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MEDICAL UNIVERSITY OF SOUTH CAROLINA

Environmental Biosciences Program
Second Quarter Report
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Principal Investigator

For

Cooperative Agreement DE-FC09-02CH11109

Submitted to the
U. S. Department of Energy

By The

Medical University of South Carolina
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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. EBPs 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 biphenyles (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 First Quarter

The table below reflects **expenditures** by budgeted category recorded for the period September through November, 2003 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 $1,347,421.

Budget Category	2nd Qrt.	YTD
	(Dollars in thousands)	
Personnel	$ 306	$ 561
Supplies	22	43
Travel	02	05
Other	15	18
Subcontract	09	09
Equipment	05	05
F & A	159	288
Total	$ 519	$ 929

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

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 a (PPARa) in the mitogenic response is unknown. It is believed that
differences in the levels or activity of PPARa between humans and rodents is important
in the relative insensitivity of human hepatocytes to traditional peroxisome proliferators.
Thus, defining the role of PPARa in the mitogenic response and delineating differences in
PPARa 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. Submitted to Toxicology. Accepted pending revisions.

2. A second manuscript entitled "Trichloroacetate and Dichloroacetate are not Complete Mitogens in Hepatocyte Cultures", J.L. Walgren, D.T. Kurtz, and J.M. McMillan, is being prepared for submission to Toxicology.

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. Western blot analysis of nuclear extracts prepared from mice treated with DCA showed a marked , but transient increase in the level of the transcription factor C/EBP beta after 6 hours of treatment.

2. Identical results were found using PPAR alpha knockout mice, indicating that the PPAr alpha receptor does not mediate this increase.

3. Western blot analysis of nuclear extracts prepared from mice treated with DCA or TCA showed a decrease in the level of the transcription factor C/EBP alpha after 48 hours of treatment; earlier time points showed the transient appearance of a higher molecular weight form of C/EBP alpha, which may represent a modification of the protein.

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 nephro-carcinogenicity 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 hepatocellularcarcinoma, 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. The supply of human kidneys continues to be a problem. We have been able to obtain only 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 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	**Effect of Genetic Variation and of Ethanol on the Formation of Trichloroacetic Acid, aPutative Hepatocarcinogenic Metabolite of TCE**
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 Km and Vmax 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 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

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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. The last 4 years I have served on a National Research Council (NRC) committee that is responsible for helping establish Acute Exposure Guideline Levels (AEGLs) for extremely hazardous chemicals.   The program was established due to the need of federal, state and local authorities for information on toxic chemicals to assist in emergency planning, notification and response. AEGLs are established to protect the general population, including potentially susceptible subpopulations, from acute inhalation exposure to toxic gases, vapors and aerosols. Different limits are set to avoid notable discomfort, serious health effects and death for exposures lasting 10 or 30 min, as well as 2, 4 and 8 hours. Animal or human data, upon which AEGLs can be based, are often limited to single exposure period (e.g., 1 hour).   Standard time-scaling methods frequently over- or underestimate AEGLs and health risks.   A second area of uncertainty involves the relevance of laboratory animal data to humans. It would appear that physiologically-based pharmacokinetic (PBPK) modeling can be quite useful for both time scaling and species-to-species extrapolations.

2. In light of the foregoing, Drs. Fisher, Keys and I worked together to use PBPK models for trichloroethylene (TCE) for time scaling and for rat-to-human extrapolations to derive AEGLs for TCE. We utilized a previously developed human model (Fisher et al., 1998) and our rat model that was recently published (Keys et al., 2003 - See Dr. Fisher's First Quarterly Report for citation). The PBPK modeling provided an accurate, scientifically-based means of time scaling AEGL values for each species.   The models were initially used to predict the blood and brain TCE concentrations associated with a given adverse effect at a particular exposure level.   The models were then utilized to forecast the TCE vapor concentrations required in rats and humans to produce the same blood/brain TCE level for each AEGL time-point (i.e., 10 & 30 min and 2, 4 & 8 hours). This approach is a substantial improvement over existing, relatively arbitrary time scaling methods (e.g., Haber's Rule).   The influence of species differences in physiology and metabolism on blood and tissue dosimetry was accounted for by the models.

