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**Environmental Biosciences Program
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Principal Investigator**

For

Cooperative Agreement DE-FC02-02CH11109

**Submitted to the
U. S. Department of Energy**

By The

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1.0 Introduction

In May 2002, the United States Department of Energy (DOE) signed Assistance Instrument Number DE-FC02-98CH11109 with the Medical University of South Carolina (MUSC) to support the Environmental Biosciences Program (EBP). This funding instrument replaces DOE Assistance Instrument Number DE-FC02-98CH10902.

EBP is an integrated, multidisciplinary scientific program, employing a range of research initiatives to identify, study and resolve environmental health risk issues. These initiatives are consistent with the Medical University's role as a comprehensive state-supported health sciences institution and the nation's need for new and better approaches to the solution of a complex and expansive array of environment-related health problems.

The intrinsic capabilities of a comprehensive health sciences institution enable the Medical University to be a national resource for the scientific investigation of environmental health issues. EBP's success in convening worldwide scientific expertise is due in part to the inherent credibility the Medical University brings to the process of addressing these complex issues.

Questions, comments or requests for further information concerning the activities under this cooperative agreement can be forwarded to Dr. Lawrence C. Mohr in the EBP office of the Medical University of South Carolina at (843) 792-1532.

1.1 Summary and Significance of Year Five Projects

Toxicology

- Trichloroethylene (TCE) is the most prevalent and widespread chemical contaminant at DOE sites. TCE is regulated as a human carcinogen based upon its hepatocarcinogenicity in a crude mouse model. Very little is known about the molecular mechanisms of carcinogenesis and the human health effects of TCE. MUSC has developed a comprehensive research program on the molecular mechanisms of disease pathogenesis and the human health effects of TCE to understand better the risks to workers at DOE sites. Through this research program, MUSC helps to ensure that TCE risk assessment and remediation activities are based upon sound science.
- PCBs and complex PCB mixtures are major environmental contaminants at DOE sites. Previous MUSC research has shown that complex mixtures of PCBs have immunotoxic effects on human lymphocytes and lymphocytes in laboratory mice. Previous work has also produced a method for the aerobic and anaerobic biodegradation of PCB mixtures by bacteria. Current research is underway to determine whether or not the bacterial biodegradation of complex PCB mixtures lowers toxicity to the immune system. This research is extremely important in demonstrating the usefulness of PCB biodegradation as a remediation technology that lowers human health risks.
- Asbestos is another major contaminant at DOE sites, and many of the workers at those sites are current or former smokers. It is well known that the risk of development of lung cancer is increased as much as 100 times in persons exposed to both asbestos and cigarette smoke. However, the molecular mechanism(s) by which cigarette smoke and asbestos exposure increase the incidence of lung cancer in humans are unknown. To address this need for research, a new project for year three will investigate the synergistic effects of cigarette smoke and asbestos exposure on the rate of programmed cell death. The data derived from this project will provide the mechanistic basis to identify biological markers that can be used in lung cancer risk assessment models for human exposure to cigarette smoke and asbestos.

Risk Assessment

- The adverse health effects of both ionizing and non-ionizing radiation are of concern to DOE and the public. Many important questions about the adverse human health effects of low-dose and low-dose rate radiation exposures remain unanswered – especially with respect to cancer risks. MUSC has developed a comprehensive research program for the study of the effects of low-dose and low-dose rate radiation exposures on human health.

- Population risk studies in areas surrounding DOE sites are of utmost importance to the department and to the citizens who live in these areas. The Savannah River Region Health Information System is a very important national, regional, and DOE resource for the study of population health effects in the area surrounding the Savannah River Site. In conjunction with the Savannah River Region Health Information System, MUSC has developed an extremely powerful Geographical Information System for population risk assessment in which databases containing health, environmental, demographic and socioeconomic data can be integrated and analyzed for population risks.

1.2 Program Expenditures

EBP Expenditure Summary First Quarter

The table below reflects **expenditures** by budgeted category recorded for the first two months, May 1, 2002 through June 30, 2002, and year-to-date, of Cooperative Agreement CH11109. Encumbrances, though not included in the figures below, include most personnel costs for the entire budget period and all F & A costs for the entire budget period, though not included in the figures below, total \$1,236,612.

<u>Budget Category</u>	<u>1st Qrt.</u>	<u>YTD</u>
	(Dollars in thousands)	
Personnel	\$ 173	\$ 173
Supplies	13	13
Travel	01	01
Other	00	00
Subcontract	00	00
Equipment	00	00
Indirect Costs	<u>116</u>	<u>116</u>
Total	\$ 303	\$ 303

2.0 Program Management and Development Office

The MUSC administration established the Program Management and Development Office to ensure the management of cooperative agreement efforts to meet the Program's goals and objectives. The Program Office responsibilities include: development and implementation of the program plan for the DOE cooperative agreement, development and implementation of major support systems necessary for managing and reporting on all EBP efforts, developing partnerships for the execution of programs with other universities and research institutions and the development of joint venture funding of environmental programs.

The Program Office reports to the Office of the Vice President for Academic Affairs and Provost. Key faculty and staff members involved in Program Management are as follows:

Principal Investigator:	Lawrence C. Mohr, Jr., M.D.
Associate Director for Program Development:	John B. Dunbar, Dr. P.H.
Associate Director for Administration and Finance:	Gail C. Brubaker, B.S.
Principal, Environmental Toxicology:	David Jollow, Ph.D.
Principal, Environmental Epidemiology and Risk Assessment:	David G. Hoel, Ph.D.
Program Coordinator:	Christina Constable, B.S.
Fiscal Analyst:	Anita G. Noisette, B.S.
Grants Coordinator:	Nora Futrell, B.S.
Administrative Coordinator:	Jill Canaday

3.0 Scientific Research

3.1 Environmental Toxicology Research Projects

3.1.1 Characterization of Species Differences in Trichloroethylene – Induced Peroxisome Proliferation and Hepatocyte Replication

Project Director:

JoEllyn M. McMillan, Ph.D.

Executive Summary

The hepatocarcinogenicity of trichloroethylene (TCE) is thought to be related to the ability of its metabolites, trichloroacetic acid (TCA) and dichloroacetic acid (DCA), to induce peroxisome proliferative and/or hepatocyte mitogenesis in B6C3F1 mice and rats. Humans are considered to be less sensitive to TCE, but their susceptibility to peroxisome proliferation and hepatocyte mitogenesis is largely unknown. The relative susceptibility of human vs. B6C3F1 mouse hepatocytes to peroxisome proliferation is of key importance for the use of mechanistic information in the reassessment of the carcinogenic risk posed by environmental TCE. Of importance, the role of the peroxisome proliferator activated receptor α (PPAR α) in the mitogenic response is unknown. It is believed that differences in the levels or activity of PPAR α between humans and rodents is important in the relative insensitivity of human hepatocytes to traditional peroxisome proliferators. Thus, defining the role of PPAR α in the mitogenic response and delineating differences in PPAR α activity in humans vs. rodents would contribute key mechanistic information for assessing the hepatocarcinogenic risk posed to humans by TCE exposure. The overall goal of this proposal is two fold: (1) to enhance our understanding of the epigenetic basis for TCE-induced hepatocarcinogenicity; and (2) to improve the assessment of relative risk of human vs. the B6C3F1 mouse hepatocarcinogenicity.

Relevance

The ability of peroxisome proliferators to induce peroxisomal and non-peroxisomal enzymes, the mitogenic activity of these compounds and their hepatocarcinogenic potential varies among species and is dependent upon the particular chemical agent being used. The proposed studies will provide valuable mechanistic data for determining the relevance of the B6C3F1 mouse model for assessing the hepatocarcinogenic potential in humans of TCE and other peroxisome proliferators. The studies will provide a quantitative comparison of the relative responsiveness of human versus mouse and rat hepatocytes to peroxisome-proliferator-induced changes in activities and levels of key proteins and mRNAs.

Objective

The hepatocarcinogenicity of TCE is believed to be related to the ability of its metabolites, TCA and DCA, to induce peroxisome proliferative and mitogenic activity in B6C3F1 mice and rats. Humans are considered to be less sensitive, but

their susceptibility to peroxisome proliferation and mitogenesis is largely unknown. The role of PPARa in peroxisomal enzyme induction in rodents is well documented. However its regulation of other non-peroxisomal genes is less understood. Differences in the levels and activity of this transcription factor have been observed between human and rodent liver. Thus determining the role of PPARa activation in both the peroxisomal and mitogenic responses in human and rodent hepatocytes is important in assessing the relative hepatocarcinogenic risk to humans of TCE exposure. To this end our specific aims are as follows.

