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**Environmental Biosciences Program
Fourth Quarter Report
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For

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**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-FC09-02CH11109 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 research program, employing a range of research initiatives to identify, study and resolve environmental health risks. These initiatives are consistent with the MUSC role as a comprehensive state-supported health sciences institution and with 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 MUSC to be a national resource for the scientific investigation of environmental health issues. EBP's success as a nationally prominent research program is due, in part, to its ability to task-organize scientific expertise from multiple disciplines in addressing these complex problems

Current research projects have focused EBP talent and resources on providing the scientific basis for risk-based standards, risk-based decision making and the accelerated clean-up of widespread environmental hazards. These hazards include trichloroethylene (TCE), polychlorinated biphenyls (PCBs), asbestos and low-dose ionizing radiation. A project is also being conducted in the use of geographical information system technology to analyze population health risks related to environmental hazards as a tool for risk-based decision-making.

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 One 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 better understand 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. In this regard, a research project 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 in which databases containing health, environmental, demographic and socioeconomic data can be integrated and analyzed for specific population health risks.

1.2 Program Expenditures

EBP Expenditure Summary Fourth Quarter

The table below reflects **expenditures** by budgeted category recorded for the period March 2004, through May 2004, and year-to-date, for Cooperative Agreement CH11109. In addition, there are encumbrances for operating expenses and F & A; these are not included in the amounts below but total approximately \$270,000. Encumbrances for this period were entered through 5/31/04. Payment for these encumbrances may take 30-60 days.

<u>Budget Category</u>	<u>4th Qrt.</u>	<u>YTD</u>
	(Dollars in thousands)	
Personnel	\$ 343	\$ 1,221
Supplies	80	148
Travel	14	23
Other	08	30
Subcontract	147	190
Equipment	06	06
F & A	<u>201</u>	<u>650</u>
Total	\$ 800	\$ 2,268

2.0 Program Management and Development Office

The mission of the Program Management Office is to ensure that all projects of the cooperative agreement achieve their stated goals and objectives and are carried out in an efficient and cost-effective manner. The executive leadership of the program has adopted a strategy-focused management approach that carefully aligns the resources and core competencies of the program with research priorities developed in coordination with DOE. Specific Program Management responsibilities include workplan development, budget formulation, task organization of multidisciplinary research teams, financial management, progress reporting and program review.

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 and Director:	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.
Co-Principal Investigator, Environmental Toxicology:	David Jollow, Ph.D.
Co-Principal Investigator, Environmental Epidemiology and Risk Assessment:	David G. Hoel, Ph.D.
Fiscal Analyst:	Anita G. Jefferson, B.S.
Administrative Coordinator:	Jill Canaday
Administrative Specialist:	Percilla E. Coaxum

3.0 Scientific Research

3.1 Environmental Toxicology Research Projects

<h5>3.1.1 <u>Characterization of Species Differences in Trichloroethylene – Induced Peroxisome Proliferation and Hepatocyte Replication</u></h5>
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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. Manuscript: Walgren, J.L., Jollow, D.J., and McMillan J.M. Induction of Peroxisome Proliferation in Cultured Hepatocytes by a Series of Halogenated Acetates. Toxicology. 197: 189-197 2004.
2. Presentation: Walgren, J.L., Jollow, D.J. and McMillan J.M. Induction of peroxisome proliferation in rat hepatocytes by a series of halogenated acetates. Poster presentation, 2004 Society of Toxicology Annual Meeting, Baltimore, MD, March 2004.

Performance Schedule and Status of Aims

We will submit the manuscripts “Effect of Halogenated Acetates on Hepatocyte Cell Death and Peroxisome Proliferation” and “Trichloroacetate and Dichloroacetate are not Complete Mitogens in Hepatocyte Cultures” for publication.

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. Real time PCR analysis indicates a marked (>8x) induction of the mRNA for c-myc occurs in rat and mouse hepatocytes treated with DCA.
2. Real time PCR analysis indicates a marked (>15x) induction of the mRNA for c-myc occurs in the livers of mice treated with DCA.
3. Western blot analysis showed that the level of C/EBP alpha protein is suppressed in the livers of mice treated with DCA.

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.