3. Accepted Manuscript:
     a. "The Acute Exposure Guideline (AEGL) Program: Application of Physiologically-Based Pharmacokinetic Modeling", by Bruckner, J.V., Keys, D.A. and Fisher, J.W., *Journal of Toxicology and Environmental Health*.

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 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.

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 two articles have been published:

    a. Keys, D.A., J.V. Bruckner, S. Muralidhara, and J.W. Fisher. Tissue Dosimetry Expansion and Validation of Rat and Mice Physiologically Based Pharmacokinetic Models for Trichloroethylene. 2003, **76,** 35-50.

    b. Lumpkin M.H., J.V. Bruckner, J.L. Campbell, C.E. Dallas, C.A. White, and J.W.Fisher. Plasma Binding of Trichloroacetic Acid in Mice, Rats, and Humans Under Cancer Bioassay and Environmental Exposure Conditions. 2003, **31**, 1203-1207.

2. A preliminary PBPK model for DCA in rats has been extended to predict blood and liver concentrations in mice based on unpublished data (DCA drinking water studies) and published data (naïve and pre-treated rat iv pharmacokinetic studies, Batelle NorthWest). An abstract has been accepted for presentation,

    a. Keys, D.A., I.R. Schultz, 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 from 8:30 AM to 11:30 AM.

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. Dose-determining experiments for our murine studies have shown that 5 mmol of TCE per kg body weight in an olive oil vehicle is the optimal concentration that can be administered on a chronic basis to Balb c/J mice without resulting in death from severe central nervous system depression or toxicity.

2. Molecular mimicry—immune response against antigens shared by the host and a virus—is a possible mechanism by which a virus can initiate an autoimmune disease. A chemical (TCE?) exposure could activate a latent viral infection. Human cytomegalovirus (HCMV) has been proposed as an amplifying factor for the three main areas of pathobiology affected in scleroderma: the immune system, the vascular system, and the fibrotic system. Our most recent results from this project show that antibodies to the HCMV late protein UL94 are associated with scleroderma and may be a marker for the severity of the disease. The following manuscript describing these results has been submitted for publication:
    a. Namboodiri AM, Rocca KM, Pandey JP. IgG antibodies to human cytomegalovirus late protein UL94 in patients with systemic sclerosis. *Autoimmunity* 2003; submitted.

3. Results of our most recent studies in lupus patients from Germany suggest that allelic variation at the exon 1 +49 locus of the CTLA-4 gene contributes to the risk of developing lupus as well as to the disease course and severity. The following manuscript describing these results has been submitted for publication:

   a. Manger K[*], Hudson LL[*], Kalden JR, Manger B, Pandey JP. *CTLA-4* gene polymorphism at position 49 in exon 1 is associated with the clinical phenotype of patients with systemic lupus erythematosus. *Ann Rheum Dis* 2003; Submitted. ([*]Equal contribution)

4. We have developed novel PCR-RFLP techniques and characterized DNA from SSc patents and controls for three polymorphic sites in the IL-10 gene. Our preliminary results show that particular alleles are associated with SSc and also influence the production anti-RNA polymerase I and III antibodies in these patients.

5. In collaboration with Drs. Cooper and Parks of NIEHS, we examined allelic variation at TNFα -308, TNFα -238, and TNFβ +252 in a population-based casecontrol study of SLE in North Carolina and South Carolina. We also considered TNF associations stratified by the presence or absence of HLA-DR2 and DR3 and the IL-1 α -889C/T polymorphism. Genotypes from 230 cases and 276 controls were examined separately for African Americans and whites. In whites, carriage of the TNFα -308A allele was significantly associated with SLE (OR=2.3; 95%CI 1.4, 3.9). There was no association of TNFα -308 genotype with SLE in African Americans. The other TNF loci examined were not independently associated with SLE in whites or African Americans. Analyses stratified by the IL-1α -889C/T polymorphism revealed mutually exclusive associations of SLE with either TNFα -308A or HLA-DR alleles, reflecting a significant interaction of TNF and IL-1 genotypes in whites. A manuscript describing these results is in preparation.