Specific Aim 1. To develop sensitive and selective approaches to measure the peroxisome proliferative and mitogenic responses in cultured liver cells

Specific Aim 2. To elucidate the mechanism for the short-term *in vivo* hepatocyte replication response

Specific Aim 3. To determine the involvement of the peroxisome proliferator activated receptor a (PPARa) in peroxisomal and cell replicative events in rodent and human hepatocytes.

Quarterly Accomplishments

1. We have completed an *in vivo* treatment protocol in male PPARa knockout mice using the compounds TCA, DCA, and WY-14,643. The livers were removed and fixed for histological examination to determine hepatocyte replication rates. The remaining liver was frozen for examination of enzyme activities, transcription factor levels and activities and changes in cell signaling molecules.
2. We are continuing to examine the effect of cholera toxin (a protein kinase A activator) on peroxisomal enzyme induction in rat, mouse, and human hepatocytes.

Performance Schedule and Status of Aims

1. We will continue to examine the importance of protein kinase A activation in induction of peroxisomal enzymes and other related enzymes in B6C3F1 mouse, rat, and human hepatocytes.
2. We will continue our studies on the comparison of the peroxisome proliferative and cytotoxic activities of halogenated analogs of trichloroacetate and dichloroacetate in cultured hepatocytes and will submit the results for publication.
3. We will complete the staining procedures to determine changes in hepatocyte replication rates in the livers from the treated PPARa knockout mice.

3.1.2 Effects of Trichloroethylene Metabolites on Hepatic Cell-Cycle Regulatory Proteins and Transcription Factors

Project Director:

David T. Kurtz, Ph.D.

Executive Summary

This project explores the hypothesis that the epigenetic carcinogenicity of TCE results from the mitogenic activity of its metabolites. Mitogenesis may occur either via the peroxisomal response or by an independent mechanism. There are two specific research objectives: to determine how TCE metabolites cause increased cell growth and division in the liver and to develop quantitative tools to allow direct comparison of the responsiveness of humans vs. the laboratory rodent. The experimental approach will utilize cultured hepatocytes the B6C3F1 mouse, Long Evans and Sprague-Dawley rats, and long-term cultures of human hepatocytes, which have retained their differentiated properties. The ability of TCE and/or its metabolites to induce: cdk mRNAs and proteins; cyclin mRNAs and proteins; CKI mRNAs and proteins; and cyclin/cdk activity will be assessed. The activation of transcription factors associated with cell division (AP1, NF kappaB, E2F) and the inactivation of transcription factors associated with the suppression of cell division (C/EBP) will also be determined. To determine the importance of the peroxisome proliferator activated receptor (PPAR) in these inductions, the studies will also be carried out on hepatocytes from PPAR alpha -/- ("knockout") mice. These studies will provide valuable insight into the molecular basis of the non-genotoxic carcinogenic effects of TCE and related hazardous compounds. Furthermore, the measurements of cell cycle regulatory protein activity, and of transcription factors associated with cell proliferation, may prove to be an accurate biomarker for hepatocarcinogenesis.

Relevance

Trichloroethylene is a widespread contaminant at DOE sites. The toxicity of this compound to humans continues to be controversial. The studies outlined above should provide specific evidence for or against the hepatotoxicity of TCE.

Objective

The scientific problem being addressed in this proposal is the molecular basis for the hepatocarcinogenicity of TCE metabolites. The general approach will be a combination of biochemical, molecular biological, and cell biological techniques. To this end our specific aims are as follows.

Specific Aim 1. To determine the molecular mechanism(s) by which TCE metabolites can serve as priming agents for mitogenesis in rodent hepatocytes and to determine if this effect can occur in human hepatocytes.

Specific Aim 2. To identify the effects of TCE metabolites on signal transduction cascades which may affect cell division in hepatocytes

Specific Aim 3. To determine the effects of TCE metabolites on the activity of hepatocyte transcription factors which regulate cell division, and whether these effects require PPAR.

Quarterly Accomplishments

1. We have shown that DCA can enhance cdk4 activity in isolated rat hepatocytes after 48 hours of treatment
2. We have shown that DCA leads to an activation of cyclinE/cdk2 activity in rat hepatocytes
3. We have prepared nuclear extracts from PPAR alpha knockout mice which have been treated with TCE, TCA, DCA, and Wyeth-14643

Performance Schedule and Status of Aims

The project is on schedule and no significant changes in the specific aims are anticipated.

3.1.3 Cellular and Molecular Actions of the Trichloroethylene Metabolite 1,2-Dichlorovinyl-L-Cystine in Renal Proximal Tubular Cells

Project Director:

Rick G. Schnellmann, Ph.D.

Executive Summary

It is well known that trichloroethylene (TCE) induces nephrotoxicity and nephrocarcinogenicity in the rat and that there is strong evidence that a metabolite, 1,2-dichlorovinyl-L-cysteine (DCVC), is responsible for these renal toxicities (1-11). However, whether the activity of the renal pathway that leads to DCVC toxicity can be used in mechanistic-based risk assessment is far from clear. Further, while it is known that laboratory animal species vary greatly in their susceptibility to TRI-induced renal toxicity, there is only limited information on the differences in the response of renal cells from susceptible versus non- or less-susceptible species to the putative renal toxic metabolite, DCVC (1,7,10). Clearly, if the cellular effects of DCVC are to be used as a basis in risk assessment for dose extrapolation from laboratory animals to man, the relevance of this pathway must be delineated. This project will address these issues by 1) examining graded degrees of acute and chronic DCVC exposure of mouse, rat, rabbit and human renal proximal tubular cells (RPTC) on distinct and integrative cellular functions, and 2) by elucidating the gene expression changes that occur following graded degrees of acute and chronic DCVC exposure of mouse, rat and human RPTC. Completion of these studies will result in the identification of distinct and integrative cellular and genetic events that occur following DCVC exposure. Further, the use of multiple species will allow species-differences to be examined, particularly in relation to genetic changes, and

will improve the basis for risk assessment with respect to nephrocarcinogenicity and nephrotoxicity of TCE.

Relevance

The risk assessment of TRI is currently based on data from the B6 mouse hepatocarcinogenicity model. However, recent epidemiological data, augmented by molecular data on TRI-associated mutations in the von Hippel-Lindau tumor suppression gene, have raised the question that renal cell carcinoma may be more relevant for humans. In view of the possibility that a future risk assessment of TRI may be based on the renal carcinoma rather than on hepatocellular carcinoma, it is essential that we understand the underlying mechanism of this neoplasia. In particular, understanding the basis of relative sensitivity between rats and humans would be important for extrapolation of rat bioassay data for humans and for the recognition of supersensitive subpopulations of humans, if such exist.

Objectives

While DCVC is considered to be the metabolite most likely responsible for TRI nephrotoxicity and nephrocarcinogenicity in rats, only a limited number of studies have addressed the molecular mechanisms underlying these toxicities. We currently have little cellular and molecular information concerning the effects of acute and chronic exposure of renal cells to DCVC. Of importance, while we know that various rodent species differ in susceptibility to TRI-induced renal toxicity and neoplasia, we know little about why they differ; specifically, how the renal cells of these species vary in their cellular response to DCVC. The concentration-dependence of these effects will be crucial in dose extrapolation from the rodent to humans and to the recognition of susceptible human populations, if such exist. The long-term objective of this project is to determine the mechanism(s) by which DCVC and related metabolites injure renal cells and the basis for the relative resistance of less susceptible species. Direct comparisons will be made with human renal cells to provide the mechanistic basis for risk assessment purposes. Experimentally, we will expose mouse-, rat-, rabbit- and human-derived RPTC to the various concentrations of DCVC, acutely and chronically, that cause minimal cell death. The expression, and the time-dependence of the expression, of distinct differentiated and integrated cell functions (e.g. transport, migration, proliferation) and gene expression (using oligonucleotide microarrays) will be determined. The following variables will be examined: DCVC concentration, single DCVC exposure, multiple DCVC exposures and time, and will be related to the expression of specific genes and differentiated functions.

Specific Aim 1. To determine the effect of graded degrees of acute and chronic DCVC exposure on injury and death (necrosis and apoptosis) in mouse, rat, rabbit and human RPTC.

Specific Aim 2. To examine the expression of distinct differentiated cell functions, migration, and proliferation following targeted DCVC exposures in mouse rat, rabbit and human RPTC.

Specific Aim 3. To determine the effect of targeted DCVC exposures in mouse, rat and human RPTC on gene expression, using gene array technology.

Quarterly Accomplishments

This is a new project starting July 1, 2002. This laboratory has extensive experience in the toxicological significance of haloalkene-derived cysteine conjugates and other toxicants in the kidney. The proposed studies on TCE-derived cysteine conjugates are thus a continuation of a long-standing interest in the fundamental mechanisms underlying haloalkene nephrotoxicity and nephrocarcinogenicity, and the risks these compounds pose to human health.

