorer leaving group than bromine. We are currently using the same chromatographic techniques described above to purify 1,2-DCE bound plasma proteins.

The change in proteinase inhibitory activity using purified α_1 -proteinase inhibitor as well as plasma was also measured after incubation with ethylene oxide, propylene oxide, styrene oxide, 1,2-DBE and 1,2-DCE using the standard assay procedures. Styrene oxide was found to be the most potent inhibitor of α_1 -PI followed by ethylene oxide and propylene oxide; 1,2-DBE and 1,2-DCE were found to be the weakest inhibitors of α_1 -PI. Since loss of α_1 -proteinase inhibitory activity in the lung has been proposed as a key factor in causing pulmonary emphysema, was also studied the effect of aldehydes (acrolein, glyoxal, and pyruvic aldehyde) and

ketones (2,3-pentadione and 2,3-butadione) found in cigarette smoking on α_1 -PI. Acrolein was found to be the most potent inhibitor followed by pyruvic aldehyde and glyoxal while 2,3-pentadione and 2-3-butadione were virtually inactive. These studies indicate that α_1 -PI can be inactivated by non-oxidative processes. These studies also suggest that the fate of α_1 -PI in smokers and nonsmokers will be different in populations exposed to chemicals. Currently, we are studying the effect of mixing chemicals with these aldehydes to see whether they cause synergistic or additive effect.

Significance

Although the present study is limited to in vitro studies with human plasma, the experiments could be extended to monitor occupationally exposed populations to these or other chemicals. Correlation between the changes in plasma proteins and the medical histories of the occupationally exposed individuals can eventually be used for medical surveillance.

Publications - Abstract(s) available

Gan JC, Ansari GAS: Non-oxidative inactivation of plasma α_1 -proteinase inhibitor by carbonyl compounds found in cigarette smoke. Res. Commun. Subs. Abuse (In Press).

DOSE/RESPONSE FOR OCCUPATIONAL STYRENE EXPOSURES

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Other Occupational Concerns
1 R01 OH02221-01A1
09/29/86 - 09/28/88
\$123,690 (\$123,690 Cum)

Summary

The proposed study is intended to investigate the linkages between exposure, absorbed dose, and cytogenetic response resulting from occupational exposures to styrene in the reinforced-plastics industry. A longitudinal study is envisioned where a cohort of 20 highly-exposed workers (and 10 controls) is followed over a period of one year. Ideally the cohort will consist of equal numbers of workers exposed either more or less continuously from day to day or in a highly intermittent fashion. Randomly-selected surveys during the period of the study will allow each worker's airborne exposures to be repeatedly measured as well as his/her blood styrene levels (as indicated by analysis of exhaled air), and levels of sister chromatid exchanges (SCEs) in peripheral lymphocytes. The goal will be to estimate the parameters of the distributions of exposures, absorbed dose, and cytogenetic response so that the sensitivity of the dose and response distributions can be investigated relative to that of exposure. This should provide insight concerning the relative importance of large transient exposures or large daily exposures and of the possible need for a STEL in regulating styrene exposures. It should also provide valuable insight into the stability of SCEs in humans exposed to genotoxic agents.

A secondary part of the study will be to apply a pharmacokinetic model for styrene, derived under constant-exposure conditions, to the situation typical of occupational exposures where air levels vary considerably over time. The input of the model will be a log normally-distributed, autocorrelated series of exposure concentrations while the output consists of the resulting concentrations of styrene in the central and peripheral compartments. A portion of the field study will gather exposure-dose data over short averaging times in order to validate the model. If it proves useful, the model will be used to estimate patterns of short-term exposures and blood styrene levels associated with the majority of the field measurements where only 4-8 hr TWAs could be measured.

Publications - None to date

FAST-GC FOR INDUSTRIAL HYGIENE MONITORING/ANALYSIS

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Other Occupational Concerns
1 R01 OH02303-01
09/28/86 - 09/28/88
\$115,903 (\$115,903 Cum)

Summary

The development of a thermally desorbable miniature passive dosimeter (MPD) for very high sensitivity STEL monitoring of organic vapors has been completed by our research group. Given an analysis time of 15-30 minutes per sample, plus quality control assays, a single series of STEL samples from one worker would tie up a gas chromatograph (GC) for a full day. Therefore, there is a need for a rapid GC method of analysis for organic vapor samples collected on passive dosimeters.

The use of GC for the real-time analysis of vapors is also severely limited by the analysis time required and the lack of sensitivity of the method. For example, for multiple components, such as at a refinery, each GC analysis will take 15-30 mins, sensitivity will be significantly degraded, and the utility of the GC method for area monitoring/alarm will be seriously compromised. Therefore, there is a need for a rapid, sensitive GC method of analysis for organic samples collected as vapors (as with a plant area monitor/alarm).

GC is also used for the analysis of CS_2 eluates of adsorbent tubes. These analyses generally takes 10-30 minutes per sample. A method that would permit the analysis of CS_2 solutions in 10 seconds would save significant laboratory resources.

Recently, a high speed GC system has been described by our research group that was demonstrated to be capable of analyzing a nine-component test mixture of organic compounds in just over 6 seconds. This is an improvement in analysis speed when compared to conventional GC of 10^2 times. Along with the improvement in speed, an expected improvement in sensitivity on the order of 10 times is expected. Therefore, this proposal is aimed at testing the following hypotheses:

1. Vapor samples collected on passive dosimeters can be thermally desorbed/analyzed using the fast GC system in 10 seconds or less with no degradation of performance when compared to conventional GC methods.
2. Air samples can be directly analyzed within 10 secs at sensitivities significantly higher than those achievable using present methods.
3. CS_2 solutions of target analytes can be analyzed in 10 seconds.

Publications - None to date

PERIPHERAL MARKERS OF MUSCARINIC RECEPTORS

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Other Occupational Concerns
1 K01 OH00054-01
09/29/86 - 09/28/89
\$32,400 (\$32,400 Cum)

Summary

Studies on the toxicity of chemicals to humans are limited by the inaccessibility of most tissues, with the exception of certain biopsies and post-mortem samples. In order to monitor chemical exposure and to predict health effects in workers and general population, it is necessary to develop, through animal experimentation, indicators of biochemical and cellular toxicity applicable to human studies. Among the thousands of chemicals used in the industrial and agricultural setting, many are known to affect the central and/or peripheral nervous system. Yet, very few peripheral indicators, accessible in humans, of nervous system toxicity are available. This proposal will focus on the muscarinic receptor, a component of the cholinergic system, which plays an important role in CNS functions as well as in the control of most of the body's organs.

The hypothesis that will be tested is that muscarinic receptors on circulating lymphocytes could represent a marker of the same receptors in the CNS, the lung and other solid tissues and that alterations of muscarinic receptors in such tissues due to environmental or genetic factors, to pharmacological treatment or to chemical exposure, will be detected by measuring receptor density, affinity and function in lymphocytes. The specific aims that this project will address are: (1) To extensively characterize muscarinic cholinergic receptors on circulating lymphocytes of rats; (2) To establish a correlation between alterations of muscarinic receptors in solid tissues, induced by treatments with cholinergic agonists and antagonists and by chronic exposure to organophosphates, and alteration of muscarinic receptors in lymphocytes.

These studies will provide important data on the biochemical and functional characteristics of muscarinic receptors on lymphocytes and on the possibility of the use of these receptors as markers of muscarinic receptors in tissues not accessible in humans. This knowledge will be useful for further studies involving the assessment of the effect of exposure of humans to chemicals affecting the nervous system.

