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

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

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

1.1 Summary and Significance of Year 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. To address this need for research, 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 for population risk assessment in which databases containing health, environmental, demographic and socioeconomic data can be integrated and analyzed for population risks.

1.2 Program Expenditures

EBP Expenditure Summary Fourth Quarter

The table below reflects **expenditures** by budgeted category recorded for the period January through March, 2003 and year-to-date, of Cooperative Agreement CH11109. Encumbrances for personnel costs, operating costs, and F & A for the entire budget period are not included in this table but total \$430,431.

<u>Budget Category</u>	<u>4th Qrt.</u> (Dollars in thousands)	<u>YTD</u>
Personnel	\$ 316	\$1,048
Supplies	34	85
Travel	02	06
Other	02	07
Subcontract	81	162
Equipment	24	44
F & A	<u>157</u>	<u>514</u>
Total	\$ 617	\$1,866

2.0 Program Management and Development Office

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

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

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

3.0 Scientific Research

3.1 Environmental Toxicology Research Projects

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

Project Director:

JoEllyn M. McMillan, Ph.D.

Executive Summary

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

Relevance

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

Objective

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

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

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

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

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

Quarterly Accomplishments

1. Studies have been initiated to elucidate the mechanism of the hepatotoxic action of the halogenated acetates.
2. A manuscript entitled "Hepatocyte Toxicity and Peroxisome Proliferation of Halogenated Acetates" is in preparation.
3. A second manuscript entitled "Trichloroacetate and Dichloroacetate are not Complete Mitogens in Hepatocyte Cultures" is also in preparation.

Performance Schedule and Status of Aims

We will submit the manuscripts "Hepatocyte Toxicity and Peroxisome Proliferation of Halogenated Acetates" 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. Wild type and PPARalpha knockout mice have been treated with Wy-14643, TCE, TCA, and DCA for varying lengths of time-livers were collected and used for the preparation of nuclear extracts and whole cell extracts.

2. DNA gel shift analysis has been used to determine that DCA and TCA have distinct effects on the pattern of transcription factors found in nuclear extracts.
3. Western blot analysis has been used to determine the effects of these compounds on cyclins, cdk's and PCNA levels.-

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-cystine (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 published one abstract during this period.

- a. Reich C.H. and Schnellmann, R.G.: Dichlorovinyl-L-cysteine (DCVC) causes G2/M cell cycle arrest in renal proximal tubular cells. *Toxicol. Sci.* 72:9 2003.
2. We have demonstrated that primary cultures of renal proximal tubular cells (RPTC) proliferate following sub-lethal injury induced by the model oxidant t-butylhydroperoxide (TBHP), but proliferate poorly following sub-lethal injury produced by the nephrotoxicant DCVC. In the current study we used both model compounds to investigate the mechanisms of the differential proliferative responses. Treatment of RPTC with DCVC (200 uM, 90min) or TBHP (250 uM, 30 min) decreased monolayer protein and DNA contents by 40% after 48 hr. TBHP injured monolayers increased their DNA content by 67 ± 14 % on day 6, while DCVC injured cells only increased their DNA content by 31 ± 7 % on day 6. Cell cycle status was determined at various times following toxicant exposure using flow cytometry. Cell cycle analysis revealed that 88% of control RPTC were in G0/G1, 6% in S and 5% in G2/M phase and that this ratio did not vary over time. In TBHP treated RPTC, 75% were in G0/G1, 15% in S and 10% in G2/M phase, and these ratios were maintained over 48 hr. Twenty-four hr after DCVC treatment, 71% of DCVC treated cells were in G0/G1, 23% in S and 6% in G2/M phase. Forty-eight hr after DCVC treatment, 43% of the cells were in the G0/G1, 23% were in the S and 34% were in the G2/M phase. These results demonstrate that RPTC monolayers regenerate following TBHP exposure through proliferation. In contrast, RPTC regenerate poorly following DCVC exposure due to cell cycle arrest in the G2/M phase.

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, a Putative Hepatocarcinogenic Metabolite of TCE

Project Director:

David McMillan, Ph.D.