Initial studies will focus on the development of methodologies to culture mouse and rabbit renal proximal tubular cells (RPTC) in primary culture that retain normal physiological functions at levels similar to that found *in vivo*. We will apply these methodologies to improve the culture of human and rat RPTC. The availability of RPTC from mice, rats, rabbits and humans will provide the basis for the comparison studies between rodent and human renal cells.

Performance Schedule and Status of Aims

Neither the performance status nor the status of aims has changed.

3.1.4 <u>Effect of Genetic Variation and of Ethanol on the Formation of Trichloroacetic Acid, a Putative Hepatocarcinogenic Metabolite of TCE</u>
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Project Director: David McMillan, Ph.D.
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Executive Summary

During this first quarter we have started a new project that will be comprised of two experimental systems. The first experimental series will be to obtain information regarding the expression of certain genes in rat primary hepatocyte cultures and human hepatocyte cell lines, in collaboration with Dr. JoEllyn McMillan. As a first step, we are interested in characterizing the expression of genes involved in the metabolism of TCE (CYP 2E1, alcohol and aldehyde dehydrogenases), and how gene expression changes in response to exposure to chloral hydrate (CH, the major oxidative metabolite of TCE) and ethanol. We are in the process of purchasing supplies, synthesizing primers and selecting commercially available gene arrays for these experiments. Secondly, we plan to develop and utilize a highly sensitive GC assay to quantify the TCE metabolites, TCA, trichloroethanol (TCOH) and DCA in rat and human hepatocytes with and without prior exposure to ethanol.

Objectives

1. To determine the kinetic constants for conversion of CH to TCA and TCOH in hepatocytes from the target species, mice and rats (including the back reaction of TCOH to CH and TCA).
2. To determine the kinetic constants for conversion of CH to TCA and TCOH in human hepatocytes.
3. To characterize the isoform composition of human hepatocytes by enzymic and DNA array technology.
4. To determine the effect of ethanol on the redox state of hepatocytes from mice, rats and humans.
5. To determine the effect of ethanol on conversion of CH to TCA and TCOH in hepatocytes from mice, rats and humans.

Quarterly Accomplishments

1. Synthesis of drug metabolizing enzyme DNA primers for genotyping experiments.
2. Obtained new quotes for purchase of GC with headspace analysis capability.

Performance Schedule and Status of Aims

The project is on schedule and no significant changes in aims have occurred.

3.1.5 Presystemic Elimination of Trichloroethylene and its Interactions with Alcohol: How Important are They at Environmental Exposure Levels?

Project Director:

James V. Bruckner, Ph.D.

Executive Summary

Although extremely high doses of trichloroethylene (TCE) are required to produce tumors in mice and rats, there is concern on the part of the EPA and others that even trace (i.e., environmental) levels may present a cancer risk to humans. The human body has a number of processes to protect against such low level toxic insults, including first-pass, or presystemic elimination. Volatile organic chemicals (VOCs) such as TCE that are absorbed from the gut are subject to metabolism by the liver and exhalation by the lungs, before they reach the arterial circulation and are distributed systemically. It has been theorized, but not demonstrated experimentally, that all of low oral doses of VOCs are removed by presystemic elimination. It will be necessary to develop very sensitive analytical techniques in order to conduct experiments with environmentally-relevant levels of TCE. Demonstration [experimentally and by physiologically-based pharmacokinetic (PBPK) modeling], that all of low oral doses of TCE are eliminated,

would have a profound effect on extrahepatic cancer and non-cancer risk assessments of TCE.

Alcohol (i.e., ethanol) and a number of other compounds are known to stimulate formation of increased amounts of cytochrome P450 2E1 (CYP2E1) in the liver. CYP2E1 is the key enzyme that initiates the oxidation of low doses of TCE to potentially mutagenic metabolites. Thus it is reasoned that drinkers metabolically activate a greater percentage of their systemically-absorbed dose of TCE to carcinogenic metabolites. Similarly, populations with genetically-determined elevations of CYP2E1 might also be anticipated to be at increased risk. The EPA uses this reasoning in their most recent health risk assessment of TCE, to support their choice of the most conservative (i.e., linear, no-threshold) mathematical model to predict cancer risks. Preliminary PBPK modeling efforts suggest that elevated CYP2E1 activity will not result increased metabolism of low, environmentally-relevant doses of TCE. Every human has CYP2E1 activity far in excess of that necessary to metabolize all of low doses. Since all of trace amounts of TCE are metabolized, it is reasonable to conclude that increased metabolic capacity due to alcohol, drugs, genetics, etc. is inconsequential. Laboratory experiments and PBPK modeling will be carried out to prove this hypothesis.

Objectives

1. Develop and validate assays of TCE and its major metabolites in biological samples, including blood, tissues and urine. The assays should be sufficiently sensitive to utilize in animal experiments employing very low doses of TCE
2. Accurately determine the capacity and dose-dependency of presystemic elimination of orally-administered TCE. Characterize the influence of dose and dosage regimen on the systemic disposition/effects of TCE and related VOCs.
3. Establish the influence (or lack thereof) of ethanol on the metabolic activation of low oral doses of TCE. Determine whether the ratio of the metabolites trichloroacetic acid (potentially carcinogenic) and trichloroethanol (non-carcinogenic) is altered by ethanol.

Quarterly Accomplishments

1. We have developed an assay for measuring trace levels of TCE in drinking water. Our initial efforts were devoted to measuring TCE levels in water, as it is a much simpler medium to extract/isolate VOCs from than are biological samples. We developed and fully validated a gas chromatography-mass spectrometry (GC-MS) method with a limit of quantitation of 5 $\mu\text{g/mL}$ (ppb). The technique is relatively rapid, sensitive and reproducible. It is described in detail in a manuscript entitled "A Validated GC-MS Assay for the Quantitation of Trichloroethylene (TCE) from Drinking Water," which we

have submitted for publication in the *International Journal of Environmental Analytical Chemistry*.

2. We have also developed an assay for measuring trace levels of TCE in target organs of animals. The liver is the major site of metabolism of TCE. The VOC is also metabolically activated in the kidneys and lungs. High, chronic doses of TCE produce tumors in all three organs of mice or rats. We therefore developed and validated a GC-MS method for quantitation of trace levels of TCE in these target tissues. Recoveries ranged from 79 to 87%. The limits of detection and quantitation were 1 and 5 ng/g (ppb), respectively. These levels are substantially lower than for other published assays. Our own method is described in detail in a manuscript entitled "Trace Level Determination of Trichloroethylene from Liver, Lung and Kidney Tissues by Gas Chromatography/Magnetic Sector Mass Spectrometry." This paper was recently accepted for publication in the *Journal of Chromatography*.

Performance Schedule and Status of Aims

Neither the performance status nor the status of aims has changed.

3.1.6 PBPK Modeling of Toxic Metabolites of Trichloroethylene in Rats, Mice and Humans: Predicting the Health Risks Posed by Low Level Exposure to TCE

Project Director:

Jeffery W. Fisher, Ph.D.

Executive Summary

Trichloroethylene (TCE) remains one of the most common ground water contaminants found in the US because of its disposal and use practices by the private sector, DOE and DoD. The projected costs for remediation of TCE in the federal sector is well over \$1 B. The health risks of TCE were recently reviewed by several scientists and published as a monologue in an Environmental Health Perspectives (EHP) Supplement (Vol. 108(2), 2000). Since the EHP publication on TCE, the US EPA released a draft 'regulatory risk assessment for TCE' to the authors of the EHP monologue and asked the authors to comment on their document. In July 2002 the US EPA convened a scientific review panel to review their most recent draft TCE document. Physiologically based pharmacokinetic (PBPK) models were used as an aid in dose-response assessment (risk assessment) for cancer and non-cancer toxicological endpoints. Five PBPK models were used on various human and rodents studies for cancer and non-cancer endpoints. Several data gaps were identified as the US EPA attempted to use the PBPK models of Fisher, Clewell and Barton. In some cases the PBPK models were inappropriately or insufficiently exercised. The objective of this project is to develop a single robust PBPK model for TCE for rodents and humans by incorporating new metabolic and kinetic data published since 1999, and by conducting limited critical metabolic and pharmacokinetic experiments in rodents to fill data gaps. The refined PBPK model for TCE and metabolites in laboratory animals and humans will be exercised in an appropriate manner,

and the results will be used to reduce the uncertainties associated with assessing the human health risks posed by low-level environmental exposure to TCE.