Publications - None to date

HIGHWAY MAINTENANCE COHORT MORTALITY STUDY

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Other Occupational Concerns
1 K01 OH00055-01
09/29/86 - 09/28/87
\$32,400 (\$32,400 Cum)

Summary

In 1978, a leukemia cluster in highway maintenance workers (HMW) in Wheaton, Minnesota was identified. Six leukemia cases occurred in the male population of Wheaton. Based on cancer rates from the Minneapolis-St. Paul component of the Third National Cancer Survey, only one would have been expected. Five of these six men were HMW. A follow-up case-control study was unable to elucidate the problem. Recently, it has been alleged that these cases were the result of benzene contamination in asphalt materials. Concern has also been expressed about exposure to 2,4,5,-T and 2,4-T as well as increased deaths due to injury. This study will examine all cause and cause specific mortality among highway maintenance workers. Specific causes of death of interest are leukemia, injuries, respiratory cancer, and pneumoconioses.

Methodology

The population identified for study is any white male who ever worked for the Minnesota Department of Transportation as a highway maintenance worker. From this group, a cohort of those working one or more years as a highway maintenance worker and at least one day after January 1, 1945 will be identified for analysis and tracing. Those lost to follow-up will be evaluated in two ways: 1) they will be assumed alive at the end of follow-up; and 2) they will be considered lost to follow-up on the last day they were known to have been alive. Follow-up will end December 31, 1984. After all tracing of the cohort has been completed, the cohort mortality experience will be compared to that of the Minnesota and United States populations of the same generational cohort of white males.

Relative mortality will be assessed by computing the observed to expected (O/E) ratio. The indirect method of standardization will be used in this analysis. Significance levels will be determined by examination of a table of significance factors for the ratio of an observed value of a Poisson variable to its expectation. In addition, corresponding to the SMR analysis, the Mantel-Haenszel estimate of the odds ratio and the Mantel-Haenszel chi square statistic will be computed. All results will be evaluated for biologic plausability.

Progress & Accomplishments

To date, 9,406 work records have been abstracted from Minnesota Department of Transportation records. Of these records, 4,851 have been identified as meeting the definition for inclusion into the cohort. There were approximately 1,531 deaths among these workers prior to December 31, 1984. The final portion of cohort tracing should be completed by early March 1987. Other results are not yet available.

Industrial hygiene monitoring has been done in order to evaluate present exposures to n-hexane, benzene, toluene, xylene, petroleum distillates, chlorobenzene, and PAHs (pyrene, benz(a)anthracene, chrysene, and benz(a)pyrene), and dodecane. These results are not yet available. Additional monitoring is to be done for herbicides and respirable silica.

Significance

Results are not yet available in order to evaluate the significance of work done to date.

Publications - None to date

ANEMOMETRY RELATED TO WORKPLACE CONTAMINANT DISTRIBUTION

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Other Occupational Concerns
5 R03 OH01825-02
05/01/84 - 04/30/87
\$11,802 (\$30,621 Cum)

Objectives

This research examines the relationship between air circulation in a test room and the spatial and temporal distributions of a contaminant gas continuously released from a fixed point source. The aim is to develop empirical measures of ventilation, based on tracer gas techniques and anemometry, that can be used to estimate contaminant distributions and contaminant removal in ventilated spaces.

Methodology

This work uses a residence time model of room airflow to relate tracer gas measurements to local contaminant distributions and tracer dispersion. This approach characterizes flows by the local age distribution of fluid elements, permitting an elegant and powerful analysis that directly relates to air concentrations at points in the room. This technique provides results that are independent of the specific mixing mechanisms, simultaneously measuring the distribution of fresh air and contaminant removal efficiency of the room ventilation system.

Contaminant distributions are studied in a 5400 ft³ test chamber having a known ventilation rate. A contaminant release is simulated by injecting a passive tracer gas at a point in the chamber. The change in tracer concentrations with time is recorded by a computer controlled multi-port air sampler that continuously monitors air concentrations at up to 16 points in the room. Numerical integration of these data provides estimates of the local contaminant distributions and age distributions of the room air.

Local contaminant levels and age distributions are directly related to diffusive and bulk transport by turbulent air movements. Measurements of the air velocity vector can provide point estimates of both transport mechanisms, so that anemometry may give rapid, useful predictions of parameters for a residence time model.

Air movement is measured with a recently developed sonic anemometer that can accurately resolve the 3-dimensional wind vector at the low velocities found in a typical room. The velocity data provides estimates of the eddy diffusivity, the magnitude and direction of contaminant transport and predictions of local age distribution parameters. Computer mapping of the velocity data provides the general pattern of air movement and dispersal in the room. The values predicted from the anemometry will be compared to those measured with the tracer gas, at different ventilation conditions and source generation rates.

Progress & Accomplishments

We have completed the necessary modifications of the test chamber and air sampling system to allow tracer studies of air mixing. In addition, a theoretical framework has been developed for interpreting the air velocity

and relating these measurements to contaminant dispersal. Testing of the computer controlled tracer injector and sampler system indicates that the residence time model provides useful data on local ventilation rates and contaminant levels. The local mean age seems to be a temporally stable measure of ventilation at a point, for a given room geometry, temperature conditions, and room air flow rate.

Significance

The ability to measure local differences in ventilation at various room locations has obvious applications for evaluating the effectiveness of room ventilation systems. Tying these measurements to local contaminant distributions provides a measuring index (i.e. air concentration) which is of primary interest to health scientists. The significance of this work arises from its longer-term potential to lead to a field deployable instrument and associated set of procedures for measuring ventilation in rooms, and determining how most efficiently to utilize or alter ventilation and source characteristics in a workplace.

Publications - None to date

SIZE DISTRIBUTION OF AIRBORNE LEAD AEROSOL IN INDUSTRY

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Other Occupational Concerns
1 R03 OH02044-02
11/01/84 - 10/31/86
\$20,650 (\$39,825 Cum)

Objectives

The overall goal of the proposed research is to assess whether a lead PEL based on size-differentiated sampling represents a more meaningful estimate of exposure. This would be accomplished by studying the impact of a variety of size distributions of lead aerosols on the predicted distribution of blood lead levels in workers exposed to lead.

Methodology

1. The lead aerosol size distribution in the workers' exposure is determined in selected operations in a battery manufacturing plant and in a brass foundry, using the Marple Series 290s personal cascade impactor.
2. The actual daily lead intake of a worker in a selected operation will be estimated with the aerosol size information obtained and with ICRP lung deposition model.
3. The Bernard model, as adapted by OSHA, for lead metabolism in the human body will be used to describe the accumulation of lead in the body and to estimate the blood lead level of a worker after a certain period of occupational exposure.
4. The probability distribution of blood lead levels of a given worker population will be estimated and compared with that predicted by OSHA.

Progress & Accomplishments

Three studies of this project were conducted. They are described as follows:

1. Field Evaluation of Particle Bounce.
This study helps determine the proper collection surface in the personal cascade impactor to be used in sampling in this project. The aim is to select a collection surface that minimizes the effect of particle bounce in the cascade impactor. Five types of collection substrates were evaluated for particle bounce in the laboratory with lead dioxide dust, and in the field with brass pouring fume and brass grinding dust. It is found that oil-saturated Teflon membranes have similar collection characteristics as the oil-saturated sintered metal, a surface for which the collection efficiency is presumed to be independent of particle loading. For subsequent sampling, we select oil-saturated Teflon membrane as the collection surface in the cascade impactor.
2. Predicted Effect of Aerosol Size Distribution on Blood Lead Distribution.
The size distribution measurements of lead aerosol determined from a brass foundry and primary lead smelter were used to simulate blood lead distributions applying a pharmacokinetic model developed by Bernard and adapted by OSHA. The predicted distribution of blood lead levels were determined using the actual size distribution of aerosol as compared to the blood lead levels predicted by OSHA.

In the furnace area of smelter and the pouring area of the foundry the predicted mean blood lead levels are higher than that predicted by the standard whereas in the smelters sintering and mixing operation the blood lead levels are less than that suggested by the standard. The data supports the conclusion that size selective sampling needs to be considered for incorporation into the OSHA lead standard.