Objective

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

1. We have obtained two kidneys in the last quarter. Both have been used to prepare renal proximal tubular cells (RPTC). We have used these preparations to improve our culture process for the human cells. Our immediate objective remains the optimization of the culture conditions for human renal cells, specifically in regard to retention of *in vivo*-like cellular characteristics that will permit the ready extrapolation of *in vitro* findings with the RPTC to the *in vivo* situation.
2. Currently, we have developed the capacity to grow the isolated human renal cells to confluency with regard to optimal glucose concentration, epidermal growth factor concentration, and general incubation conditions.
3. Characterization continues in regard to maintenance of the cultures post experimental procedures using glucose uptake and metabolism as a prime metabolic determinant.

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.

Executive Summary

During this second quarter we have continued to perform studies on the effect of ethanol co-exposure on chloral hydrate metabolism using rat hepatocyte cultures. We have quantified the formation of trichloroacetate (TCA) and trichloroethanol (TCE-OH) with respect to time of incubation, protein concentration and chloral hydrate concentration, both in the presence and absence of ethanol. We have constructed double-reciprocal plots to determine the K_m and V_{max} for each metabolite. These experiments have allowed us to optimize the experimental methods such that we can begin, in this quarter, to examine chloral hydrate metabolism in human hepatocytes that have been genotyped for alcohol and aldehyde dehydrogenases.

Relevance

The utility of PBPK modeling of blood TCA levels as a dose metric for liver exposure to TCA after TCE ingestion is well accepted. Unfortunately, the relationship between TCE exposure and liver levels (AUC and peak concentrations [which may vary independently]) are complex and are very likely to show major differences among human sub-populations. These differences may underlie enhanced susceptibility (or resistance) by both genetic and environmental factors. The interaction of the genetic and environmental factors may further alter the relationship between applied dose of TCE and liver exposure to TCA. The proposed studies will be used in collaboration with projects 5 and 6 to improve the reliability and applicability of PBPK modeling in the assessment of risk of humans to TCE.

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.

Specific Aim 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).

Specific Aim 2. To determine the kinetic constants for conversion of CH to TCA and TCOH in human hepatocytes.

Specific Aim 3. To characterize the isoform composition of human hepatocytes by enzymic and DNA array technology.

Specific Aim 4. To determine the effect of ethanol on the redox state of hepatocytes from mice, rats and humans.

Specific Aim 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. Conducted experiments to quantify the formation of TCA and TCE-OH in rat and human hepatocytes.
2. Conducted experiments to examine the effect of ethanol exposure on the formation of TCA and TCE-OH.

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.

Relevance

As described above, this research project is directly relevant to current and proposed EPA regulatory standards for drinking water contamination by TCE. The EPA concludes, through both its cancer and non-cancer risk assessments (EPA, 2001), that exposure to even minute levels of TCE is associated with low-level human risks. It is concluded that certain subpopulations with genetically- or drug-induced elevations of P4502E1 (the enzyme responsible for formation of toxic metabolites of TCE) will be at significant risk. Preliminary research with other well-metabolized chemicals indicates that this is not true. The proposed research with alcohol should definitively establish this for TCE. The second low-dose phenomenon to be investigated here will be presystemic, or first-pass elimination. The liver and lungs act in concert to eliminate ingested VOCs before they reach the systemic/arterial circulation. It is postulated that virtually all of trace levels of TCE in drinking water are removed, before they reach and present a hazard to extrahepatic target organs such as the lungs and kidneys. Experiments have been designed and a PBPK model will be developed in collaboration with Dr. Fisher to characterize the capacity of this protective mechanism under different TCE exposure conditions.

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.

Specific Aim 1. To determine the capacity and dose-dependency of presystemic elimination of ingested TCE and to delineate the relative contribution of the liver and lungs.

Specific Aim 2. To establish the influence (or lack thereof) of ethanol on the metabolic activation of environmentally-encountered doses of TCE.

Specific Aim 3. To determine whether the ratio of the metabolites trichloroacetic acid (TCA) (potentially carcinogenic) and trichloroethanol (TCOH) is altered by co-ingestion of ethanol.