Executive Summary

During this fourth quarter we have continued studies to examine the relationship between metabolism of TCE and genotype of human liver alcohol and aldehyde dehydrogenases. We have begun to examine trichloroacetate (TCA, toxic metabolite) and trichloroethanol (non-toxic metabolite) formation in liver homogenates using our GC assay as noted in the previous report. We have examined chloral hydrate metabolism using rat liver homogenate and hepatocytes. We can detect the formation of the following metabolites: TCA, DCA and trichloroethanol. Experiments on chloral hydrate metabolism using the human livers are planned for the next quarter.

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. Methods have been developed in our lab to genotype human liver in regard to their isoforms of alcohol and aldehyde dehydrogenases.
2. We have examined chloral hydrate metabolism in rat liver microsomes and hepatocytes.

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. The initial phase of studies of presystemic elimination of TCE has been completed. Adult, male Sprague-Dawley (S-D) rats were administered oral doses of 10 or 50 mg TCE/kg by gavage (oral bolus), by constant gastric infusion over 2 hours, and by intravenous injection. Apparent clearance decreased as the bolus dose increased. Bioavailability increased from 18.3% (10 mg/kg) to 46.4% (50 mg/kg), indicative of saturation of first-pass metabolism in this dosage range. The most Striking Finding was that TCE could NOT be detected in the bloodstream in animals receiving 10 or 50 mg/kg by gastric infusion. The limit of quantitation of our gas chromatography assay in this study was ~ 20 ng/ml. It should be noted that these two doses are several orders of magnitude higher than are received from drinking water. On the basis of our findings, it is reasonable to conclude that trace levels of TCE ingested in food and water will be completely removed from the systemic circulation by the liver and lungs. Thus, risks of noncancer effects and cancer resulting from metabolic activation *in situ* should be negligible in extrahepatic organs. More detailed experiments will be conducted, in conjunction with PBPK modeling by Drs. Fisher and Keys, to define the exposure conditions under which presystemic elimination is protective of humans. We also monitored levels of trichloroacetic acid (TCA) and trichloroethanol (TCOH), TCE's two major metabolites, in the blood of the rats dosed with 10 and 50 mg/kg by gavage and by gastric infusion. Blood concentrations of TCA and TCOH were 2- to 3-fold lower at each dosage level in the gastric infusion groups. These findings suggest that hepatic metabolism of TCE is significantly lower in animals ingesting the chemical over a prolonged period. If this is the case, risks of liver cancer from TCA and other TCE metabolites should be also be lower. Gastric infusion approximates actual human exposures where TCE is ingested in divided doses of the course of the day. In contrast, the U.S. EPA's cancer risk estimates for TCE are based upon high-dose gavage studies of mice and rats, in which the animals received their daily lifetime doses as a single oral bolus.
2. These experiments are included in the following manuscript that has been submitted to *Drug Metabolism and Disposition* for publication:
 - a. White, C.A., Muralidhara, S., Hines, C. and Bruckner, J.V.: Effect of rate of oral administration on the bioavailability and metabolism of trichloroethylene and trichloroethane.
 - b. TCE toxicokinetic data were provided to Drs. Fisher and Keys for development and validation of a PBPK model for TCE in mice and rats. The rat experimental data were quite extensive, in that both blood and tissue time-courses were measured in animals dosed with TCE by

inarterial injection, gavage (oral) and inhalation. These are the most extensive and comprehensive TCE pharmacokinetic profiles that have been assembled. As described in Dr. Fisher's report, these findings were presented at the recent Society of Toxicology meeting and incorporated into a manuscript being submitted for publication in *Toxicological Sciences*.