3. Sampling in a Brass Foundry and A Battery Manufacturing Plant.

A minimum of 10 personal cascade impactor samples were collected in four operations each in two factories. The four operations in the brass foundry were cutting, grinding, pouring, and furnace. The four operations in the battery manufacturing plant were pasting, grid casting, cast-on-strap unloading and high-voltage welding.

Preliminary analysis shows that the size distribution and the total concentration of lead aerosol varies markedly according to the operation in the brass foundry whereas the differences are minimal in the battery plant. In the battery plant, the data show that lead aerosol in all four operations have MMDs greater than 10 μ m and total lead concentrations much lower than observed in brass foundry operations.

Significance

In the second study described above, we have demonstrated that size selective sampling should be considered for incorporation into the OSHA lead standard. Since personal samples give more representative estimates of workers' exposure than the area sample does, a detailed analysis will be done on the personal samples collected to determine the magnitude of the difference in the predicted blood lead distributions based on a size selective sampling and on OSHA's assumptions. This research may enable the development of a lead exposure sampling scheme whose end result is better protection for lead exposure workers.

Publications

Hinds WC, Liu WV, Froines JR: Particle Bounce in a Personal Cascade Impactor: A Field Evaluation. Am Ind Hyg Assoc J 46(9):517-523, 1985.

SCBA STRESSORS: EFFECTS OF CARBON DIOXIDE BREATHING

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Other Occupational Concerns
1 R03 OH02179-01A1
09/29/86 - 09/28/87
\$18,476 (\$18,476 Cum)

Objectives

Recently initiated, this small grant was designed to analyze data previously collected on the effects of breathing an elevated but subclinical level of carbon dioxide (CO_2) -- similar to what might occur in self-contained breathing for quantifying the effects of environmental stressors and neurotoxins on human psychomotor performance and (2) analyze data from a CO_2 experiment designed to provide information useful for recommending federal guidelines for the maximum allowable concentration of CO_2 in SCBAs.

Methodology

In a $2 \times 2 \times 2 \times 4$ (Gas x Exercise x Stimulus-Response Compatibility x Time-On-Task) repeated measures analysis of variance design, six highly practiced, young, healthy male subjects each performed 20,000 trials on a serial four-choice reaction time task. Independent variables were CO_2 (room air vs. 4.0% CO_2 with 50% O_2), exercise (rest vs. work at 70% $\text{VO}_{2\text{ max}}$ for 15 min), a task manipulation of stimulus-response compatibility (high vs. low), and mental fatigue as measured by time-on-task (subdivision of the 20 min testing period into 5-min intervals). Principal dependent measures included total number of trials performed, serial choice reaction time, dwell time, response time, percentage errors, and an inventory of clinical symptoms.

Progress & Accomplishments

Prior analyses by the author omitted the exercise variable because of the complexities in this methodology. Previous results showed main effects of each factor and an interaction of CO_2 inhalation with stimulus-response compatibility which, according to the logic of the Additive Factors Method, suggests that breathing CO_2 impairs central nervous system information process activity in the response selection stage of processing. Identifying a level at which cognition is impaired by breathing CO_2 is meaningful; however, workers seldom breathe this gas while sedentary. The picture is incomplete without knowledge of how CO_2 affects us during work.

Significance

Determining the effects of exercise and carbon dioxide inhalation on information processing is essential to advancing our understanding of CO_2 as a neurotoxin and recommending revisions of federal standards (e.g., 30CRF11).

Publications - None to date

MEASUREMENT OF WORKER'S EXPOSURE TO STYRENE

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Other Occupational Concerns
1 R03 OH02180-01
05/01/86 - 04/30/87
\$22,164 (\$22,164 Cum)

Objectives

The research is investigating several factors which affect estimates of inhalation exposure to styrene which are made from personal monitor measurements. The initial objectives are to: 1) document the magnitude of the variability in the breathing zone concentrations of styrene within the reinforced plastics industry; and 2) study the relationship between exposure estimates made with personal samplers as they are commonly used and the inhalation exposure to styrene measured close to the nose. A third objective is to determine how the aerosols generated during the spraying process may contribute to the exposure. In addition, we will investigate the stability of samples of styrene collected on charcoal which are stored prior to analysis.

Methodology

Measurements are being made on workers in a boat manufacturing facility to compare the styrene concentration measured at commonly used personal sampler locations with the concentration measured at the nose. Additional information on sources and processes is being collected in order to understand some of the factors which contribute to the variability of the measurements. Spray booth experiments are being carried out both in the field and in a laboratory testing facility. The stability of the styrene on charcoal is being studied by generating a uniform styrene atmosphere and collecting simultaneous samples, some of which are analyzed immediately and some stored for various time periods.

Progress & Accomplishments

A field study was conducted in a boat-manufacturing facility in Massachusetts. The production process involves spraying a mixture of styrene, catalysts and chopped fiberglass onto a shell which has been formed on a mold. A major purpose of the study was to document the magnitude of the variability in breathing zone concentrations of styrene during the spraying process. Concentration variations around individual workers were obvious, with the highest concentration found at the chest. The concentration measured by the nose sampler was about 76% of that measured by the chest sampler. A more detailed evaluation of the data is in progress. Also, the factors which contribute most significantly to these differences are being investigated.

To examine the effect of aerosols, airborne styrene levels were measured during four spraying experiments in an application testing laboratory. Two experiments involved the spraying of catalyzed polyester resin solution, which represents a typical process in the reinforced plastics industry. In the other two experiments, uncatalyzed polyester resin solution was sprayed in order to simulate a paint spraying process. Comparison of the samples collected with uncatalyzed and catalyzed resin permitted evaluation of the

stability of the collected aerosols. The results of the four experiments showed that aerosols represented $30\% \pm 3\%$ of the total styrene air concentration for the conditions used in this study. These results suggest that: 1) aerosol droplets represent a significant percentage of the total air concentration of styrene during resin spraying operations; 2) many of the particles generated during resin spraying operations are respirable; 3) the aerosol droplets may undergo physical and chemical changes such as vaporization and polymerization, which will affect the nature of respiratory exposure encountered and the dose received. Although the current study concentrated on the reinforced plastics industry where extensive exposure to styrene occurs, these results should be considered in other industrial situations involving spraying of volatile organic solvents. Exposure to volatile organic contaminants should not be assumed to be attributable to vapors only.

Significance

It is essential to know the reliability of the measurements used to estimate inhalation exposures in the reinforced plastic industry and to understand the factors which influence both the measurement and the true exposure. Worker exposure to volatile organic contaminants is evaluated by collecting breathing zone samples. This may not provide a true exposure estimate due to concentration variations within the breathing zone. Furthermore, exposure to volatile contaminants usually is attributed to vapors alone. This study demonstrated that aerosols, generated during the spraying of polyester resin solution, can contribute significantly to the exposure to volatile organic contaminants.

Finally, organic contaminants are usually collected by adsorption on a solid sorbent such as charcoal or silica gel. Sample stability prior to the analysis is essential in obtaining accurate exposure estimates. Many organic compounds are reactive and can undergo chemical reactions such as oxidation, hydrolysis, polymerization, etc. leading to underestimation to the true exposure of the species collected. The stability of styrene vapors on charcoal substrate has not yet been shown, particularly when peroxide, a polymerization initiator, is present.

Publications

Malek RF, Daisey JM, Cohen BS: The effect of aerosol on estimates of inhalation exposure to airborne styrene. Am Ind Hyg Assoc J 47:524-529, 1986.