Quarterly Accomplishments

1. Trichloroacetic acid (TCA), a major oxidative metabolite of trichloroethylene (TCE), is one of two major TCE metabolites believed responsible for causing liver cancer in mice. Ideally, scientists and risk assessors would like to know what tissue dose of TCA is produced by TCE doses in different species. Unfortunately, there is a paucity of tissue data and a limited amount of blood level data. It is not clear whether blood TCA concentrations accurately reflect liver concentrations. Therefore, we undertook a study of the dose-, time- and species-dependency of internal TCA levels as dosimeters of TCE exposure.
2. Blood and tissue concentrations of TCA were monitored for up to 48 hours following oral administration of dosages of 25, 50, 100, 500 and 1,000 mg TCE/kg to male Sprague-Dawley rats. TCA maximum observed concentration (C_{max}) and area under the concentration versus time curve (AUC) values were determined for blood, liver, kidney, brain, lung and fat. These rat data were compared to data from male B6C3F1 mice similarly dosed by Jeff Fisher in a previous investigation. TCA levels in blood exceeded levels in each tissue in both mice and rats. The blood levels rose to a greater extent, with increase of TCE dose into the cancer bioassay dosage range, than did liver or kidney levels in each species. This dose-dependent partitioning of TCA into blood apparently results from accumulation of the metabolite bound to plasma proteins. Blood free (unbound) TCA C_{max} s and AUC most closely resembled total liver levels. Thus the use of free rather than total blood TCA AUCs would result in a narrower, more precise and more scientifically rigorous range of human cancer risk estimates from mouse liver bioassay data.
3. The aforementioned findings have been incorporated into a manuscript that will soon be submitted for publication in *Toxicology and Applied Pharmacology*. The blood and tissue time-course data are presently being considered by Jeff Fisher and Harvey Clewell in their effort to consolidate/harmonize a PBPK TCE model for the EPA.

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.

Relevance

The scientific issues related to determining the health risks posed by low levels of TCE in the environment are relevant to many other solvents found in water supplies. If sound science and extrapolation methodology can be demonstrated for this chemical, then other

chemicals can be evaluated in a similar manner. This could lead to a potential saving of multiple millions of dollars in unnecessary clean-up costs.

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, LH, DA Putt, WT Brashear, R Abbas, J Parker and JW 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 posed 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.

Specific Aim 1. To harmonize current PBPK models used by the US EPA into one PBPK model for TCE and metabolites by incorporating newly published and unpublished data in humans and rodents.

Specific Aim 2. To examine the metabolism of TCE in rodents with emphasis on the dose-dependence of conversion of TCE to DCVC.

Specific Aim 3. To re-examine the dose-dependence of conversion of TCE to DCA in laboratory animals.

Specific Aim 4. To perform a cancer and non-cancer risk assessment for TCE using the harmonized single PBPK model for TCE and metabolites.

Quarterly Accomplishments

1. The following poster was presented by Dr. Fisher at the Annual Meeting of the Society of Toxicology in Baltimore, MD: Keys, D.A., I.R. Schultz, D.A. Mahle, R.D. Stenner and J.W. Fisher. "A Quantitative Description of Suicide Inhibition of Dichloroacetic acid in Rats and Mice." Presentation Date & Time: March 25, 2004.
2. The following paper has been submitted for publication to *Toxicological Sciences*, Keys, D.A., I.R. Schultz, D.A. Mahle and J.W. Fisher. "A Quantitative Description of Suicide Inhibition of Dichloroacetic acid in Rats and Mice."
3. Dr. Fisher attended meeting at MUSC on 28 April with investigators from MUSC and DOE contract managers to discuss research on TCE.
4. Research into development of an analytical method to measure dichloroacetic acid as a metabolite of trichloroethylene is still underway. Artificial production of dichloroacetic acid from trichloroacetic acid is still of concern for biological samples.

Performance Schedule and Status of Aims

No research on the DCVC pathway is scheduled in the near future (Specific aim-2). Neither the performance status nor the status of other aims has 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. Studies on murine microchimerism and TCE exposure are in progress. Three groups of mice (two groups of retired breeders and one group of virgin mice) have been exposed to 12 injections of TCE or vehicle alone. Retired breeder mice have been bred to male mice with either identical or disparate H-2 genotypes. We are currently performing histology and X and Y chromosome-specific fluorescent *in situ* hybridization on tissue sections from one group of retired breeders. In addition, we are designing real-time PCR methods to measure levels of fetal DNA in peripheral blood following TCE treatment.
2. The following manuscript, which describes the interactive effects of CTLA-4 and GM genes on SLE, has been accepted for publication:
 - a. Parks CG, Hudson LL, Cooper GS, Dooley MA, Treadwell EL, St. Clair EW, Gilkeson GS, Pandey JP. CTLA-4 gene polymorphisms and systemic lupus erythematosus in a population-based study. *Lupus* 2004; In press.
3. We have genotyped 255 SSc patients and controls for promoter-region SNPs of the IL-10 gene. In Caucasians subjects, we have found a significant difference in the distribution of genotypes between patients and controls at all four loci examined. Furthermore, IL-10 genotypes appear to influence the production of autoantibodies to RNA polymerase I and III. Characterization of Japanese subjects for the IL-10 gene as well as the protein is in progress.
4. Initiated collaboration with Dr. Joe Craft, Chief of Rheumatology, Yale University, to delineate the relative contribution of genes and environment in the etiology of SLE. Dr. Craft has measured several parameters of immune response in these SLE patients and we plan to determine whether Ig GM and KM genes regulate the expression of these immune parameters.
5. Continued to investigate the role immunoglobulin and cytokine genes in other immunologically mediated diseases, resulting in the following publications:

- a. Satoh T, Pandey JP, Okazaki Y, Yasuoka H, Kawakami Y, Ikeda Y, Kuwana M. Single nucleotide polymorphisms of the inflammatory cytokine genes in adults with chronic immune thrombocytopenic purpura. *Br J Haematol* 2004; 124:796-801.
- b. Vejbaesya S, Tanwandee T, Pandey JP. Immunoglobulin GM and KM genotypes in hepatitis C virus infection. *J Med Virol* 2004; 73:384-386.
- c. Skov M, Pandey JP, Pressler T, Høiby N, Koch C. Immunoglobulin allotypes and IgG subclass antibody response to *Aspergillus fumigatus* in cystic fibrosis patients. *J Cystic Fibrosis* 2004; In press.

Performance Schedule and Status of Aims

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

<h3>3.1.8 <u>Immunological Effects of Trichloroethylene Exposure</u></h3>
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<p>Project Director: Gary S. Gilkeson, M.D.</p>
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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.

Relevance

There is growing concern about the effects of *in utero* 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 affects on the immune system that can then be assayed in humans exposed *in utero* to TCE. We will also hope to develop potential assays for TCE exposure using the now available anti-TCE adduct polyclonal sera.

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. The DTH B6C3F1 study was completed and a first run of the tumor cell challenge was completed.
2. An abstract was submitted to the SETAC meeting on May 31st:
The Delayed-Type Hypersensitivity Response is a Biomarker for Trichloroethylene Exposure. DE Keil, LM Hessmann, J Miller, JG Eudaly, GG Gilkeson and MM Peden-Adams.

Performance Schedule and Status of Aims:

No changes have been made in the status of aims.

Year 1- June 2002-2003: Specific Aim 1 is completed and a manuscript is in preparation.

Year 2-June 2003-2004: Specific Aim 2 has begun but was delayed due to the availability of the auto-immune prone mice. The mice are only available from Jackson Laboratories and were back order for 9 months. The mice have arrived, have been bred and dosed, and pups are currently being dosed. Autoantibody production assessments and urinary protein determinations began in the pups in April 2004 and are being assessed monthly.

3.1.9 Molecular Mechanism of Pathogenesis in a Model of Trichloroethylene-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

Congenital heart defects involve developmental miscues in the division of the heart into chambers and its connection to the rest of the circulatory system. These processes are mediated by endocardial cells that undergo epithelial-mesenchymal transformation (EMT), i.e. they lose cell:cell adhesion, migrate into the previously acellular basement membrane known as the cardiac jelly that separates the myocardium and endocardium, and there differentiate into the endocardial cushion tissue (ECT), a progenitor of cardiac valves and septa. The goal of this project is to determine the effect of TCE on the development of the ECT. Using whole embryo culture, we have shown that TCE affects both the number and distribution of endocardially-derived cells within the cardiac jelly in a dose-dependent fashion.

In the current quarter we have continued experiments to demonstrate the feasibility of delivering TCE and TCE metabolites *in ovo* to developing chicks. We found good survival of embryos to day 11 after the addition of TCA in 10 - 50 μ L of buffer placed directly on the surface of the egg. This approach is inadequate for testing TCE due to its high volatility. We also have had good success microinjecting diluent into the vasculature of embryos *in ovo*. Approximately 30% of chick embryos injected (10 - 50 μ L) at day 5 develop normally to day 10 embryos *in ovo*. This will allow us to assure that each embryo receives a predictable concentration of TCE (or TCA, etc) in a manner more consistent with a mammalian embryo. The next step is to attempt injecting younger embryos (day 3) when the cushions are still in their initial stages of formation.