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. TCE Rat PBPK Model (Drs. Keys, Fisher and Bruckner)
 - a. Successful description of the liver dosimetry of TCE in the expanded rat and mouse TCE model required the splitting of the "well-stirred" liver model into two compartments, a shallow liver and a deep liver.
 - b. A novel application of a jackknife cross-validation, a statistical model validation technique, to the TCE PBPK model was performed.
 - c. A poster titled, "Low-dose Validation of a Physiologically Based Pharmacokinetic Model for Trichloroethylene in the Rat" was presented at the Society of Toxicology Annual Meeting in Salt Lake City, March 11, 2003.
 - d. A manuscript titled "Tissue Dosimetry Validation of Rat and Mouse Physiologically Based Pharmacokinetic Models for Trichloroethylene" is in the final stages of preparation for submission to *Toxicological Sciences*.
2. DCA PBPK Model (Drs. Keys and Fisher)
 - a. Unpublished DCA kinetic datasets in mice and rats from drinking water studies along with newly available literature studies are being compiled for use in a PBPK model for DCA.
 - b. A PBPK model is being developed that accounts for suicide inhibition of DCA metabolism by zeta glutathione, the primary enzyme responsible for degradation of DCA.

- c. Amy Dixon, a Ph.D. student, is validating a MS method to detect DCA in whole blood and liver. The limit of detection is expected to be near 1 ng/ml blood and 3 ng/g tissue. To re-examine the dose-dependence of conversion of TCE to DCA in laboratory animals, experiments are being planned in which mice will be dosed with TCE to obtain empirical information on the liver levels of DCA.
- 3.. TCA PBPK Model. (Dr. Lumpkin (former Ph.D. student), Drs., Jim Bruckner, Cham Dallas, Jeff Fisher)
- a. The dosimetry and risk assessment implications of TCA in mice, rats, and humans when species-specific serum protein binding is accounted for in the PBPK models was explored (Manuscript in preparation). Trichloroacetic acid (TCA) is considered a principle metabolite responsible for TCE-induced liver cancer in mice.
 - b. Manuscript submitted to *Drug Metabolism and Disposition*, titled, “Plasma Binding of Trichloroacetic Acid in Mice, Rats and Humans Under Cancer Bioassay and Environmental Exposure Conditions”.

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. The following manuscript, which describes our recent findings concerning the immunogenetic (CTLA-4 genotypes) risk factors in SSc, has been submitted for publication:
 - a. Hudson LL, Silver RM, Pandey JP. Ethnic differences in CTLA-4 genotype associations with systemic sclerosis. *J Rheumatol* 2003; Submitted.
2. In an effort to shed light on the mechanisms underlying autoimmunity, we have been investigating the epistatic effects of several genes on autoimmune responses to particular autoantigens. The following manuscript describes the recent results of our collaborative project with Drs. Hurme (Finland) and Prohászka (Hungary):
 - a. Pandey JP, Prohászka Z, Veres A Füst G, Hurme M. Epistatic effects of genes encoding immunoglobulin GM allotypes and interleukin-6 on the production of autoantibodies to 60-kDa and 65-kDa heat-shock proteins. *Genes and Immunity* 2003; Submitted.
3. The following abstract, which describes our recent findings concerning the immunogenetic risk factors in SLE, has been submitted for presentation at the 36th annual meeting of the *Society for Epidemiologic Research*, June 11-14, 2003, Atlanta, GA:
 - a. Parks CG, Hudson LL, Cooper GS, Pandey JP. Epstein-Barr virus seroprevalence in systemic lupus erythematosus: interaction with cytotoxic T lymphocyte antigen-4 genotypes.
4. To understand the immunogenetic mechanisms underlying the endothelial cell damage—considered the primary event in the pathogenesis of scleroderma—we measured autoantibodies to endothelial cell epitopes in 378 SSc patients and controls. The subjects were also characterized for several immunoglobulin and HLA class II genes. The statistical analysis of the data is in progress.
5. As part of our collaborative project with Dr. Glinda Cooper, Epidemiology Branch, NIEHS, we analyzed the CTLA-4 and GM genotype data for the SLE patients and controls. Our preliminary analysis shows an interactive effect of these two unlinked genetic systems on susceptibility to SLE: Particular CTLA-4 genotypes are risk for SLE in subjects heterozygous for GM loci but not in those who are homozygous for these markers. A manuscript describing these results is in preparation.