MECHANISMS OF CYTOSKELETAL INJURY BY HEAVY METALS

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Other Occupational Concerns
1 R03 OH02321-01
09/29/86 - 09/28/87
\$24,837 (\$24,837 Cum)

Summary

Serious health problems can occur in workers exposed to metals in the workplace atmosphere. Chronic or acute exposure to fumes containing Hg, or Pb can result in anemia and neurological damage, while acute exposure to Cd, or As can result in emphysema. Long-term exposure to As ultimately can lead to lung cancer, while long-term exposure to Cd can result in severe renal damage. It is unclear which intracellular targets of these metals have a direct effect on the expression of their systemic toxicity. This project represents initial studies concerning the cytoskeleton as a target of intracellular damage by these metals. It is intended to establish a basis for future studies relating cytoskeletal damage to systemic toxicity. All these metals bind avidly to sulfhydryl groups. However, Cd, Hg, and Pb can also bind to and activate calmodulin. In contrast, As cannot activate calmodulin. Microtubules contain sulfhydryl groups that must remain reduced for assembly to occur, and to maintain the assembled state. In addition, microtubule dynamics are regulated by calcium, via activation of calmodulin. Thus Cd, Hg, and Pb may affect microtubules by sulfhydryl binding, or calmodulin binding while the effects of As would be due to sulfhydryl binding. Changes in the amount of total cellular protein and cytoskeletal protein sulfhydryl groups during metal exposure will be monitored with the DTNB colorimetric assay and the ³H-NEM labelling assay. Activation of calmodulin by Ca, Cd, Hg, and Pb will be studied by determining the effects of calmodulin inhibitors on the effects of Ca and other metals on microtubules. Calmodulin binding of metal ions will be determined by an enhancement of tubulin assembly in the presence of calmodulin by the chelating action of calmodulin. Finally, binding and activation of calmodulin is shown by the slower mobility of activated calmodulin in nondenaturing PAGE. Calmodulin dependent phosphorylation of cytoskeletal proteins is analyzed with SDS-PAGE and autoradiography.

Publications - None to date

FORMALDEHYDE MOLECULAR STATES IN THE GAS PHASE

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Other Occupational Concerns
7 R03 OH02377-01*
09/28/84 - 05/31/87
\$8,000 (\$23,000 Cum)

* Previously supported under Grant Number R03 OH02045.

Objectives

The research is to investigate the molecular species of formaldehyde present in the gas phase. Goals of the research are to: (1) validate an experimental protocol wherein formaldehyde vapors at equilibrium with formalin solution were found to consist of methylene glycol, methylal and the first three oligomers of polyoxymethylene glycol monomethyl ethers; and (2) determine the equilibrium constant for the formation of methylene glycol from formaldehyde and water in the vapor state.

Methodology

Validation of the gas phase trimethylsilylation method of analysis for formaldehyde condensate vapors has been conducted through vapor phase derivatization of chemically similar compounds. A mixture of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and N,N-dimethylformamide was placed in an evacuated vial and the vapors of alcohols, glycols and glycolic ethers were then introduced. Contents of the vial were analyzed by capillary gas chromatography with flame ionization detection. Yields were estimated through comparison of analytical results with the concentrations predicted from vapor pressure calculations.

The equilibrium constant for the formation of methylene glycol from formaldehyde and water in the gas phase will be determined over a range of temperatures of environmental and toxicological interest. Vapors of water and formaldehyde will be reacted in a dynamic dilution system and the effluents from the system analyzed by a matrix of analytical methods that collectively may distinguish monomeric formaldehyde, s-trioxane and the polyoxymethylene glycols.

Progress & Accomplishments

Validation of the gas phase derivatization of polar organics by BSTFA has been accomplished with the results listed in Table 1 below. The yields for most of the compounds studied were near quantitative and highly reproducible.

A monomeric formaldehyde generator has been constructed based on the acid catalyzed depolymerization of s-trioxane vapors. Gas chromatographic analysis of the generator effluent indicates complete conversion of s-trioxane to monomeric formaldehyde. Subsequent analysis of the effluent contents by the matrix of analytical methods for gas phase formaldehyde has yet to be performed. The gas phase reaction/dilution system for formaldehyde and water is being constructed.

TABLE 1. Yields for Gas Phase Trimethylsilylation of Polar Organics by BSTFA.

Compound	x	S	
Methanol	87.5	2.9	3
Ethanol	91.8	3.3	4
Isopropanol	94.9	4.9	4
Phenol	68.4	1.1	3
Ethylene glycol	69.7	8.9	6
1,2-Propanediol	86.0	2.7	3
1,3-Butanediol	111.0	1.5	4
2-Methoxyethanol	79.4	13.2	11
2-Ethoxyethanol	93.4	11.2	5

Significance

Gas phase trimethylsilylation of polar organics by BSTFA has essentially quantitative yields for many of the compounds analyzed. When viewed by chemical class, i.e. alcohols, glycols and glycolic ethers, the method averaged about eighty-five percent yield for each class with greater yields for the higher molecular weight compounds in each class. Results indicate that the method is good for approximation of the concentration of polar organics in the gas phase including polyoxymethylene glycols and polyoxymethylene glycol monomethyl ethers in formalin headspace. In addition, s-trioxane depolymerization under nitrogen gas is the recommended method for generation of monomeric formaldehyde since the absence of water in this system prevents the formation of formaldehyde oligomers.

Publications - None to date

SAFETY AND SENSITIVITY: ETHICAL ISSUES IN IDENTIFYING AND PROTECTING
HIGH-RISK PERSONS IN THE WORKPLACE

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and Geography
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Other Occupational Concerns
RII 8217297
08/01/84 - 10/31/85
\$40,000 (\$40,000 Cum)

Objectives

The central question addressed by this project was: How should society respond to the differential sensitivity of workers to technological hazards in the workplace? The following objectives were formulated to answer this question: 1. a review of scientific progress in conceptualizing and identifying differential sensitivity, considering the implications of these developments for policies and practices in the workplace; 2. an inventory and analysis of ethical issues posed by differential susceptibility to hazards on the job; 3. a detailed analysis of the alternative responses made in three cases--lower back injury, smoking linked with asbestos exposure, and lead exposure; 4. development of methodologies, based in theories of social justice, for assessing equity in occupational exposure to risk; 5. development of ethical guidelines and strategies that could incorporate project results in occupational health and safety policies and practices.

Methodology

The research tasks undertaken in pursuing the above objectives were: 1. a state-of-the-art review of recent developments bearing on the definition and identification of worker susceptibility to hazards, searching major data bases as well as scientific, medical, and trade journals; 2. an inventory of ethical issues, as identified in the literature, in practitioner experience, and in our case studies; 3. clarification of ethical issues by investigating relevant criteria (such as the source of sensitivity, who or what is protected, life-stage at which identification occurs, nature of the identification program, nature and predictability of the harm, nature of responses to differential sensitivity); 4. analysis of alternative responses using case studies--lower back injuries, smoking/asbestos, cotton dust exposure, alpha-1-antitrypsin deficiency, reproductive hazards--to explore the risk issues and the ethical problems involved in management responses; 5. the formulation of draft ethical guidelines for defining, identifying, and responding to differential susceptibility to occupational hazards.

Progress & Accomplishments

The objectives of this project have been largely realized. In some respects they have also become more ambitious than originally projected. The scientific and technological developments, and their implications, have been surveyed; a conceptual model of susceptibility has been developed and described; case studies, treating scientific, risk, and policy issues as well as alternative responses, have been completed for lower back pain, byssinosis and smoking, asbestos and smoking, reproductive hazards, and alpha-1-antitrypsin deficiency; ethical issues have been explored in four

chapter drafts as well as a tentative set of ethical guidelines. Each case study identifies and evaluates the ethical problems associated with differential susceptibility but also attempts to reach "closure" by suggesting how management should proceed. A solid analytical base supports the general ethical guides which we have formulated. A final task, in process, is to develop a decision path, or flow chart, to direct the application of the guides.