Much progress has been achieved over the last 5 years in understanding the quantitative aspects of metabolism of TCE in humans and rodents and in understanding the toxic and carcinogenic potential of the acid metabolites that are formed from metabolism of TCE. PBPK models have progressed from models that simply describing the parent chemical to PBPK models that contain sub models describing the formation and kinetics of metabolites such as trichloroacetic acid (TCA) , trichloroethanol, chloral hydrate and in some cases, dichloroacetic acid. Colleagues of mine and I have developed and published most of the PBPK models for TCE and metabolites in humans and rodents with financial support from the USAF, US EPA and Strategic Environmental Research and Development Program (SERDP). The US EPA used early-unpublished versions of our most recent PBPK models for mice and humans in their current draft risk assessment document.

Objectives

1. Harmonize current PBPK models used by the US EPA into one PBPK model for TCE and metabolites. Incorporate newly published and unpublished data in humans and rodents. New data sets include published and unpublished rat data on first pass metabolism of TCE from the laboratory of Dr. Jim Bruckner at the University of Georgia, published human and unpublished rat data on glutathione conjugation of TCE [(S-(1,2-Dichlorovinyl) Glutathione (DCVG)] obtained by Dr. Larry Lash at Wayne State University, and published Epidemiology studies performed in Europe, where urinary excretion of TCA was quantified.
2. Conduct laboratory studies to refine PBPK model predicted dose metrics in laboratory animal and humans that will be used in the formulation of the final product of this project, namely a TCE human health risk assessment. Determine the stoichiometric yield of DCVG for relevant doses of TCE in rats. Information on DCVG will provide data to develop the DCVG pathway in a PBPK model for TCE and to offer plausible dose-metrics that can be associated with the risk of kidney cancer in humans. Colleagues and I have time course data for DCVG in humans exposed to TCE vapors [Lash, L. H., D. A. Putt, W. T. Brashear, R. Abbas, J. Parker and J. W. Fisher. 1999. Identification of S-(1,2-Dichlorovinyl) Glutathione in the Blood of Human Volunteers Exposed to Trichloroethylene. *J. Toxicol. Environ. Health Part A*, 56, 1-21].
3. Conduct laboratory studies to evaluate how much dichloroacetic acid (DCA) is formed metabolically from TCE. This minor metabolite remains an important risk assessment issue because of its carcinogenic potency and the requirement that the US EPA account for cumulative risks. DCA is the number one by-product from chlorination of water. Thus, to account for the health risks poised by TCE in drinking water, the health risks from exposure to DCA itself must be quantified and accounted for in the health risk assessment of TCE.

4. Perform a cancer and non-cancer risk assessment for TCE using the harmonized single PBPK model for TCE and metabolites. The risk assessment will rely on 'mode of action' hypotheses and theoretical assumptions for low dose extrapolations. Relevant human data sets will be incorporated into the analyses.

Quarterly Accomplishments

Funding was accessible at UGA by mid July 2002. Dr. Debra Keys has accepted employment working on this research project starting 23 August 2002. Dr. Keys obtained her degree in Biomathematics from UNC. She conducted her dissertation research at CIIT under the advisement of Dr. Rory Conolly in first pass metabolism. She is highly qualified person to work on this project. Dr. Fisher has read a rough draft of the Science Advisory Panel's review of the EPA new draft document on TCE. He is evaluating key uncertainties in the PBPK modeling identified by the panel. Dr. Fisher plans on meeting with Dr. Bruckner and Jollow to discuss the first year's goals in PBPK modeling.

Performance Schedule and Status of Aims

New start. Schedule and status not changed.

3.1.7 Trichloroethylene Exposure and Host Genetic Factors in Autoimmune Diseases

Project Director:

Janardan P. Pandey, Ph.D.

Executive Summary

Several environmental chemicals have been associated with autoimmune diseases; however, in most cases, a definitive role for environmental agents in the initiation or exacerbation of autoimmune diseases is not firmly established. In particular, very little is known about the effects of the host genetic factors on the ability of environmental agents to initiate, perpetuate, or prevent autoimmune diseases. Identification of disease-associated single nucleotide polymorphisms (SNPs) will aid in fine-mapping the disease susceptibility genes. Moreover, the elucidation of the genomic response to environmental toxicants— toxicogenomics—may be helpful in identifying individuals with increased susceptibility to environmental agents. Understanding the role of environmental chemicals and the genetic factors in the induction of autoimmune diseases will aid in designing new tools for diagnosis and prophylaxis of these diseases. In addition to the possible identification of genes for systemic sclerosis (SSc), systemic lupus erythematosus (SLE), and myositis, the proposed investigation will contribute to the construction of haplotype maps of SNPs on chromosomes 2,6,10, and 14 that may be used for studies involving other diseases whose causative genes are known to be on these chromosomes. As emphasized at a recent NIH-sponsored meeting, building haplotype maps is the next phase of the human genome project.

Relevance

Understanding the role of chemicals like TCE and the host genetic factors in the induction of autoimmune diseases will be helpful in designing new tools for diagnosis and prophylaxis of these diseases. Identification of the disease-associated genetic markers may shed further light on the role of these polymorphic genetic systems in autoimmunity.

Objective

The overall long-term goal of this project is to identify the genetic and environmental factors which contribute to the pathways to autoimmunity. In particular, we would like to determine how certain genes of the immune system and those involved in the bioactivation of particular environmental toxicants interact in causing autoimmune diseases. We also plan to develop a murine model for use in dissecting the biological mechanisms underlying environmentally associated autoimmunity. Specifically, we would like to determine whether the exposure of mice to TCE causes activation of microchimeric cells and the appearance of dermal inflammation and fibrosis similar to that of graft-versus-host disease, a condition with remarkable similarities to SSc.

To the above end, during this cooperative agreement period, using a case-control study design, the proposed study will address the following specific aims:

Specific Aim 1. (a) To further estimate the magnitude of the association between TCE/silica exposure and SSc, SLE, and myositis and (b) to determine if the effect is modified by the prevalence of disease-specific autoantibodies — anti-topoisomerase I, anticentromere, and anti-RNA polymerase I and III in SSc; anti-Sm in patients with SLE; and anti-tRNA synthetases in myositis.

Specific Aim 2. To compare the distribution of particular genetic markers (HLA, TNF-a, TNF-b, IL-1b, IL-1RA, IL-10, CTLA-4, DNASE1, cytochrome P450IIE1, GM, and KM) and the recently-identified SNPs closely linked to them, among TCE/silica exposed SSc, SLE, and myositis patients with (a) non-exposed patients and (b) non-autoimmune controls.

Specific Aim 3. To compare the association of autoantibodies with the immunogenetic markers among TCE/silica-exposed and nonexposed SSc, SLE, and myositis patients.

Specific Aim 4. To develop a murine model for use in examining the role of microchimeric cells and TCE exposure in SSc pathogenesis.

Specific Aim 5. To construct transgenic mice with different combinations of CTLA-4 genotypes and expose them to TCE to determine the possible interactive effects of CTLA-4 alleles and TCE exposure in producing dermal inflammation and fibrosis.

Quarterly Accomplishments

1. The following editorial has been accepted for publication:
 - a. Pandey JP, LeRoy EC. A Profibrotic Polymorphism (of TGFb1) in Systemic Sclerosis. *Annals of Rheumatic Diseases* 2002. (In press).
2. A manuscript, which presents some recent results of our ongoing collaboration with Frederick W. Miller, M.D., Ph.D., Chief, Environmental Autoimmunity Group, NIEHS, has been accepted for publication:
 - a. Shamim EA, Rider LG, Pandey JP, O'Hanlon TP, Jara Quezada LJ, Samayoa E, Burgos-Vargas R, Vazquez-Mellado J, Alcocer-Varela J, Kutzbach AG, Malley JD, Targoff IN, de la Torre IG, Miller FW. Idiopathic Inflammatory Myopathy Phenotypes and Genotypes Differ in Mestizos and Caucasians Emphasizing Ethnogeographic Influences in the Genetics and Clinical Expression of Myositis. *Arthritis Rheum* 2002. (In press).
3. A manuscript, which describes the results of our recent studies on the role of CTLA-4 genes in SLE pathogenesis, has been accepted for publication:
4. Hudson LL, Rocca K, Song YW, Pandey JP. *CTLA-4* gene polymorphisms in systemic lupus erythematosus: a highly significant association with a determinant in the promoter region. *Human Genetics* 2002; In press
5. Initiated a collaboration with Professor Czirják László, Head of the Department of Immunology and Rheumatology, University of Pécs, Hungary. Professor Czirják has a computerized database and serum/DNA bank of patients with scleroderma, Raynaud's phenomenon, polyarthritis, and other connective tissue diseases. He also has data on organic solvent exposure for some of these patients. He will ship us DNA samples for genotyping various candidate genes for these diseases. This collaborative arrangement will be productive for both parties and save us considerable funds.
6. During the previous quarter, we planned the following:
 - a. Characterization of SLE patients and controls from NIEHS for CTLA-4 genes.
 - b. Submit manuscripts describing 1) the role of CTLA-4 genes in SSc pathogenesis, 2) the role of TNF and IL-1 genes in the etiopathogenesis of SLE.