Performance Schedule and Status of Aims

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

3.1.8 <u>Immunological Effects of Trichloroethylene Exposure</u>

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

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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 Lejune, 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. Poster presented at the Society of Environmental Toxicology and Chemistry Meeting (November 2003).
   a. Immunological Function is Altered after Lifetime Exposure to Trichloroethylene. Keil DE, Heesemann L, Adams C, Meyers K, EuDaly A, Smythe J, EuDaly J, Gilkeson G, Peden-Adams MM

Performance Schedule and Status of Aims

Specific Aim 1 is completed. Specific Aim 2 has begun but was delayed due to the availability of the autoimmune prone mice. The mice are only available from Jackson Laboratories and were back ordered for 9 months. The mice have recently arrived and were placed in breeder pairs on December 10, 2003.

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

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. In previous quarters, we found that the number and distribution of endocardially-derived cells within the cardiac jelly is altered by TCE in a dose-dependent fashion, in particular the migration of cells into the cardiac jelly was inhibited. The fact that cell migration is inhibited by TCE strongly suggests that the distribution of the cell surface and extracellular matrix proteins that mediate cell adhesion and migration will also be altered by TCE. This quarter we initiated studies to: 1) Determine whether these effects can also be induced by known TCE metabolites, and 2) Identify proteins involve in cell adhesion and migration whose expression is altered by TCE.

1. In the current quarter we tested the ability of the TCE metabolite TCA to affect the morphogenesis of the ECT. These studies are important because it is unlikely that human embryos are ever directly exposed to TCE even when their mothers ingest or are exposed to TCE. Rather the embryos are exposed to TCE metabolites such as TCA, DCA, TCE-OH, and chloral hydrate. Over 100 stage 14 embryos were cultured for 24 hours in the presence of 10 to 500 ppm TCA. No overt toxicity was observed with the lower concentrations (10, 100, and 250 ppm), however, 500 ppm was lethal. Our initial analysis of 10 ppm TCA-treated embryos shows that, as with TCE, cell migration into the cardiac jelly may be inhibited. Analysis of the remaining samples will determine if this effect is concentration dependent and if there are fewer cushion cells. Thus, these preliminary experiments suggest that the TCE metabolites to which mammalian embryos are exposed are at least as effective as TCE itself in causing defects in cardiac morphogenesis. In the upcoming quarter we will perform similar studies with other TCE metabolites to further compare TCE and its metabolites in terms of their effects on ECT morphogenesis.

2. In the current quarter we examined the effect of TCE on the expression of the cell-cell adhesion molecule NCAM in the ECT. Ordinarily, the cells of the endocardial endothelium express NCAM. Those cells that lose adhesion to their neighbors and migrate into the cardiac jelly express lower, but still detectible, levels of NCAM. Interestingly, in TCE-treated embryos we found little or no NCAM on cells migrating into the cardiac jelly. This result is exciting because it demonstrates that TCE can affect the level of a specific adhesion molecule in cells. Whether other adhesion molecules are also affected will be examined in the upcoming quarter. We will need to learn the distribution patterns of several adhesion molecules in TCE-treated embryos in order to form a comprehensive picture of how TCE-induced changes in the expression of specific adhesion molecules lead to alterations in patterns of cell migration and morphogenesis.

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

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. As reported previously, we have shown by quantitative real-time PCR that the mRNA levels of multidrug resistance 1 (MDR1) gene, which encodes for P-glycoprotein (P-gp) is significantly up regulated in the asbestos-cigarette smoke resistant A549 cells (A549/CR-SMK) when compared to parental A549 cells. The expression of MRP1 levels did not appear to change in these cells.