These results have been presented at several scientific meetings and symposia, including a full double session on "Managing Differential Susceptibility in the Workplace" chaired by Roger Kasperson at the annual meeting of the Society for Risk Analysis in October 1985.

Significance

The primary usefulness of the results is two-fold: 1. to identify the types of ethical problems which arise and show how they may be analyzed, and 2. to provide guidelines for management which may be employed (or adapted) by those responsible for decisions. In addition, it alerts other interested parties, some directly affected (e.g., workers), of their rights and needed protection.

It is our hope that this will have clear significance as these problems emerge in occupational health settings.

The entire project will be brought together in a book, tentatively entitled Managing High-Risk Workers: Scientific, Ethical, and Policy Problems, with extensive and fully documented treatment of the issues.

Publications - None to date

AWARD SUMMARY OF ACTIVE GRANTS DURING FY86

	COMPETING GRANTS		TOTAL GRANTS	
	NO. OF AWARDS	AMOUNT OF AWARDS	NO. OF AWARDS	AMOUNT OF AWARDS
Grants from FY86 Budget (\$6.221M)				
Research Project Grants (R01)	11	\$1,294,812	44	\$5,258,665
Career Development Grants (K01)	2	\$64,800	12	\$378,943
Small Grants (R03)	11	\$240,484	18	\$365,498
Other Grants	2	\$58,629	3	\$145,143
Subtotal	26	\$1,658,725	77	\$6,148,249*
Grants from FY85 Budget (\$6.50M)				
Research Project Grants (R01)	3	\$348,160	21	\$1,920,888
Career Development Grants (K01)	0	\$0	2	\$51,840
Small Grants (R03)	2	\$46,052	12	\$172,166
Other Grants	0	\$0	0	\$0
Subtotal	5	\$394,212	35	\$2,144,894
Grants from FY84 Budget (\$6.50M)				
Research Project Grants (R01)	2	\$81,391	8	\$847,316
Career Development Grants (K01)	1	\$32,400	1	\$32,400
Small Grants (R03)	0	\$0	6	\$135,411
Other Grants	1	\$40,000	1	\$40,000
Subtotal	4	\$153,791	16	\$1,055,127
Awards from all Years				
Research Project Grants	16	\$1,724,363	73	\$8,026,869
Career Development Grants	3	\$97,200	15	\$463,183
Small Grants	13	\$286,536	36	\$673,075
Other Grants	3	\$98,629	4	\$185,143
TOTAL	35	\$2,206,728	128	\$9,348,270

*Less than the FY86 budget because some of the funds were used to supplement awards made in FY85.

DISTRIBUTION OF GRANTS BY PROGRAM AREA

Program Area	Competing in FY86		Total Grants		
	No. of Awards	Amount of Awards	No. of Awards	Amount of Awards	Amt. Per.
Occupational Lung Diseases	5	\$265,691	23	\$1,471,097	16%
Musculoskeletal Injuries	3	\$112,183	9	\$679,398	7%
Occupational Cancers	4	\$327,248	12	\$1,099,595	12%
Traumatic Injuries	0	\$0	2	\$132,861	1%
Cardiovascular Diseases	0	\$0	2	\$140,782	2%
Reproductive Disorders	2	\$40,067	12	\$959,220	10%
Neurotoxic Disorders	1	\$166,870	13	\$1,191,937	13%
Noise-Induced Hearing Loss	0	\$0	7	\$542,531	6%
Dermatologic Disorders	0	\$0	8	\$290,299	3%
Psychologic Disorders	0	\$0	4	\$359,521	4%
Control Technology Research	0	\$0	4	\$257,962	3%
Respirator Research	2	\$134,082	12	\$1,016,938	11%
Other Occupational Concerns	9	\$612,584	20	\$1,206,129	13%
TOTAL	26	\$1,658,725	128 *	\$9,348,270	100%

* Exceeds FY86 budget of \$6,221,000 because total includes all grants that were active at any time during FY86.

A map of the United States divided into eight numbered regions (I-VIII) for a geography quiz. The regions are defined by the following state boundaries:

- Region I:** Includes Washington, Oregon, California, Nevada, Idaho, Utah, Arizona, New Mexico, Texas, Oklahoma, Kansas, Nebraska, South Dakota, North Dakota, Minnesota, Iowa, Missouri, Arkansas, Louisiana, Mississippi, Alabama, Georgia, Florida, South Carolina, North Carolina, Virginia, West Virginia, Maryland, Delaware, Pennsylvania, New Jersey, New York, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire, and Maine.
- Region II:** Includes Montana, Wyoming, Colorado, New Mexico, Texas, Oklahoma, Kansas, Nebraska, South Dakota, North Dakota, Minnesota, Iowa, Missouri, Arkansas, Louisiana, Mississippi, Alabama, Georgia, Florida, South Carolina, North Carolina, Virginia, West Virginia, Maryland, Delaware, Pennsylvania, New Jersey, New York, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire, and Maine.
- Region III:** Includes Montana, Wyoming, Colorado, New Mexico, Texas, Oklahoma, Kansas, Nebraska, South Dakota, North Dakota, Minnesota, Iowa, Missouri, Arkansas, Louisiana, Mississippi, Alabama, Georgia, Florida, South Carolina, North Carolina, Virginia, West Virginia, Maryland, Delaware, Pennsylvania, New Jersey, New York, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire, and Maine.
- Region IV:** Includes Montana, Wyoming, Colorado, New Mexico, Texas, Oklahoma, Kansas, Nebraska, South Dakota, North Dakota, Minnesota, Iowa, Missouri, Arkansas, Louisiana, Mississippi, Alabama, Georgia, Florida, South Carolina, North Carolina, Virginia, West Virginia, Maryland, Delaware, Pennsylvania, New Jersey, New York, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire, and Maine.
- Region V:** Includes Montana, Wyoming, Colorado, New Mexico, Texas, Oklahoma, Kansas, Nebraska, South Dakota, North Dakota, Minnesota, Iowa, Missouri, Arkansas, Louisiana, Mississippi, Alabama, Georgia, Florida, South Carolina, North Carolina, Virginia, West Virginia, Maryland, Delaware, Pennsylvania, New Jersey, New York, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire, and Maine.
- Region VI:** Includes Montana, Wyoming, Colorado, New Mexico, Texas, Oklahoma, Kansas, Nebraska, South Dakota, North Dakota, Minnesota, Iowa, Missouri, Arkansas, Louisiana, Mississippi, Alabama, Georgia, Florida, South Carolina, North Carolina, Virginia, West Virginia, Maryland, Delaware, Pennsylvania, New Jersey, New York, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire, and Maine.
- Region VII:** Includes Montana, Wyoming, Colorado, New Mexico, Texas, Oklahoma, Kansas, Nebraska, South Dakota, North Dakota, Minnesota, Iowa, Missouri, Arkansas, Louisiana, Mississippi, Alabama, Georgia, Florida, South Carolina, North Carolina, Virginia, West Virginia, Maryland, Delaware, Pennsylvania, New Jersey, New York, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire, and Maine.
- Region VIII:** Includes Montana, Wyoming, Colorado, New Mexico, Texas, Oklahoma, Kansas, Nebraska, South Dakota, North Dakota, Minnesota, Iowa, Missouri, Arkansas, Louisiana, Mississippi, Alabama, Georgia, Florida, South Carolina, North Carolina, Virginia, West Virginia, Maryland, Delaware, Pennsylvania, New Jersey, New York, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire, and Maine.