Using the whole embryo culture model, we also extended our analysis of TCE effects to proepicardial morphogenesis which is important for both continued valvuloseptal morphogenesis as well as maturation of the myocardium. Two preliminary effects of TCE were observed. The proepicardium appears to be both enlarged and to begin growing over the surface of the myocardium sooner with TCE treatment. The position of the proepicardium also appears to be shifted relative to the inlet and outlet limbs of the heart. This latter observation could be due to several reasons including looping differences or displacement of the proepicardium. These studies will be continued in the coming quarter to increase our sample size.

In order to more objectively assess the visually obvious anomalous pattern of cushion mesenchyme distribution in TCE treated embryos we have enlisted the aid of a spatial statistician, Dr. Elizabeth Hill, in the Department of Biometry at MUSC. Her evaluation of these data will continue in the next quarter.

Performance Schedule and Status of Aims:

In the coming quarter we will continue our analysis of TCA-treated embryos and initiate parallel studies with TCE-OH. Specimens from these two alternative treatments will be compared to TCE-treated embryos in terms of the number and localization of migrating cells in the cardiac jelly and in terms of the immunohistochemical localization patterns of specific proteins involved in cell-cell and cell-extracellular matrix adhesion.

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

Alice Boylan, M.D.

Co-Director:

Besim Ogretmen, 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.

Specific Aim 1. To determine the roles of Bcl-2 and p21 proteins in the development of resistance to asbestos and cigarette smoke-induced apoptosis, and in the development of increased tumorigenic potential in A549 cells.

Specific Aim 2. To determine the roles of Bcl-2 and p21 proteins, and other possible molecular markers of resistance (using gene-chip analysis) to asbestos alone, cigarette smoke alone, and asbestos plus cigarette smoke in combination, in normal human airway epithelial cells.

Specific Aim 3. To determine whether increased Bcl-2 and loss of p21 proteins are potential biomarkers of the development of bronchogenic carcinomas and lung cancer, response to chemotherapy, and overall survival in exposed and unexposed patients.

Quarterly Accomplishments

1. This project has accomplished all of its stated objectives and will be brought to a conclusion at the end of this reporting period.
2. We are in the process of preparing a manuscript which will describe our findings on the mechanisms of resistance to cell death in response to chronic exposure to asbestos and cigarette smoke in human lung cancer cells. The control experiments with exposing cells chronically to non-cancerous type of asbestos did not result in the induction of anti-apoptotic Bcl-xL in the lung cancer cells, supporting specific role for asbestos in the development of resistance. This demonstrates that anti-apoptotic Bcl-xL is a molecular biomarker of biological effect that has utility for assessing the risk of lung cancer following asbestos exposure.

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 Immunotoxicological Assessment of Non-degraded and Biodegraded PCB Mixtures

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. In addition, the molecular mechanism by which PCBs can effect the immune response is poorly understood. Biological effects of PCBs in animals have mostly been attributed to coplanar congeners, although effects of *ortho* congeners also have been demonstrated. In our studies, Aroclors and individual PCB congeners were evaluated for their effect on splenocyte viability and lipopolysaccharide (LPS)-induced splenocyte proliferation. The results suggested that mixtures of PCBs and/or noncoplanar congeners might be more immunotoxic than individual planar or mono-*ortho*-coplanar congeners. Since di- or multi-*ortho*-substituted congeners are found in high concentrations in human breast milk, we extended these initial studies to specifically investigate the relationship of immunotoxicity and chlorine substitution pattern. Results of these studies suggest individual congeners with chlorines in two or more *ortho* positions, regardless of surrounding chlorine substitutions, preferentially inhibit LPS-induced splenocyte proliferation as compared with non- or mono-*ortho*-substituted congeners. In addition, a widespread process in anaerobic sediments contaminated with PCBs, reductive dechlorination, could directly impact PCB substitution patterns by removing chlorines primarily from the *meta* and *para* positions, resulting in a modulation of toxic response. We have evaluated the toxicity of anaerobic PCB dechlorinated cultures using microbial communities from three PCB-contaminated soils. Our results indicate that preferential meta- and para-dechlorination was evident and that preferential *ortho*-substituted PCB congeners remained. In toxicity assays, these dechlorinated PCB mixtures were as or more toxic than the parent compound suggesting that *ortho*-substituted PCBs which preferentially bioaccumulate in the environment significantly contribute PCB toxicity. Our studies suggest a novel mechanism for the preferential inhibition of LPS-induced splenocyte proliferation by *ortho*-substituted congeners via an interruption of cell cycle progression through the decreased expression of the cell cycle regulatory protein, cyclin D2, which acts at a G₀/G₁ restriction checkpoint that allows progression into S phase. In addition, anaerobic reductive dechlorination, a naturally occurring biological process that produces a PCB mixture with a high concentration of