- c. Characterization of myositis patients and controls from NIEHS for GM and KM genes.
- d. Determine the role of polymorphic genes encoding transforming growth factor b-1 — a cytokine involved in the production and degradation of extracellular matrix — in susceptibility to scleroderma.

Performance Schedule and Status of Aims

Neither the performance schedule nor the status of aims has changed.

3.1.8 Immunological Effects of Trichloroethylene Exposure

Project Director: Gary S. Gilkeson, M.D.

Executive Summary

In previous periods of funding, we have evaluated immunological function after chronic exposure to TCE via drinking water in adult lupus-prone (NZB/NZW) and normal inbred strains of mice (B6C3F1). Furthermore, we have developed a polyclonal rabbit serum that binds to TCE/protein adducts. In this series of experiments, TCE accelerated the onset and severity of lupus-like disease in NZB/NZW mice. Significant increases in autoantibody production also developed in the B6C3F1 strain of mice, suggesting potential development of autoimmune effects even in ‘normal’ mice. We believe these data, when combined with data from other laboratories, indicate that TCE is an environmental inducer/accelerator of autoimmune disease. Based on recent concerns regarding chronic exposure of TCE to families living at or near Camp Lejeune, NC, we propose to utilize our mouse models to evaluate the impact of TCE during early developmental periods of the immune system and assess predisposition or initiation of autoimmune disease. Thus, immune status should be evaluated after full life exposures to TCE to include stages of *in utero* development, neonatal development, puberty, and early adulthood. There is growing concern about the effects of *in utero* and childhood exposure to environmental toxins. It is of obvious importance to determine if *in utero* exposure to common environmental toxins has effects on the developing immune system. These experiments in normal and lupus-prone mice will provide insight into potential effects on immunological function and the development of autoimmune disease that can be used in assessment of risk for the human population, and in particular, children.

Objective

The purpose of this project is to define the impact of TCE exposure on immunological function, with particular emphasis on autoimmune disease.

Specific Aim 1. Determine the immunological effects of *in utero* and early life exposure to low-levels of TCE (1,000 ppb and 10,000 ppb in the drinking water) in a non-autoimmune prone mouse strain (B6C3F1), with particular emphasis on the detection of autoimmune manifestations.

Specific Aim 2. Determine the effects on autoimmune disease development/progression in NZB/NZW mice exposed *in utero* and during early life to low-levels of TCE (1,000 ppb and 10,000 ppb in the drinking water). Effects attributed to *in utero* and early life exposure will compliment earlier studies with adult mice as the same strains of mice and levels of TCE will be utilized. Furthermore, the proposed study will also permit direct comparisons between the immune effects of male and female mice exposed to TCE during these early developmental periods.

Quarterly Accomplishments

1. C57 female mice and C3H male mice were bred while being exposed to TCE. B6C3F1 pups were born and are being tested at 3 and 8 weeks of age.
2. Results are being analyzed in order to submit an abstract in September to the Toxicologist.

Performance Schedule and Status of Aims

The project is on schedule and no changes have been made in the status of aims.

3.1.9 Molecular Mechanism of Pathogenesis in a Model of Trichlorethylene-Induced Congenital Heart Disease: Roles of Growth Factors, Extracellular Matrix (ECM) Proteins, and Matrix Metalloproteinases (MMPs)

Project Director:

Stanley Hoffman, Ph.D.

Executive Summary

Increased numbers of heart defects occur in children born where the water supply is contaminated with trichloroethylene (TCE), suggesting that TCE is teratogenic in humans. TCE has been reported to have teratogenic effects on chick and rat embryo hearts, without apparent effect on other organs. Heart malformations usually involve structures that form by epithelial-mesenchymal transformation (EMT). In this process which is repeated several times during heart development, a subset of cells in an epithelial sheet detach and migrate into the underlying basement membrane where they then differentiate in a novel direction. For example, endocardial cells undergo EMT and differentiate into valves and septa. In our laboratory, we use a cell line, QCE-6 cells, and explants of embryonic heart tissue to model EMT. This cell line has allowed us to identify biochemical changes that accompany and control EMT. Of particular interest are developmental changes in proteins present in the ECM and enzymes involved in the remodeling of the ECM that are capable of regulating cell behavior. Specifically,

chondroitin sulfate proteoglycans may be critical components of the ECM because they are present in dynamically changing distributions in the developing heart and have been shown to regulate cell-cell and cell-ECM adhesion and subsequent intracellular signaling. MMPs are a major family of enzymes involved in the remodeling of the ECM. Moreover, our recent studies demonstrate that blocking MMP activity blocks both EMT and accompanying cell differentiation.

Relevance

The purpose of this project is to identify the molecular mechanisms associated with normal EMT and heart development that are affected by TCE. This information will provide a basis to assess the teratogenic potential of TCE for humans and, if TCE is indeed teratogenic in humans, to determine whether some individuals may show super-susceptibility. These studies may also suggest better methods to recognize and treat cardiac malformations induced by TCE.

Objectives

1. Determine whether TCE or its metabolites affect the morphology of the developing chicken and rodent hearts *in vivo*.
2. Determine when in development TCE-induced heart defects first appear and in what region of the heart.
3. Determine whether the morphological defects induced by TCE can be correlated with concomitant biochemical defects, particularly in components of the ECM involved in EMT including MMPs and chondroitin sulfate proteoglycans.
4. Determine whether experimentally reversing the effects of TCE on the composition and function of the ECM will also reverse its effects on heart morphology.

Quarterly Accomplishments

In the current quarter, we have initiated efforts to demonstrate the effects of TCE on heart development *in vivo*. To ensure the success of these studies, these studies are being performed in collaboration with Drs. Edward Krug and Noboru Mishima in the Department of Cell Biology here at MUSC. Drs. Krug and Mishima both have years of experience in studying heart development and are adept in many specialized skills that will be required in these studies including the manipulation of early chicken embryos and the ability to recognize subtle morphological defects in the developing heart. Two methods of treating chicken embryos with TCE or its metabolites are being worked on. In one method that we have learned from the literature, mineral oil containing TCE is injected into the air space in the egg. In a second method, we are using whole embryo culture outside

of the egg shell. This method has the advantages that: 1) the concentrations of TCE and metabolites that are present are more readily varied and quantified and 2) a mixture of TCE and metabolites similar to what human embryos may experience where the water supply is contaminated with TCE may be generated by co-culturing embryos with hepatocytes in the presence of TCE. It has the disadvantage that the culture must be initiated at a specific time in development and can only be continued for two days. Therefore, it will be necessary to scan developmental stages using this assay in order to find the stage when heart development is most sensitive to TCE and its metabolites. In the experiments that are now underway, TCE treatment is begun at stage 14 (the time when the EMT that turns the heart from a simple tube into a four-chambered structure is beginning).

EMT can be modeled in an *in vitro* assay in which cells from explants of early heart tissue migrate into a three-dimensional collagen gel. In recent experiments, we could not find an effect of TCE on EMT using this assay. We have not yet used this assay in conjunction with TCE metabolites. In any case, the *in vivo* experiments described above are the obvious gold standard for determining whether TCE and/or its metabolites can act as a cardiac teratogen *in vivo*. Nevertheless, we will continue to put some effort into the *in vitro* assay because this assay, if successful, would be particularly useful for biochemical studies on the molecular mechanisms underlying the putative teratogenic effect of TCE on heart development.

Performance Schedule and Status of Aims

As indicated above, experiments on TCE (and its metabolites) as a cardiac-specific teratogen in chicken embryos *in vivo* (Objective 1) are underway. These experiments will probably go on for at least a year as they will probably have to be repeated and optimized many times. The remaining objectives cannot be performed until we become more sophisticated in successfully performing the experiments that make up Objective 1.

3.1.10 Biomarkers of Synergism Between Asbestos and Cigarette Smoke for Development of Bronchogenic Carcinoma and Lung Cancer

Project Director:

Alice Boylan, M.D.

Co-Director:

Besim Ogretman, Ph.D.