6. During the previous quarter, we planned the following:
 - a. Characterization of the SLE patients and controls from Germany for CTLA-4 and GM genes.
 - b. Submit a manuscript describing the interactive effects of CTLA-4 and GM genes in susceptibility to SLE.
 - c. Submit a manuscript describing the role of IL-10 genes in the etiology of SSc.
 - d. Submit a manuscript describing the genetic control of autoimmunity to endothelial cell epitopes.
 - e. Characterization of DNA from SSc patients (exposed to TCE) and matched controls, to be obtained from the University of Pécs, Hungary, for several genes of the immune system.

Performance Schedule and Status of Aims

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

3.1.8 Immunological Effects of Trichloroethylene Exposure

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

Executive Summary

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

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 effects 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. Final studies with 8-week old B6C3F1 mice were completed. Serum samples have been archived and autoantibody levels will be assessed in the next quarter.
2. Publication- Abstract:
 - a. Lifetime Exposure to Trichloroethylene (TCE) Modulates Immune Function. C. Adams, D.E. Keil, K. Meyers, A. EuDaly, J. Smythe, J. EuDaly, G.Gilkeson, M.M. Peden-Adams (2003). *The Toxicologist*, 70(1): 375.
3. A poster was presented at the 2003 Society of Toxicology (SOT) meeting.
 - a. C. Adams, D.E. Keil, K. Meyers, A. EuDaly, J. Smythe, J. EuDaly, G.Gilkeson, M.M. Peden-Adams (2003). Lifetime Exposure to Trichloroethylene (TCE) Modulates Immune Function.

Performance Schedule and Status of Aims

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

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

Project Director:

Stanley Hoffman, Ph.D.

Executive Summary

Increased numbers of heart defects occur in children born where the water supply is contaminated with trichloroethylene (TCE), suggesting that TCE is teratogenic in humans. TCE has been reported to have teratogenic effects on chick and rat embryo hearts, without apparent effect on other organs. Heart malformations usually involve structures that form by epithelial-mesenchymal transformation (EMT). In this process which is repeated several times during heart development, a subset of cells in an epithelial sheet detach and migrate into the underlying basement membrane where they then differentiate in a novel direction. For example, endocardial cells undergo EMT and differentiate into valves and septa. In our laboratory, we use a cell line, QCE-6 cells, and explants of embryonic heart tissue to model EMT. This cell line has allowed us to identify biochemical changes that accompany and control EMT. Of particular interest are developmental changes in proteins present in the ECM and enzymes involved in the remodeling of the ECM that are capable of regulating cell behavior. Specifically, chondroitin sulfate proteoglycans may be critical components of the ECM because they are present in dynamically changing distributions in the developing heart and have been shown to regulate cell-cell and cell-ECM adhesion and subsequent intracellular signaling. MMPs are a major family of enzymes involved in the remodeling of the ECM. Moreover, our recent studies demonstrate that blocking MMP activity blocks both EMT and accompanying cell differentiation.

Relevance

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

Objectives

1. Determine whether TCE or its metabolites affect the morphology of the developing chicken and rodent hearts *in vivo*.
2. Determine when in development TCE-induced heart defects first appear and in what region of the heart.

3. Determine whether the morphological defects induced by TCE can be correlated with concomitant biochemical defects, particularly in components of the ECM involved in EMT including MMPs and chondroitin sulfate proteoglycans.
4. Determine whether experimentally reversing the effects of TCE on the composition and function of the ECM will also reverse its effects on heart morphology.

Specific Aim 1. To compare the effects of TCE on heart development in chicken, mice, and rats in order to find the most convenient and robust system for further characterization of the molecular mechanisms altered by TCE.

Specific Aim 2. To determine when in development TCE-induced heart defects first appear and in what region of the heart.

Specific Aim 3. To determine the effects of TCE treatment on the developmental expression patterns of developmental markers, signaling molecules, MMPs, and chondroitin sulfate proteoglycans.

Specific Aim 4. To determine whether TCE inhibits the migration into a collagen gel of QCE-6 cells and cells from embryonic heart tissue explants.

Specific Aim 5. To determine whether TCE treatment affects the expression, molecular forms, and distributions of chondroitin sulfate proteoglycans in QCE-6 and explant cultures.