2. Since our last report, first, we confirmed the expression of P-gp in A549/CR-SMK cells by Western blotting using mouse monoclonal antibody, which detects human P-gp. Then, we focused our attention to determine the mechanisms involved in the up-regulation of MDR1 mRNA levels in these resistant cells. To examine whether an increase in the promoter activity of MDR1 gene is responsible for its up-regulation, we cloned the core promoter region of MDR1 gene upstream of luciferase reporter gene in PGL2-basic vector. After transient transfections of both A549 and A549/CR-SMK cells with PGL2/MDRpromoter vector, the luciferase activity was detected as a measure of MDR1 promoter activity in these cells. The transfection efficiency of PGL2/MDRpromoter vector in each cell line was normalized by co-transfection with a plasmid containing beta-galactosidase cDNA. Interestingly, the data (in at least three independent experiments with duplicate samples) showed that the promoter activity of MDR1 is nearly identical in both A549 and A549/CR-SMK cells, demonstrating that the regulation of its promoter is not involved in the up-regulation of its mRNA levels in response to chronic treatment of asbestos and cigarette smoke-induced resistance.

3. Next, we will examine whether the stability of MDR1 mRNA is increased in A549/CR-SMK cells, which may lead to increased mRNA levels of MDR1 in these cells. These experiments will be very important to determine the molecular mechanisms by which resistance to asbestos and cigarette smoke leads to overexpression of MDR1 gene in human lung cancer cells. We are also in the process of preparing our manuscript which will describe these results.

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. 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 immuotoxicity from noncoplanar congeners that bioaccumulate. When compared with AhR expression data, these results suggest this immunotoxic response is not mediated through the AhR pathway.

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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.

Specific Aims 1.   To analyze the effects of anaerobic dechlorination and ortho/non-ortho substitution patterns on the immunotoxicity of PCB mixtures.

Specific Aim 2.   To analyze the effects of aerobic biodegradation of specific intermediate and end products of the aerobic PCB degradation pathway on the immunotoxicity of PCB mixtures.

Specific Aim 3.   To examine the effects of exposure to dechlorinated Aroclors and ortho/non-ortho congeners on CYP gene expression in hepatocytes and lymphocytes.

Quarterly Accomplishments

1. Submitted Manuscript:
The following manuscript was submitted for review to the Journal of Immunology but was not accepted for publication. We are currently in the process of revising the manuscript for submission to Toxicology.
   a. 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.

Performance Schedule and Status of Aims

To date, we are ~ 95% complete with the original specific aims of this proposal. We are in the process of revising the current aims to further investigate the mechanism of immunotoxicity associated with PCB exposure, specifically investigating signal transduction pathways associated with cell proliferation.

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 that 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.

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Quarterly Accomplishment

1. The Janus mouse data continues to be analyzed. The priority is with the new rat data from Italy. Dose response functions have been estimated for both mammary and pancreatic cancer. We are beginning to explore genetic susceptibility by using litter -mate data.

2. Publication:
   a. Carnes BA, Grahn D, Hoel D. Mortality of Atomic Bomb Survivors Predicted from Laboratory Animals. Radiation Research 160, 159-167 (2003).

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 will be analyzed.

Performance Schedule and Status of Aims

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

3.2.3 <u>Health Risks of Low Dose Plutonium Exposure</u>

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 quantitive 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 requested from DOE. Hopefully, the data will soon be available for analysis and modeling. We have had encouraging feed-back from the DOE Data Repository about obtaining detailed data.

Performance Schedule and Status of Aims

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

3.2.4   Population Risk Studies Using Geographic Information System Technology

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. Summer research students completed a GIS Study project. The project involved a geographic location of a source and spatial analysis of exposure. The project was a training activity.

2. Water sources and Parkinson's Disease cases were plotted and analyzed resulting in an abstract submission to the 2004 Environmental Systems Research Institute (ESRI) conference:
   a. "Descriptive Epidemiology of Parkinson's Disease in South Carolina". A new color map printer was installed to enhance the capabilities of epidemiological map production related to population health risks. This analysis showed no increased risk of Parkinson's Disease in geographical areas close to the Savannah River Site.

3. Planned manuscripts and abstracts include: 1) an assessment of population disease rates in comparison to the disease rates of former production workers at SRS; 2) an assessment of specific environmental exposures and the development of Parkinson's Disease; 3) Specific factors related to cancer rates among populations that live in geographical areas close to the SRS.

Performance Schedule and Status of Aims

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