Executive Summary

It has long been known that workers occupationally exposed to asbestos who also smoke carry a very marked increase incidence of bronchogenic carcinoma and lung cancer. The interaction is clearly synergistic; however, the mechanism of this synergism is unknown. We presently lack biomarkers of early stages of the disease process and biomarkers that would distinguish which workers are most susceptible to this synergism. Current information suggests that cigarette smoke increases the uptake of asbestos fibers into

airway walls and that there is a more than additive increase in hydroxyl radical damage to cellular DNA. Such DNA-damaged cells would normally die by an apoptotic mechanism. The hypothesis under study here is that cigarette smoke and asbestos also synergistically increase resistance to apoptosis by changing the level of expression of apoptosis-related genes (e.g., bcl-2, bcl-x, bax and p53) in normal and/or cancerous human lung epithelial cells that are resistant to these agents.

Relevance

Occupational exposure to asbestos is of major concern for worker health. Exposure to small amounts of asbestos can cause bronchogenic carcinoma and lung cancer in susceptible individuals. This risk is synergistically increased by exposure to tobacco smoke. The availability of biomarkers for exposure, early stage of response and enhanced susceptibility would greatly enhance the risk assessment and risk management of workers exposed to asbestos.

Objectives

1. To determine expression of apoptosis-related genes in cells that have developed resistance to asbestos and cigarette smoke, and correlate these findings with levels of expression in airways from exposed and unexposed persons.
2. To determine the pathways involved in the development of resistance to asbestos-induced apoptosis that contribute to development of a malignant phenotype.
3. To determine the effect of asbestos exposure on the K-ras pathway, we will employ a mouse tumor models that conditionally expresses the K-ras transgene in the lung.

Quarterly Accomplishments

1. In this quarter, we have performed more experiments comparing gene expression in A549 cells resistant to asbestos with the sensitive parental cell line using microarray analysis. The experiments were repeated three times for statistical analysis. We consistently found significant down-regulation of heat-shock protein 70 in cells resistant to asbestos. We also found, these cells had significant upregulation of Niemann Pick Disease protein and caveolin-2, which are related to fatty acid metabolism. Finally, we found a three-fold increase in BCRA-2 expression. We are in the process of confirming these changes in gene expression using Northern and Western blot analysis. Repeated attempts at developing an airway epithelial cell line from primary cultures that is resistant to asbestos and or cigarette smoke have not been successful. We have obtained new immortalized airway epithelial cell lines to determine if the changes found in A549 cells are seen consistently with the development of asbestos resistance. In addition, we have received human approval to begin analysis of bronchial biopsy specimens from normal airways and primary lung cancers for expression of the apoptotic

proteins found to have altered expression in our previous work. We plan to correlate the patient's occupational and smoking histories with the patterns of expression found.

2. We have begun the production of transgenic mouse colonies that express murine K-Ras4b^{G12D} under the control of doxycycline in type II pneumocytes. These mice were generously provided by our collaborator Galen Fisher who showed in previous work that induction using doxycycline for two months led to the development of adenomas and adenocarcinomas in the lung of all mice. However, removal of doxycycline caused a rapid fall in levels of mutant K-Ras RNA associated with apoptotic regression of all tumors. Once the colony is established we plan to expose mice to low levels of asbestos prior to induction with doxycycline. We will evaluate the mice for changes in tumor burden and changes in apoptotic regression. This will provide a tool to determine the effect of asbestos exposure on proteins involved in the K-ras pathway.

Performance Schedule and Status of Aims

The project is on schedule as proposed, and there are no changes in the status of aims.

3.1.11 <u>Immunotoxicological Assessment of Non-degraded and Biodegraded PCB Mixtures</u>
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Project Director:

Lucille London, Ph.D.

Executive Summary

The long-term goal of our laboratory is to understand the biological process by which complex mixtures of contaminants can be degraded in the environment and to apply that knowledge to better understand potential human health effects associated with exposure. We will focus on the biodegradation of complex mixtures of polychlorinated biphenyls (PCBs) and their subsequent immunotoxicological effects. The potential for existing and newly emerging bioremediation technologies to treat complex waste sites is based upon their ability to remove these chemicals from contaminated environments. However, there has been little attempt to correlate disappearance of contaminated material with a discernible decrease in the health hazards associated with biotreated materials. Little is known about the immunotoxicity of the partial degradation products of PCBs; in particular whether the spectrum of effects may be different from that of the parent compounds. This project is focused on the assessment of the toxicity of PCB mixtures after specific dechlorination patterns achieved after anaerobic dechlorination in the laboratory. The project will assess the ability of both non-degraded and biodegraded PCB mixtures to modulate the murine B cell mitogenic response to lipopolysaccharide. The ability of B cell to secrete antibody in the presence of PCBs will also be evaluated. In addition, we will evaluate the effect of PCBs on the induction of apoptosis in lymphocytes (both B and T cells). We hypothesize that the immunotoxicity of biodegraded PCBs will be lower than the immunotoxicity of the commercial mixtures

since the biodegradation process results in the dechlorination of the PCB mixture and an association of increased immunotoxicity *in vivo* and in our *in vitro* proliferation assay correlates with more heavily chlorinated aroclors. An understanding of how the toxicity of specific PCB mixtures change after bioremediation in the laboratory will help determine the potential toxicity associated with PCB contamination in the environment.

Relevance

Previous studies in the laboratory have shown that Aroclor mixtures inhibit lipopolysaccharide (LPS)-induced splenocyte proliferation and antibody secretion at similar concentrations. We have extended these studies to include PCB congeners from all three classes, focusing on those congeners which accumulate in breast milk. Our results demonstrate selectively higher immunotoxicity from noncoplanar congeners that bioaccumulate. When compared with AhR expression data, these results suggest this immunotoxic response is not mediated through the AhR pathway.

Objectives

1. Using two *in vitro* B cell specific assays, proliferation and immunoglobulin secretion, determine whether chlorine position on the PCB molecule is important for PCB induced immunotoxicity.
2. Using two *in vitro* B cell specific assays, proliferation and immunoglobulin secretion, determine whether anaerobic dechlorination effects the pattern of toxicity observed between parent (non-dechlorinated) and dechlorinated compound.
3. Determine whether PCB Aroclor, individual congeners, or anaerobic dechlorinated PCBs induce apoptosis in B cells.

Quarterly Accomplishments

1. No significant decrease in the induction of surface IgM or IgK was observed after stimulation of 70Z/3 cells with LPS in the presence of 1, 10, 12.5, 15, and 17.5 ppm Aroclor 1242.
2. At 20ppm Aroclor 1242, a significant decrease in viability was observed on 70Z/3 cells induced with LPS. We will determine whether this decrease in viability is due to apoptosis and if so, by which apoptotic pathway. (not enough live cells at 20ppm)
3. Poster presentation at national meeting of the American Society of Microbiology:
 - a. Ashley Smithwick, John Quensen, Andrew Smith, Lucille London, and Pamela Morris. Impact of Anaerobic Dechlorination on PCB Immunotoxicity. American Society for Microbiology, Salt Lake City, UT, May 19-23, 2002.

4. Western analysis of aryl hydrocarbon receptor (AhR) expression following exposure to Aroclor 1242 and fractions of multi-*ortho* and mono- and non-*ortho* substituted PCB congeners reveals no activation of the AhR following exposure to any of the three mixtures.
5. We have completed an analysis of noncoplanar congeners that accumulate in breast milk. A manuscript is currently in preparation and will be sent out for review by August 1, 2002.

Performance Schedule and Status of Aims

No significant deviation from performance schedule or specific aims is anticipated.

3.2 Environmental Epidemiology and Risk Assessment Projects

3.2.1 Low Dose Radiation: Statistical Models of Childhood Leukemia Risk

Project Director:

David G. Hoel, Ph.D.

Executive Summary

The relationship between low-dose ionizing radiation exposure and the development of childhood leukemia is a matter of international interest and concern. Recent studies in the United Kingdom have generated worldwide interest in the question of whether or not there are increased rates of childhood leukemia in the vicinity of nuclear facilities. Similar studies have been conducted in several locations throughout the world. These include the Pickering Ontario Site (Canada), The Kruemmel Site (Germany), and the Savannah River Site (SRS, USA). An increased rate of childhood leukemia was found in areas near the Kruemmel Site. An increased rate of childhood leukemia was also found in the vicinity of the Pickering Ontario reactor site. The results of this study barely failed to achieve statistical significance. There was no increased incidence of childhood leukemia found in the SRS region; however, data was not collected in this region until 1991. Because the results of the Pickering Ontario study were only slightly insignificant, the incident of childhood leukemia in the vicinity of this site warrants further research.

Each of the above three sites is similar in that tritium is the primary environmental exposure of concern. The purpose of the project is to perform a comprehensive, detailed epidemiological study of the incidence of childhood leukemia in the vicinity of the Pickering Ontario reactor site. In addition, a joint analysis of combined data from all three sites will be analyzed in order to better resolve the question of childhood leukemia in the vicinity of tritium-releasing nuclear facilities.