Specific Aim 6. To determine whether experimentally reversing the effects of TCE on chondroitin sulfate proteoglycans and MMPs also reverses the inhibitory effects of TCE on cell migration and differentiation.

Quarterly Accomplishments

1. 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 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. Our present goal is to determine the effect of TCE on the development of the ECT.
2. We have verified our initial observations of TCE-mediated alterations in atrioventricular canal (AV) endocardial cushion tissue morphology in an increased number of carefully stage-paired embryos and at different doses of TCE. The previously observed reduction in number of migrating mesenchymal cells in the ECT and the increase in cell:cell association were shown to be dose-dependent (25, 100, and 250 ppm). Interestingly, the endocardium along the

atrial-to-ventricular axis exhibited a differential susceptibility to 100 ppm TCE (effects on ECT proximal to the ventricle were less pronounced than at mid-level or towards the atrial extremity). The reason for this remains unclear but it could potentially result in an increased level of anomalies in particular valvular or septal structures (e.g. valve leaflets, papillary muscle, ventricular septum). *In ovo* experiments will be necessary to assess this possibility due to limitations in the duration of the embryo culture method.

3. We have also initiated studies to evaluate the sensitivity to TCE of other epithelial to mesenchymal transformation (EMT) events in the developing heart. The proepicardium is a cluster of epithelial cells that arises from the posterior aspect of the primitive ventricle shortly after the initial formation of ECT in the AV canal. It migrates over the surface of the heart as an epithelial sheet which subsequently undergoes EMT to generate progenitors of ventricular fibroblasts and coronary vessels as well as cells that enter the already existing endocardially derived ECT. Our preliminary observations indicate that the overall size of the proepicardium is reduced by 100 and 250 ppm TCE. This is consistent with a similar reduction in the number of mesenchymal cells in the ECT suggesting that TCE may alter the proliferative capacity of these cells. Assessing the effects of TCE on epicardial EMT will require the initiation of cultures using slightly older embryos than we have used to evaluate ECT morphology (i.e. stage 16 rather than stage 14). These embryo cultures will also be suitable to evaluate potential TCE effects on endocardial EMT in the outlet of the heart.

Performance Schedule and Status of Aims:

Immunohistochemical analyses of cell adhesion proteins and extracellular matrix compositional changes will be pursued in the next quarter. We have collected sufficient stage-paired embryos at all TCE concentrations to initiate these studies.

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

Project Director:

Alice Boylan, M.D.

Co-Director:

Besim Ogretman, Ph.D.

Executive Summary

It has long been known that workers occupationally exposed to asbestos who also smoke carry a very marked increase incidence of bronchogenic carcinoma and lung cancer. The interaction is clearly synergistic; however, the mechanism of this synergism is unknown. We presently lack biomarkers of early stages of the disease process and biomarkers that would distinguish which workers are most susceptible to this synergism. Current information suggests that cigarette smoke increases the uptake of asbestos fibers into airway walls and that there is a more than additive increase in hydroxyl radical damage to cellular DNA. Such DNA-damaged cells would normally die by an apoptotic mechanism. The hypothesis under study here is that cigarette smoke and asbestos also synergistically

increase resistance to apoptosis by changing the level of expression of apoptosis-related genes (e.g., bcl-2, bcl-x, bax and p53) in normal and/or cancerous human lung epithelial cells that are resistant to these agents.

Relevance

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

Objectives

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

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. In this quarter, we focused on the development of the animal model to examine our in vitro results in in vivo conditions. In this model, we are proposing to study the synergistic roles of asbestos and cigarette smoke (alone and in combination) in the development of K-Ras-induced lung cancers in transgenic mice. We got mice bi-transgenic for K-ras and the lung promoter. We have harvested whole lung