ortho-substituted congeners, also blocked cells at the G₀/G₁/S interface by the inhibition of cyclin D2 expression. These results address the need to consider non-coplanar congeners within the context of risk assessment and further support the notion that "the use of Ah-receptor binding and its associated biological effects to assess the total toxicity of PCBs may no longer be defensible because of the actions produced by the non-coplanar congeners." Continued analysis of the molecular mechanisms of action of non-coplanar PCB congeners and their contribution to risk assessment connected with low-level exposures is warranted. An understanding of how the toxicity of specific PCB mixtures change after bioremediation and the mechanism behind this change in the laboratory will help determine the potential toxicity associated with PCB contamination in the environment.

Relevance

An inherent problem in environmental remediation is whether clean-up activities might alter, or even enhance, the toxicity of environmental chemicals for exposed humans. This study will address this important issue and investigate relevant mechanisms of immunotoxicity using a common contaminant (PCB mixtures) and a sensitive biological response, the immune system.

Objectives

We will examine potential immunotoxicity using two well defined assays: (1) *in vitro* proliferation of primary splenocyte cultures using either, the non-specific mitogen, LPS, or using anti-Ig cross linking of the B cell surface antigen receptor; and (2), *in vitro* differentiation of either the pro-B cell line, 70Z/3 with LPS which induces surface Ig expression, or LPS stimulation of the mature B cell line, WEHI, which induces B cell class switching and Ig secretion. In this manner, induction of proliferation in primary cell culture will be evaluated through either the B cell surface antigen receptor, Ig, or non-specifically through the use of the B cell mitogen, LPS. In addition, the use of *in vitro* derived cell lines will allow the analysis of the effect of PCBs on B cell differentiation.

Specific Aims 1. To analyze the effects of various PCB mixtures and individual congeners on NF- κ B regulation and expression including I κ B- α degradation.

Specific Aim 2. To analyze the effects of various PCB mixtures and individual congeners on apoptotic pathways including the Fas:FasL pathway, the BCL2 mitochondrial pathway, and the induction of early and late phase caspase enzyme activity

Specific Aim 3. To analyze the effects of various PCB mixtures and individual congeners on cell cycle dependent proteins including the retinoblastoma gene product (pRb), the cyclin dependent kinases (cdks), and their regulators.

Quarterly Accomplishments

Manuscript Accepted and In Press:

The following manuscript was accepted for publication in Toxicology.
Smithwick, LA, Quensen III, JF, Smith A, Kurtz DT, London, L Morris, PJ.
Ortho substituted and microbially degraded polychlorinated biphenyls inhibit B cell Proliferation through a mechanism associated with a decreased expression of the cell cycle regulatory protein, Cyclin D2.

Experimental Progress:

A. Three experimental protocols have been initiated.

First, a short-term time course of 0, 5 and 10 minutes and 6 hrs was initiated with spleen cells obtained from C57bl/6 mice. Experimental conditions included for each time point media alone, LPS alone, LPS plus Aroclor 1242 at 1 and 25 ppm; LPS plus 2,2-PCB at 1 and 25 ppm, and 4, 4 PCB at 1 and 25 ppm. Nuclear and cytoplasmic protein extracts have been prepared from each time point. Protein concentration has been determined.

Second, a long-term time course of 18, 24, 48, and 72 hours was initiated with spleen cells obtained from C57bl/6 mice. Experimental conditions included for each time point media alone, LPS alone, LPS plus Aroclor 1242 at 1 and 25 ppm; LPS plus 2,2-PCB at 1 and 25 ppm, and 4, 4 PCB at 1 and 25 ppm. Nuclear and cytoplasmic protein extracts have been prepared from each time point. Protein concentration has been determined.