Relevance

This project will provide important new information on the risk of developing childhood leukemia in the vicinity of nuclear facilities. The development of a multidimensional time-space risk model will provide a new statistical tool for the analysis of disease clusters related to environmental exposures.

Objective

The objective of this project is to examine various statistical models to determine whether there is excess risk of leukemia to children exposed to low levels of ionizing radiation residing near Pickering Nuclear Facility in Ontario, Canada.

Specific Aim 1. To continue our collaborative research relationship with the Ontario Cancer Registry (OCR).

Specific Aim 2. To use smoothed standard mortality ratios and proportional hazard models to compare childhood leukemia near Pickering and three control areas.

Specific Aim 3. To use spatial statistics to determine whether childhood leukemia rates decrease towards background rates with increasing distance from Pickering Nuclear Facility.

Specific Aim 4. To conduct a meta-analysis to determine an overall risk of childhood leukemia near nuclear facilities.

Quarterly Accomplishments

1. All data for the meta-analysis has been collected and entered into a database and are currently being analyzed.
2. IRB approval for the Pickering study has been ascertained from the Medical University of South Carolina, the University of Toronto, and OCR. Currently, OCR is abstracting and preparing the data.

Performance Schedule and Status of Aims

Neither the performance schedule nor the status of aims has changed.

3.2.2 Low Dose Radiation: Toxicological Models of Cancer Risk

Project Director:

David G. Hoel, Ph.D.

Executive Summary

The use of experimental animals in radiation risk estimation is especially important for those situations when human data are inadequate or unavailable. This is particularly true for neutron exposures and low-dose rate exposures to gamma and x-ray. The purpose of this project is to apply biological based models to radiation risk estimation using experimental data.

Basic biological/mathematical models of radiation induced double strand chromosome breaks and misrepair have been developed and applied to the estimation of radiation risk of chronic myelogenous leukemia (CML), which is understood to be the result of a single specific translocation. Using this biomathematical modeling, it has been shown that CML risk estimates are considerably less than what is obtained from extrapolating to low doses some highly variable epidemiological data. Using the idea of susceptible stem cells it is also shown that the dose response is nonlinear at low doses. In addition, computer algorithms have been developed for biological based two stage mutation cancer models (Moolgavkar) for the analysis of lifetime mouse studies.

Relevance

By comparing the Moolgavkar risk models with the *in vivo* experimental data from the Argonne National Laboratory, the investigators will not only increase understanding of cancer development following low-dose radiation exposure, but also add biological credibility. This approach will provide a method for answering the important environmental question of whether risks are decreased with decreasing dose-rate, a key issue for chronic radiation control of workplace exposures.

Objective

The objective of this project is to determine the effects of dose-rate and radiation type on the development of various cancer types following low-dose radiation exposures. Two-stage biologically based Moolgavkar risk models will be used for analysis. Using previously validated data, assumptions made about the biological effects of ionizing radiation can be used in the two-stage model to predict dose-rate effects on the development of various cancers following low-dose exposures.

Specific Aim 1. To use the large Argonne National Laboratory Janus mouse study to answer basic questions concerning dose-rate and radiation type effects on cancer. This involves over forty thousand mice exposed acutely and chronically at several doses and using either gamma or neutron.

Quarterly Accomplishments

1. A low dose subset of experimental mortality data from experiments conducted at the Argonne National Laboratory on the effects of exposure of B6CF₁ mice to whole-body irradiation, gamma rays (< 300cGy) or fission neutrons (< 30cGy), were analyzed to assess the shape of the dose response and the effects of fractionation. The Cox proportional hazards model was used as an empirical model, while the two-stage clonal expansion model was used as the biologically based cancer model in which information on the carcinogenesis process is incorporated into the model. The two models resulted in similar descriptions of the dose response curves, cancer risks, neutron relative biological effectiveness and dose rate effectiveness factor associated with exposure to ionizing radiation. Both models suggest that a dose-response curve linear in dose provides an adequate fit to the data. Fractionation reduced the effectiveness of gamma radiation.
2. High dose studies were also analyzed. The mice were grouped based on their sex, exposure pattern (single exposure or 60 once-weekly exposures) and total accumulated dose of exposure with doses ranging from 0 cGy to 1839 cGy for gamma exposure and 0 cGy to 226 cGy for neutrons. The two-stage model and the Cox proportional hazards model were again used to compare the results of an empirical model with a model based on biological information on the carcinogenesis process. Both the neutron and gamma dose response curves

appear linear at the lower doses (less than 30-40 cGy), before the nonlinearities become evident. The findings suggest a reduction in the effectiveness of gamma irradiation with fractionation, while the effectiveness of neutrons increases with fractionation.

3. Two manuscripts have been prepared and have been sent to Radiation Research for publication.

Performance Schedule and Status of Aims

Neither the performance schedule nor the status of aims has changed.

3.2.3 Low Dose Radiation: Epidemiological Risk Models

Project Director:

David G. Hoel, Ph.D.

Executive Summary

The data used for estimating health risk from low LET radiation (e.g. x-ray, gamma) has been obtained from the A-bomb survivor cohort. This group, along with some cohorts of high dose medically exposed individuals makes up our source of information. Two important issues are of current concern: 1) Does the risk of cancer follow a linear dose-response at low-doses?, 2) Are individuals exposed at older ages (i.e. greater than 45 years) more susceptible to developing cancer than expected?

We have shown that the cancer risks at low-doses based upon the A-bomb data over estimates cancer risk. We have incorporated errors in dosimetry into the analysis of cancer risk and are proceeding to evaluate the risk at low doses of radiation exposure.

Relevance

Using Japanese bomb survivor data, the investigators seek to refine our understanding of the mathematical relationship between health outcomes (cancer) data and exposures to low-dose radiation. The issue of whether the relationship is linear or non-linear continues to be controversial. This project will address this very important scientific issue.

Objective

The shape of the dose-response function for radiation-induced carcinogenesis in humans has depended primarily on data obtained from the Japanese A-bomb survivors. This project will re-examine these data with respect to the linearity of cancer risks from low dose (1-10 rem) radiation exposures. An analysis of A-bomb survivor data for solid tumors and leukemia indicates that there is a non-linear relationship to carcinogenesis following low-dose radiation exposure. Uncertainty in the dose estimates, including underestimation of neutrons and a relative biological effectiveness (RBE) that varies with dose are being

incorporated into this low-dose analysis. This comprehensive and focused analysis of epidemiological data from Japanese A-bomb survivors will greatly increased our understanding of the true epidemiological relationship between cancer risks and low-dose radiation exposure. In addition, DOE worker data which has been reported as providing the scientific basis for an increased susceptibility from exposure at older ages will be evaluated and contrasted with the A-bomb data.

Specific Aim 1. To carefully perform statistical modeling of the available epidemiological data from the A-bomb survivor cohort and the DOE worker cohort in order to increase our understanding of the cancer risk related to low-dose radiation exposure and the effect of older age on the magnitude of this risk.

Specific Aim 2. Epidemiological data from the A-bomb survivor cohort is being used to develop the biomathematical model of cancer risk. The previously published models for dose uncertainty and neutron exposure are being incorporated into our analysis. The DOE worker data from CEDER (DOE's data repository) will be used to evaluate the effect of older age cancer risk following low-dose radiation exposure. The entire set of available worker data will be modeled in order to evaluate the older age issue. The results of the worker analysis will then be compared to the analysis of the acutely exposed A-bomb survivors.

Quarterly Accomplishments

1. Cancer incidence and mortality data from the cohort of Japanese atomic bomb survivors has been adjusted for uncertainty that exists in the dose estimates, systematic error in the neutron dose estimates, and a dose-dependent relative biological effectiveness. Once the adjustments were incorporated in the dose estimates the data was analyzed to allow for the possibility of a threshold dose response. The dose response models that were fit to the data were the same models used in the original papers. A threshold term was included in the model with possible threshold values ranging from 0 to 0.35 Sv. These analyses suggest that for the A-bomb solid tumor and leukemia incidence data a threshold term significantly improves the fit to the purely linear or linear quadratic model. The results from the mortality data suggests that the leukemia data agree more with the threshold model than the linear quadratic model although the linear quadratic model is statistically equivalent, while the solid tumor data does not suggest any improvement with a threshold.
2. A manuscript describing the results has been submitted to Health Physics.

Performance Schedule and Status of Aims

Neither the performance schedule nor the status of aims has changed.

3.2.4 Low Dose Radiation: Epidemiological Models in Airline Flight Crews Exposed to Cosmic Radiation and Electromagnetic Fields

Project Director:

Joyce S. Nicholas, Ph.D.