RNA from exposed and non exposed (doxocyclin) mice at various time points. This will allow us to look at a variety of genes associated with K-ras induction and tumor development. In the model, some cells are obviously spared from tumor development. Therefore, to see if the proliferative stage is important in determining which cells go on to develop tumors after induction of k-ras we exposed mice to dox when the lungs are rapidly developing. Exposure of the fetus at day 18 led to still birth. Exposure at birth: one died at 2 days and the rest died at one week. However, exposure of mice at 3 weeks after birth-the animals lived and there did appear to be an increase in the number of tumor foci. We have also injected the mice with Brdu to see if proliferating cells are only seen in areas of foci formation. The initial staining of the lungs for Brdu was poor and this is being repeated. In the next phase of these experiments, we will investigate the roles of asbestos and cigarette smoke (alone or in combination) in the proliferative potential of K-ras-induced lung cancers, and in the development of resistance to apoptotic cell death in these lung cancers. We are also in the process of obtaining slides from patients with and without asbestosis to examine whether overexpression of Bcl-2 protein, which we observed in our cell culture model in cells which developed resistance to cell death following chronic exposure to asbestos, is consistent in the clinical samples.

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

Objectives

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

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 endproducts 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. We asked whether the observed inhibition of LPS-induced splenocyte proliferation is a result of an induction of apoptosis? Apoptosis was evaluated by both DNA fragmentation assays and induction of specific apoptotic genes. No significant difference in the percentage of DNA fragmentation was observed in the presence or absence of LPS whether or not Aroclor 1242 was present. In addition, the induction by LPS of bcl-xL, which suppresses apoptosis, was not inhibited in the presence of Aroclor 1242. Therefore, the inhibition of proliferation observed in the presence of PCBs is not due to the selective induction of apoptosis.
2. We asked whether the observed inhibition of LPS-induced splenocyte proliferation by PCBs (Aroclor 1242) was linked to an interruption of NFkB signaling which is important for both proliferation and immunoglobulin secretion. Our data suggests that NFkB activation and subsequent IkBa degradation after LPS induction of splenocyte proliferation is not affected by Aroclor 1242. In addition, exposure of splenocytes to Aroclor 1242 in the absence of LPS does not induce IkBa degradation or NFkB signaling.
3. Next we asked whether PCBs induce cell cycle arrest. Using flow cytometry and cell cycle stage analysis by staining with propidium iodide, we found that Aroclor 1242 prevents the progression of LPS-induced splenocytes into G₂ phase of the cell cycle.
4. Manuscript Accepted:
 - a. Smithwick, L.A., A. Smith, J.F. Quensen III, A. Stack, L. London, P.J. Morris. Inhibition of LPS-Induced Splenocyte Proliferation and Antibody Secretion by Ortho- Substituted Polychlorinated Biphenyl Congeners.
Toxicology: *In Press*

Performance Schedule and Status of Aims

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

3.2 Environmental Epidemiology and Risk Assessment Projects

3.2.1 Low Dose Radiation: Statistical Models of Childhood Leukemia Risk

Project Director:

David G. Hoel, Ph.D.

Executive Summary

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

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

Relevance

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

Objective

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

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

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

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

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

Quarterly Accomplishment

We have received the cases of childhood leukemia from the Ontario Cancer Registry. We are awaiting the arrival of the small area census data after which we will be able to complete Specific Aims 1-3. The meta-analysis of the data from the existing studies of childhood leukemias residing near nuclear facilities has been completed. A draft of the findings has been completed and is under review before submitting it to a scientific journal for publication.

Performance Schedule and Status of Aims

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

3.2.2 Low Dose Radiation: Toxicological Models of Cancer Risk

Project Director:

David G. Hoel, Ph.D.

Executive Summary

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

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

Relevance

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

Objective

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

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

Quarterly Accomplishment

Using the Janus mouse data Cox proportional hazard models have been fit to the lung cancer data for both gamma and neutron exposures. We will now use the results to examine dose and dose-rate effects on lung cancer as well as the value of the relative biological effectiveness factors for neutrons. Further we are examining the dose-response for lung cancer from the low-dose acute neutron exposures in order to see if the “bystander effect” as predicted from *in vitro* cell transformation assays after low-dose alpha exposure is correct.

Performance Schedule and Status of Aims

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

3.2.3 Low Dose Radiation: Epidemiological Risk Models

Project Director:

David G. Hoel, Ph.D.