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Grant Number	Principal Investigator	Current Funding	Year	Page
2 R01 OH00823-07	Abou-Donia, Mohamed B., Ph.D.	\$166,870	86	121
2 R01 OH00835-08	Swartz, William J., Ph.D.	\$37,852	86	100
5 R01 OH00851-06	Spencer, Peter S., Ph.D.	\$176,554	85	123
5 R01 OH00923-05	Yu, C. P., Ph.D.	\$58,351	85	10
5 R01 OH00952-07	Scheving, Lawrence E., Ph.D.	\$134,013	86	125
5 R01 OH00978-06	Trudell, James R., Ph.D.	\$58,318	85	212
5 R01 OH00982-03	Bigos, Stanley J., M.D.	\$190,230	84	53
5 R01 OH01035-04	Graham, William G., M.D.	\$287,892	86	12
5 R01 OH01122-06	Kauffman, Charles W., Ph.D.	\$99,184	86	91
2 R01 OH01152-07	Henderson, Donald, Ph.D.	\$179,847	86	144
5 R01 OH01281-03	Juchau, Mont R., Ph.D.	\$114,466	84	102
5 R01 OH01301-03	Willeke, Klaus, Ph.D.	\$71,790	84	190
2 R01 OH01331-04A1	Bakale, George, Ph.D.	\$160,935	86	69
5 R01 OH01413-03	Peters, John M., M.D.	\$189,478	84	104
5 R01 OH01485-03	Liu, Benjamin, Ph.D.	\$98,842	85	192
5 R01 OH01511-02	Matanoski, Genevieve, M.D.	\$144,728	84	71
5 R01 OH01520-03	Thysen, Benjamin, Ph.D.	\$190,839	86	106
5 R01 OH01522-02	Puschett, Jules B., M.D.	\$80,999	85	214
5 R01 OH01531-02	Carlson, Gary P., Ph.D.	\$56,939	85	216
5 R01 OH01533-02	Gierthy, John F., Ph.D.	\$41,759	85	158
5 R01 OH01542-03	Wierda, Daniel, Ph.D.	\$71,480	85	218
5 R01 OH01556-03	Menter, Julian, Ph.D.	\$55,233	84	161
5 R01 OH01557-04	Moss, Andrew P., Ph.D.	\$72,582	85	72
5 R01 OH01595-03	Hinds, William C., Sc.D	\$62,011	85	194
5 R01 OH01603-03	Nichols, Larry D., Ph.D.	\$131,948	85	196
5 R01 OH01605-03	Swift, David, Ph.D.	\$69,061	85	14
5 R01 OH01608-03	DuBois, Arthur B., M.D.	\$57,754	85	198
5 R01 OH01617-02	Raven, Peter B., Ph.D.	\$94,583	85	200
5 R01 OH01630-04	Uyeki, Edwin M., Ph.D.	\$87,586	86	220
5 R01 OH01644-03	Underhill, Dwight W., Sc.D.	\$76,656	86	202
5 R01 OH01646-03	Corn, Morton, Ph.D.	\$119,866	85	204
5 R01 OH01650-03	Merchant, James A., M.D.	\$86,645	85	206
5 R01 OH01910-03	Hemminki, Kari J., Ph.D.	\$74,642	86	108
5 R01 OH01928-02S1	Sulzer-Azaroff, Beth, Ph.D.	\$33,677	85	94
1 R01 OH01929-01A1	Cook, Thomas M.	\$68,026	86	56
5 R01 OH01932-03	Perkins, Jimmy L., Ph.D.	\$72,733	86	183
1 R01 OH01956-01	Mital, Anil, Ph.D.	\$44,558	84	57
1 R01 OH01957-01	Stemhagen, Annette, Dr.P.H.	\$36,833	84	222
5 R01 OH01962-03	Schultz, Albert B., Ph.D.	\$138,739	85	58
5 R01 OH01968-02	Barnett, Rosalind C., Ph.D.	\$195,134	86	175
5 R01 OH01970-03	Hammad, Yehia Y., Sc.D.	\$41,644	86	16
5 R01 OH01972-03	De Caprio, Anthony P., Ph.D.	\$46,290	86	127
5 R01 OH02003-02	Abou-Donia, Mohamed B., Ph.D.	\$192,793	86	129
5 R01 OH02005-03	Harber, Philip I., M.D.	\$82,761	86	207
5 R01 OH02020-03	Sickles, Dale W., Ph.D.	\$64,881	86	131
5 R01 OH02021-02	Stedman, Donald, Ph.D.	\$87,649	86	163
5 R01 OH02024-03	Drury, Colin G., Ph.D.	\$108,627	86	177
5 R01 OH02027-03	Corbett, Michael, Ph.D.	\$73,722	86	74
5 R01 OH02029-03	Donham, Kelley J., D.V.M.	\$232,099	86	18
1 R01 OH02057-01	Kannel, William B., M.D.	\$121,342	85	96

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Grant Number	Principal Investigator	Current Funding	Year	Page
5 R01 OH02065-02	Spencer, Peter S., Ph.D.	\$147,311	86	133
5 R01 OH02066-02	Levine, Steven P., Ph.D.	\$120,938	85	223
5 R01 OH02067-03	Swanson, G. Marie, Ph.D.	\$258,679	86	76
5 R01 OH02076-02	Carter, Dean E., Ph.D.	\$129,194	86	20
5 R01 OH02084-02	Walsh, Patrick R., M.D.	\$165,058	86	60
1 R01 OH02104-01A1	Asal, Nabih R., Ph.D.	\$123,205	86	78
1 R01 OH02108-01	Pellizzari, Edo D., Ph.D.	\$141,155	86	225
1 R01 OH02114-01	Merrill, William W., M.D.	\$132,884	86	22
5 R01 OH02116-02	Johnson, Christopher M., Ph.D.	\$54,569	86	24
1 R01 OH02122-02	Fischbein, Alf, M.D.	\$101,605	86	80
5 R01 OH02128-03	Clark, William W., Ph.D.	\$132,641	86	146
5 R01 OH02132-02	Esmen, Nurtan A., Sc.D.	\$84,540	86	185
5 R01 OH02148-02	Cleary, Stephen F., Ph.D.	\$125,896	86	110
1 R01 OH02149-01	Ansari, Ghulam A. S., Ph.D.	\$101,559	86	227
1 R01 OH02154-01	Evans, John S., Sc.D.	\$85,453	86	209
1 R01 OH02185-01	Aposhian, H. Vasken, Ph.D.	\$75,132	86	26
5 R01 OH02191-02	Boekelheide, Kim, M.D.	\$118,855	86	112
1 R01 OH02221-01A1	Rappaport, Stephen M., Ph.D.	\$123,690	86	229
7 R01 OH02264-01	Kennedy, Thomas P., M.D.	\$86,126	86	28
1 R01 OH02303-01	Levine, Steven P., Ph.D.	\$115,903	86	230
5 R01 OH02317-02	Hamernik, Roger P., Ph.D.	\$134,217	86	148
7 R01 OH02351-01	Draper, William M., Ph.D.	\$69,031	85	82
7 R01 OH02384-01	Letz, Richard E., Ph.D.	\$157,787	85	135

Career Development Grants (K01)

5 K01 OH00002-03	Reiser, Karen M., M.D.	\$32,400	86	30
5 K01 OH00007-03	Shatos, Marie A., Ph.D.	\$32,400	86	32
5 K01 OH00011-02	Sokas, Rosemary K., M.D.	\$19,440	85	98
5 K01 OH00017-03	Guy, Richard H., Ph.D.	\$31,174	86	164
5 K01 OH00018-03	Kreiss, Kathleen, M.D.	\$32,400	86	33
5 K01 OH00019-03	Lantz, Robert C., Ph.D.	\$26,524	86	34
5 K01 OH00022-03	Cohen, Beverly S., Ph.D.	\$32,400	86	36
5 K01 OH00028-02	White, Roberta F., Ph.D.	\$32,400	86	179
1 K01 OH00031-01	Obrams, Gunta I., M.D.	\$32,400	84	84
5 K01 OH00035-03	Prichard, Howard M., Ph.D.	\$30,563	86	114
5 K01 OH00042-03	Talbott, Evelyn O., Ph.D.	\$32,400	86	150
1 K01 OH00054-01	Costa, Lucio, Guido, Ph.D.	\$32,400	86	231
1 K01 OH00055-01	Parker, David L., M.D.	\$32,400	86	232
5 K01 OH00059-02	Caruso, Rita L., Ph.D.	\$31,482	86	116
7 K01 OH00064-02	Letz, Richard E., Ph.D.	\$32,400	85	136