Executive Summary

Airline flight crews are chronically exposed to low-levels of ionizing cosmic radiation and to electromagnetic fields generated by the aircraft's electrical system. Similar low dose, low rate radiation exposures may occur among workers at DOE sites. The objective of this project is to ascertain and analyze the relationship between health outcomes and exposure to a combination of low-dose cosmic radiation and electromagnetic fields using epidemiologic and exposure data obtained from an international cohort of airline flight crews. The need to examine the combined exposure to cosmic radiation and electromagnetic fields is established by observed risk ratios for certain cancers (especially breast cancer) among flight crews that are higher than would be expected on the basis of ionizing radiation alone.

Relevance

This effort will make it possible to evaluate the health effects of chronic exposure to a source of low-dose radiation (cosmic radiation) to a human population. The cooperation of key professional societies and airlines continues to be a noteworthy accomplishment.

Objective

The objective of this project is to ascertain and analyze the relationship between health outcomes and exposure to a combination of low-dose cosmic radiation and electromagnetic fields using epidemiologic and exposure data obtained from an international cohort of airline flight crews.

Specific Aim 1. To continue our collaborative research relationships with the Airline Pilots Association International (ALPA) and the Fedex Pilots Association (FPA).

Specific Aim 2. To quantify occupational exposure among flight crews to galactic cosmic radiation and to magnetic fields using direct measurements, calculations, and/or biomarker assessments.

Specific Aim 3. To determine the age- and gender-specific prevalence of disease among flying personnel across airlines and to explore the potential association of disease with occupational exposure.

Specific Aim 4. To assess women's health issues including pregnancy outcomes among flying personnel.

Specific Aim 5. To try to obtain data required for a standardized mortality study.

Quarterly Accomplishments

1. The following abstracts have been accepted for publication and presentation:
 - a. Nicholas JS, Butler GC, Davis S, Bryant E, Hoel DG, Mohr Jr LC. Chromosomal aberrations and ionizing radiation in airline pilots. Epidemiology. Accepted. To be presented at the Joint meeting of the International Society of Exposure Analysis (ISEA) and the International Society for Environmental Epidemiology (ISEE). Linking exposures and health: innovations and interactions. Vancouver, BC Canada. August 11-15, 2002.
 - b. Nicholas JS, Butler GC, Padgett S#, Hoel DG, Mohr LC Jr. Women on the flight deck: disease prevalence among female airline pilots in four US airlines. The Annals of Epidemiology. Accepted. To be presented at the 2002 Meeting of the American College of Epidemiology, Albuquerque, New Mexico, September 22-24, 2002.
2. The following studies have been completed and manuscripts are being prepared:
 - a. Fluorescent in situ hybridization study (FISH) study.
 - b. Fatigue and disease incidence study in 5 airlines.
3. All data from the study of health and ALPA women pilots (all ALPA member airlines) have been entered into a database and are ready for analysis.
4. Data from the study of health among commercial cargo carrier pilots, conducted in collaboration with the Fedex Pilot's Association (FPA), are being entered into a database and a second survey to female pilots has been developed and is being mailed. The FPA recently merged with ALPA, so the study will continue under ALPA-FEDEX.
5. Progress is being made toward the acquisition of data required for a standardized mortality study.

Performance Schedule and Status of Aims

Neither the performance schedule nor the status of aims has changed.

3.2.5 Health Risks of Low Dose Plutonium Exposure

Project Director:

David G. Hoel, Ph.D.

Executive Summary

Human data on health risks associated with internal exposure to radionuclides (by inhalation and/or ingestion) is limited. With regard to plutonium exposures, there have been two DOE worker studies and, more recently, several rudimentary studies of Russian nuclear workers. One of the DOE worker cohorts (Los Alamos) contains data that may be very useful in understanding the carcinogenic effects of low-dose plutonium exposure. In contrast to the paucity of human data, there is a considerable amount of experimental data related to the development of cancer in rats and dogs following plutonium inhalation. A statistical model of cancer risk following low-dose plutonium exposure is becoming increasingly important with respect to planned DOE material disposition activities, both domestic and international. For example, plans to eliminate surplus U.S. plutonium during the next two decades, through the irradiation of mixed oxide fuel and the conversion of a certain portion of the material to an immobilized waste form, represent significant program initiatives, the effects of which should be incorporated into evolving statistical models. Similarly, U.S. activities aimed at implementation of the U.S. – Russia Plutonium Management and Disposition Agreement, related to the elimination of Russian surplus plutonium, will be incorporated as part of the effort, and related to prior studies of the Mayak workers which have consistently shown a higher level of lung, liver and bone cancer in comparison to U.S. workers. Pulmonary fibrosis is also a risk from the inhalation of plutonium.

Relevance

The processing and storage of plutonium requires a quantitative understanding of the health risks of plutonium, particularly in the low-dose range. Furthermore, DOE workers who may be exposed to plutonium should be monitored with a state-of-the-art medical surveillance program that includes the use of validated biomarkers.

Quarterly Accomplishments

This is a new project and data on plutonium exposed animals is being identified for risk analysis purposes.

Performance Schedule and Status of Aims

Neither the performance schedule nor the status of aims has changed.

3.2.6 Population Risk Studies Using Geographic Information System Technology

Project Director:

Daniel Lackland, Dr.P.H.

Executive Summary

We have developed the infrastructure resources and expertise necessary to conduct epidemiological assessments. Our sources include the following:

Savannah River Region Health Information System (SRRHIS)

The geographic cancer registry incorporates 25 counties around the Savannah River Site. Cancer incidence data obtained in a high quality manner is an essential component of epidemiological investigations.

A direct link to this resource has been established in which cancer cases are geographically identified and incorporated in the data analysis. SRRHIS provides the cancer-related component of the assessment system. Cancer incidence and mortality rates are associated with various aspects of population.

Geo-coding System

The ability to ascertain and analyze health-related, environmental, and socio-economic data for small areas, such as a census block, is an essential component of epidemiological investigation. A Geographic Information System (GIS) defines geographic study areas by organizing small areas such as census blocks. The system consists of computerized databases structured to a defined geographic area combining the tools for thematic map generation, proximity analysis, buffer zone identification and map overlay comparisons.

A critical component of any GIS is the ability to “address match” other databases into the system. An efficient GIS with a high match record must incorporate a system to add new addresses and changes, which requires an elaborate system of updates. In addition to collecting new data, epidemiological investigations are greatly enhanced with the use of existing data, saving money and time. Such databases, however, must be comprehensive and include multiple health outcomes, co-morbidities, indicators of socio-economic status, environmental exposures and population demographics and characteristics.

The analytical assessment of disease patterns constitutes a critical stage in the investigation of the environmental etiology of disease. The assessment involves the use of resources such as the GIS and multiple databases. Analyses involve a complex and sophisticated quantitative methodology.

Existing Databases

The Project has established access links to various health and environmental data bases including the SC Medicaid and Medicare data bases, hospital discharge and billing data, census TIGER files, as well as data and tissue specimens from cohort studies such as the Evans County Heart Study. The Project also maintains the capability to collect new data and tissue samples.

Objectives

1. To develop a comprehensive population risk assessment system and associated protocols.
2. To complete several epidemiology risk assessments using the resources of the comprehensive system.
3. To establish and maintain a state-of-the-art information system that interfaces with the agencies and custodians of health, environmental, geographic demographic and economic databases.

Quarterly Accomplishments

1. The Project work resulted in the acceptance of the following manuscripts:
 - a. Lackland DT, Egan BM, Syddall HE and Barker DJP. Associations between birthweight and antihypertensive medication in black and white Medicaid recipients. *Hypertension*; 2002; 39:179-183.
 - b. Silverstein MD, Nietert PJ, Ye X, Lackland DT. Racial disparities in the incidence of lung cancer: The Savannah River Region Health Information System Cancer Registry, 1991-1995. *Journal of Health Care for the Poor and Underserved*. (In press).
2. Other progress on publications is as follows:
 - a. Ferguson P, Lackland DT, Hall RK, Hoel D and Mohr L. Higher birthweights may be related to increased risk of aggressive breast cancer in young African-American females.
3. Other progress includes:
 - a. Five summer students are working with the project:
 - b. Megan Singleton – Spelman College
 - c. Chris Yako – Vanderbilt University
 - d. John Knapp – Porter-Gaude High School
 - e. Kristen Newson – SC Governor’s School for Science and Mathematics
 - f. Liwen Jin – SC Governor’s School for Science and Mathematics
4. Grant preparation for proposal to NIEHS assessing the environmental factors associated with the Fetal Origins of Adult Disease hypothesis.
5. NIEHS grant review - Special Emphasis Panel (2002) – National Institute of Environmental Health Sciences. “Review of Conference Grant Applications”.

Performance Schedule and Status of Aims

Neither the performance schedule nor the status of aims has changed.