Third, a preliminary experiment using the pro-B cell line 70Z/3 was initiated with a time course of 18, 24, 48 and 72 hours. Experimental conditions included for each time point media alone, LPS alone, LPS plus Aroclor 1242 at 1 and 25 ppm.. Nuclear and cytoplasmic protein extracts have been prepared from each time point. Protein concentration has been determined.

B. We have confirmed with a limited number of samples that our method for generating cytoplasmic and nuclear extracts is adequate. Using western blot analysis with antibodies specific for protein found predominately in the cytoplasm (HSP-90--cytosolic) or nucleus (Oct-1 nucleic) was evident. Actin as a positive control for loading efficiency was also verified.

C. We have begun to modify a 'non-stripping' procedure on our western blot nitrocellulose to be able to use the same blot multiple time.

D. In addition, we have begun preliminary western blots to determine the efficiency of our antibodies.

Performance Schedule and Status of Aims

We have revised the specific aims as reported in quarterly report #3 to further investigate the mechanism of immunotoxicity associated with PCB exposure, specifically investigating signal transduction pathways associated with cell proliferation. Work on these aims are currently in progress.

3.2 Environmental Epidemiology and Risk Assessment Projects

3.2.1 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 Accomplishment

The Janus mouse data from Argonne and the rat data from Italy continue to be analyzed. Methods for analyzing both benign and malignant tumors are being evaluated.

Performance Schedule and Status of Aims

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

3.2.2 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 individual's 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 Accomplishment

Detailed human data on radium-exposed workers has been obtained. Risk analysis of internally emitted alpha particles in humans is being analyzed.

Performance Schedule and Status of Aims

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

3.2.3 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 radionucleides (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 risk models. U.S. data will be 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; factors related to this risk will be assessed through the analysis of available animal and human data.

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.

Objectives

1. The general problem we are considering is the evaluation and protection of the health of DOE workers in their handling of plutonium at the SRS and other DOE facilities. The project will begin by developing risk models of the health effects of low dose exposures and the design of an appropriate medical surveillance system.
2. The first step will be a quantitative evaluation of the human and animal data so that we have good productive risk models.
3. Secondly, we will develop a medical and environmental surveillance system which includes the use of film badges for measuring external radiation dose and urine analyses for the measurement of internal plutonium levels.

Specific Aim 1. To develop a medical surveillance system for DOE workers. This includes methods for the medical and environmental surveillance of the workers as well as up to date quantitative health risk models of plutonium exposure.

Quarterly Accomplishment

Data on animals exposed to plutonium has been obtained from DOE. We have data on exposed dogs from ITRI in New Mexico. Dulaney Wilson traveled to the state of Washington to evaluate the DOE animal repository.

Performance Schedule and Status of Aims

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

<h3>3.2.4 <u>Population Risk Studies Using Geographic Information System Technology</u></h3>

Project Director:

Daniel Lackland, Dr.P.H.

Executive Summary

We have developed the infrastructure resources and technical 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 overly 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.

Specific Aims

Specific Aim 1. To continue to develop and enhance the Geographic Information System as a tool for the conduct of population risk studies.

Specific Aim 2. To continue the analysis of population cancer risks in the vicinity of the SRS.

Specific Aim 3. To assess population health risks in relation to plutonium transportation; assess health risks of former workers at the SRS.

Quarterly Accomplishments

1. Water sources and Parkinson's Disease cases were plotted and analyzed resulting in an abstract submission and acceptance to the 2004 Environmental Systems Research Institute (ESRI) conference: "Descriptive Epidemiology of Parkinson's Disease in South Carolina". There was no increase in the incidence of Parkinson's Disease in geographic areas adjacent to the Savannah River Site. In addition to the oral presentation, a manuscript will also be submitted at the time of the conference.
2. Updated GIS data is being obtained for areas of trichloroethylene (TCE) contamination in South Carolina. This data will be used in an investigation of population health risks potentially related to TCE exposure.
3. Attended GIS conference in Miami regarding novel uses of GIS and the study of health.
4. Planned manuscripts and abstracts include: 1] an assessment of population disease rates and the disease rates of former workers at the Savannah River Site; 2] an assessment of environmental exposures and Parkinson's Disease in the vicinity of the Savannah River Site; 3] The geographic comparisons of adverse health outcomes and the availability of primary medical care among populations in the vicinity of the Savannah River Site; 4] cancer rates and the location of cancer prevention services associated with populations in the vicinity of the Savannah River Site.

Performance Schedule and Status of Aims

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