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Grant Number	Principal Investigator	Current Funding	Year	Page
<u>Small Grants (R03)</u>				
5 R03 OH01684-02	Kriebel, David, M.S.	\$13,132	85	38
5 R03 OH01715-02	Ip, Michael Po Chee, Ph.D.	\$24,750	85	40
5 R03 OH01718-02	Gerhardt, Kenneth J., Ph.D.	\$22,500	84	152
5 R03 OH01755-02	Marras, William S., Ph.D.	\$22,630	84	62
5 R03 OH01761-02	Spencer, Peter S., Ph.D.	\$28,125	84	137
5 R03 OH01825-02	Spear, Robert C., Ph.D.	\$11,802	85	234
1 R03 OH01826-01A1	Shalat, Stuart L., M.S.	\$24,450	85	139
5 R03 OH01827-02	Magee, Carol A., M.P.H.	\$5,080	85	117
5 R03 OH01829-02	Casali, John G., Ph.D.	\$19,500	85	154
5 R03 OH01830-02	Guy, Richard H., Ph.D.	\$19,590	84	166
5 R03 OH01833-02	Thompson, David C., M.S.	\$19,600	84	86
5 R03 OH01852-02	Armstrong, Thomas J., Ph.D.	\$6,000	85	64
5 R03 OH01865-02	Kreiss, Kathleen, M.D.	\$22,966	84	42
5 R03 OH01905-02	Nolan, Robert P., B.A.	\$8,775	85	44
5 R03 OH01990-02	Kowal, Charles D., M.D.	\$10,642	86	168
5 R03 OH02038-02	Sun, True-Jenn, M.S.	\$5,715	85	140
5 R03 OH02044-02	Froines, John R., Ph.D.	\$20,650	86	236
5 R03 OH02071-02	Brix, Kelley A., M.D.	\$23,360	85	181
5 R03 OH02091-02	Rheins, Lawrence A., M.D.	\$22,650	86	170
5 R03 OH02095-02	Dusenbery, David B., Ph.D.	\$14,748	86	142
5 R03 OH02098-02	Guernsey, Judith R., Ph.D.	\$20,723	86	46
5 R03 OH02101-02	Flynn, Michael R., Sc.D.	\$14,175	86	187
5 R03 OH02141-02	Feth, Lawrence L., Ph.D.	\$21,426	86	156
1 R03 OH02179-01A1	Vercruyssen, Max, Ph.D.	\$18,476	86	238
1 R03 OH02180-01	Cohen, Beverly S., Ph.D.	\$22,164	86	239
1 R03 OH02182-01	Dorn, C. Richard, D.V.M.	\$21,602	85	173
1 R03 OH02189-01	Ashizawa, Eiko A., M.P.H.	\$24,675	86	48
1 R03 OH02229-01	Yates, James W., Ph.D.	\$20,693	86	66
1 R03 OH02238-01	Talaska, Glenn G., Ph.D.	\$21,206	86	88
1 R03 OH02243-01	Connor, Thomas H., Ph.D.	\$21,902	86	90
1 R03 OH02258-01	Gandy, Jay, Ph.D.	\$17,556	86	119
1 R03 OH02265-01	Witek, Theodore J., Dr.P.H.	\$23,000	86	51
1 R03 OH02287-01	Chung, Min K., Ph.D.	\$23,464	86	68
1 R03 OH02321-01	Perrino, Brian A.	\$24,837	86	241
1 R03 OH02345-01	Sublet, Virginia Hill	\$22,511	86	120
7 R03 OH02377-01	Utterback, David F., Ph.D.	\$8,000	85	242

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1 R13 OH02117-01	Lockey, James E., M.D.	\$10,000	86	52
<u>Small Business Grants (R43, R44)</u>				
1 R43 OH02312-01	Stetter, Joseph R., Ph.D.	\$48,629	86	211
5 R44 OH01951-03	Bruce, Robert D., M.S.	\$86,514	86	188
<u>National Science Foundation Grant</u>				
1 RII 8217297	Kasperson, Roger E., Ph.D.	\$40,000	84	244

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Abou-Donia, Mohamed B., Ph.D.	Neuro	5 R01 OH02003-02	129
Ansari, Ghulam A. S., Ph.D.	Other	1 R01 OH02149-01	227
Aposhian, H. Vasken, Ph.D.	Lung	1 R01 OH02185-01	26
Asal, Nabih R., Ph.D.	Cancer	1 R01 OH02104-01A1	78
Bakale, George, Ph.D.	Cancer	2 R01 OH01331-04A1	69
Barnett, Rosalind C., Ph.D.	Psycho	5 R01 OH01968-02	175
Bigos, Stanley J., M.D.	Muscu	5 R01 OH00982-03	53
Boekelheide, Kim, M.D.	Repro	5 R01 OH02191-02	112
Carlson, Gary P., Ph.D.	Other	5 R01 OH01531-02	216
Carter, Dean E., Ph.D.	Lung	5 R01 OH02076-02	20
Clark, William W., Ph.D.	Noise	5 R01 OH02128-03	146
Cleary, Stephen F., Ph.D.	Repro	5 R01 OH02148-02	110
Cook, Thomas M.	Muscu	1 R01 OH01929-01A1	56
Corbett, Michael, Ph.D.	Cancer	5 R01 OH02027-03	74
Corn, Morton, Ph.D.	Respir	5 R01 OH01646-03	204
De Caprio, Anthony P., Ph.D.	Neuro	5 R01 OH01972-03	127
Donham, Kelley J., D.V.M.	Lung	5 R01 OH02029-03	18
Draper, William M., Ph.D.	Cancer	7 R01 OH02351-01	82
Drury, Colin G., Ph.D.	Psycho	5 R01 OH02024-03	177
DuBois, Arthur B., M.D.	Respir	5 R01 OH01608-03	198
Esmen, Nurtan A., Sc.D.	Contr	5 R01 OH02132-02	185
Evans, John S., Sc.D.	Respir	1 R01 OH02154-01	209
Fischbein, Alf, M.D.	Cancer	1 R01 OH02122-02	80
Gierthy, John F., Ph.D.	Derma	5 R01 OH01533-02	158
Graham, William G., M.D.	Lung	5 R01 OH01035-04	12
Hamernik, Roger P., Ph.D.	Noise	5 R01 OH02317-02	148
Hammad, Yehia Y., Sc.D.	Lung	5 R01 OH01970-03	16
Harber, Philip I., M.D.	Respir	5 R01 OH02005-03	207
Hemminki, Kari J., Ph.D.	Repro	5 R01 OH01910-03	108
Henderson, Donald, Ph.D.	Noise	2 R01 OH01152-07	144
Hinds, William C., Sc.D.	Respir	5 R01 OH01595-03	194
Johnson, Christopher M., Ph.D.	Lung	5 R01 OH02116-02	24
Juchau, Mont R., Ph.D.	Repro	5 R01 OH01281-03	102
Kannel, William B., M.D.	Cardio	1 R01 OH02057-01	96
Kauffman, Charles W., Ph.D.	Trauma	5 R01 OH01122-06	91
Kennedy, Thomas P., M.D.	Lung	7 R01 OH02264-01	28
Letz, Richard E., Ph.D.	Neuro	7 R01 OH02384-01	135
Levine, Steven P., Ph.D.	Other	1 R01 OH02303-01	230
Levine, Steven P., Ph.D.	Other	5 R01 OH02066-02	223
Liu, Benjamin, Ph.D.	Respir	5 R01 OH01485-03	192
Matanoski, Genevieve, M.D.	Cancer	5 R01 OH01511-02	71
Menter, Julian, Ph.D.	Derma	5 R01 OH01556-03	161
Merchant, James A., M.D.	Respir	5 R01 OH01650-03	206
Merrill, William W., M.D.	Lung	1 R01 OH02114-01	22
Mital, Anil, Ph.D.	Muscu	1 R01 OH01956-01	57
Moss, Andrew P., Ph.D.	Cancer	5 R01 OH01557-04	72
Nichols, Larry D., Ph.D.	Respir	5 R01 OH01603-03	196

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Perkins, Jimmy L., Ph.D.	Contr	5 R01 OH01932-03	183
Peters, John M., M.D.	Repro	5 R01 OH01413-03	104
Puschett, Jules B., M.D.	Other	5 R01 OH01522-02	214
Rappaport, Stephen M., Ph.D.	Other	1 R01 OH02221-01A1	229
Raven, Peter B., Ph.D.	Respir	5 R01 OH01617-02	200
Scheving, Lawrence E., Ph.D.	Neuro	5 R01 OH00952-07	125
Schultz, Albert B., Ph.D.	Muscu	5 R01 OH01962-03	58
Sickles, Dale W., Ph.D.	Neuro	5 R01 OH02020-03	131
Spencer, Peter S., Ph.D.	Neuro	5 R01 OH02065-02	133
Spencer, Peter S., Ph.D.	Neuro	5 R01 OH00851-06	123
Stedman, Donald, Ph.D.	Derma	5 R01 OH02021-02	163
Stemhagen, Annette, Dr.P.H.	Other	1 R01 OH01957-01	222
Sulzer-Azaroff, Beth, Ph.D.	Trauma	5 R01 OH01928-02S1	94
Swanson, G. Marie, Ph.D.	Cancer	5 R01 OH02067-03	76
Swartz, William J., Ph.D.	Repro	2 R01 OH00835-08	100
Swift, David, Ph.D.	Lung	5 R01 OH01605-03	14
Thysen, Benjamin, Ph.D.	Repro	5 R01 OH01520-03	106
Trudell, James R., Ph.D.	Other	5 R01 OH00978-06	212
Underhill, Dwight W., Sc.D.	Respir	5 R01 OH01644-03	202
Uyeki, Edwin M., Ph.D.	Other	5 R01 OH01630-04	220
Walsh, Patrick R., M.D.	Muscu	5 R01 OH02084-02	60
Wierda, Daniel, Ph.D.	Other	5 R01 OH01542-03	218
Willeke, Klaus, Ph.D.	Respir	5 R01 OH01301-03	190
Yu, C. P., Ph.D.	Lung	5 R01 OH00923-05	10

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Caruso, Rita L., Ph.D.	Repro	5 K01 OH00059-02	116
Cohen, Beverly S., Ph.D.	Lung	5 K01 OH00022-03	36
Costa, Lucio, Guido, Ph.D.	Other	1 K01 OH00054-01	231
Guy, Richard H., Ph.D.	Derma	5 K01 OH00017-03	164
Kreiss, Kathleen, M.D.	Lung	5 K01 OH00018-03	33
Lantz, Robert C., Ph.D.	Lung	5 K01 OH00019-03	34
Letz, Richard E., Ph.D.	Neuro	7 K01 OH00064-02	136
Obrams, Gunta I., M.D.	Cancer	1 K01 OH00031-01	84
Parker, David L., M.D.	Other	1 K01 OH00055-01	232
Prichard, Howard M., Ph.D.	Repro	5 K01 OH00035-03	114
Reiser, Karen M., M.D.	Lung	5 K01 OH00002-03	30
Shatos, Marie A., Ph.D.	Lung	5 K01 OH00007-03	32
Sokas, Rosemary K., M.D.	Cardio	5 K01 OH00011-02	98
Talbott, Evelyn O., Ph.D.	Noise	5 K01 OH00042-03	150
White, Roberta F., Ph.D.	Psycho	5 K01 OH00028-02	179

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Ashizawa, Eiko A., M.P.H	Lung	1 R03 OH02189-01	48
Brix, Kelley A., M.D.	Psycho	5 R03 OH02071-02	181
Casali, John G., Ph.D.	Noise	5 R03 OH01829-02	154
Chung, Min K., Ph.D.	Muscu	1 R03 OH02287-01	68
Cohen, Beverly S., Ph.D.	Other	1 R03 OH02180-01	239
Connor, Thomas H., Ph.D.	Cancer	1 R03 OH02243-01	90
Dorn, C. Richard, D.V.M.	Derma	1 R03 OH02182-01	173
Dusenbery, David B., Ph.D.	Neuro	5 R03 OH02095-02	142
Feth, Lawrence L., Ph.D.	Noise	5 R03 OH02141-02	156
Flynn, Michael R., Sc.D.	Contr	5 R03 OH02101-02	187
Froines, John R., Ph.D.	Other	5 R03 OH02044-02	236
Gandy, Jay, Ph.D.	Repro	1 R03 OH02258-01	119
Gerhardt, Kenneth J., Ph.D.	Noise	5 R03 OH01718-02	152
Guernsey, Judith R., Ph.D.	Lung	5 R03 OH02098-02	46
Guy, Richard H., Ph.D.	Derma	5 R03 OH01830-02	166
Ip, Michael Po Chee, Ph.D.	Lung	5 R03 OH01715-02	40
Kowal, Charles D., M.D.	Derma	5 R03 OH01990-02	168
Kreiss, Kathleen, M.D.	Lung	5 R03 OH01865-02	42
Kriebel, David, M.S.	Lung	5 R03 OH01684-02	38
Magee, Carol A., M.P.H.	Repro	5 R03 OH01827-02	117
Marras, William S., Ph.D.	Muscu	5 R03 OH01755-02	62
Nolan, Robert P., B.A.	Lung	5 R03 OH01905-02	44
Perrino, Brian A.	Other	1 R03 OH02321-01	241
Rheins, Lawrence A., M.D.	Derma	5 R03 OH02091-02	170
Shalat, Stuart L., M.S.	Neuro	1 R03 OH01826-01A1	139
Spear, Robert C., Ph.D.	Other	5 R03 OH01825-02	234
Spencer, Peter S., Ph.D.	Neuro	5 R03 OH01761-02	137
Sublet, Virginia Hill	Repro	1 R03 OH02345-01	120
Sun, True-Jenn, M.S.	Neuro	5 R03 OH02038-02	140
Talaska, Glenn G., Ph.D.	Cancer	1 R03 OH02238-01	88
Thompson, David C., M.S.	Cancer	5 R03 OH01833-02	86
Utterback, David F., Ph.D.	Other	7 R03 OH02377-01	242
Vercruyssen, Max, Ph.D.	Other	1 R03 OH02179-01A1	238
Witek, Theodore J., Dr.P.H.	Lung	1 R03 OH02265-01	51
Yates, James W., Ph.D.	Muscu	1 R03 OH02229-01	66

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Stetter, Joseph R., Ph.D.	Respir	1 R43 OH02312-01	211
Bruce, Robert D., M.S.	Contr	5 R44 OH01951-03	188
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Johns Hopkins University	Matanoski, Genevieve, M.D.	71
Louisiana State University Medical Center	Swartz, William J., Ph.D.	100
Mayo Foundation	Johnson, Christopher M., Ph.D.	24
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Harvard School of Public Health	Flynn, Michael R., Sc.D.	187
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University of Cincinnati	Rheins, Lawrence A., M.D.	170
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Hoover Keith & Bruce Inc	Bruce, Robert D., M.S.	188
<u>National Science Foundation Grant</u>		
Clark University	Kasperson, Roger E., Ph.D.	244

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