Executive Summary

The data used for estimating health risk from low LET radiation (e.g. x-ray, gamma) has been obtained from the A-bomb survivor cohort. This group, along with some cohorts of high dose medically exposed individuals makes up our source of information. Two important issues are of current concern: 1) Does the risk of cancer follow a linear dose-

response at low-doses?, 2) Are individuals exposed at older ages (i.e. greater than 45 years) more susceptible to developing cancer than expected?

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

Relevance

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

Objective

The shape of the dose-response function for radiation-induced carcinogenesis in humans has depended primarily on data obtained from the Japanese A-bomb survivors. This project will re-examine these data with respect to the linearity of cancer risks from low dose (1-10 rem) radiation exposures. An analysis of A-bomb survivor data for solid tumors and leukemia indicates that there is a non-linear relationship to carcinogenesis following low-dose radiation exposure. Uncertainty in the dose estimates, including underestimation of neutrons and a relative biological effectiveness (RBE) that varies with dose are being incorporated into this low-dose analysis. This comprehensive and focused analysis of epidemiological data from Japanese A-bomb survivors will greatly increased our understanding of the true epidemiological relationship between cancer risks and low-dose radiation exposure. In addition, DOE worker data which has been reported as providing the scientific basis for an increased susceptibility from exposure at older ages will be evaluated and contrasted with the A-bomb data.

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

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

Quarterly Accomplishment

1. Work on Specific Aims 1&2 are now beginning. The appropriate worker databases are being consider for analysis.
2. The manuscript Baker GS, Hoel DG, "Corrections in the Atomic Bomb Data to Examine Low Dose Risk has been revised and resubmitted to Health Physics.

Performance Schedule and Status of Aims

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

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

Project Director:

Joyce S. Nicholas, Ph.D.

Executive Summary

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

Relevance

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

Objective

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

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

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

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

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

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

Quarterly Accomplishments

1. Invited presentation
 - a. Nicholas JS. Flight Deck Occupational Health and Exposures Research: Update. Air Line Pilots Association International, Human Performance Committee, Aeromedical Committee Chairpersons/Coordinators Meeting. Denver, Colorado, February 22-23, 2003.
2. Abstract submitted
 - a. Nicholas JS, Butler GC, Hoel DG, Mohr LC Jr. Breast cancer in female airline pilots. 15th Conference of the International Society for Environmental Epidemiology, Perth, Australia, September 24-26, 2003.
3. Manuscript submitted
 - a. Nicholas JS, Butler GC, Davis S, Bryant E, Hoel DG, Mohr LC Jr. Stable Chromosome Aberrations and Ionizing Radiation in Airline Pilots. Aviation, Space and Environmental Medicine. Submitted March 2003.
4. Manuscript being prepared for submission
 - a. Padgett SL, Nicholas JS, Hoel DG, Maize JC, McGee DL, Mohr LC Jr, Simpson KN. Skin cancer among commercial airline pilots: rates, predictors, and potential impact.
5. Progress on FedEx study: Data is currently being analyzed.
6. Progress on mortality study: Application to NDI has been approved.

Performance Schedule and Status of Aims

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

3.2.5 Health Risks of Low Dose Plutonium Exposure

Project Director:

David G. Hoel, Ph.D.

Executive Summary

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

Relevance

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

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

Graduate student Dulaney Wilson has chosen the development of quantitative models of plutonium health effects as her PhD dissertation topic. Her basic background reading has been completed and a first draft of a background report on health effects has been completed. The report includes results from beagle studies and all epidemiology studies. What is particularly important are the cancer effects recently reported from the former Soviet Mayak facility. High doses of plutonium have been shown to induce liver, bone and lung cancer among these workers as predicted from the animal studies. What is important is: a) Are the dose-response functions linear? b) Do the low dose non-linear results of the “bystander effects” of alpha exposure to in vitro transformation assays valid for humans? c) Do the animal studies quantitatively predict the human effects?

Performance Schedule and Status of Aims

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

3.2.6 Population Risk Studies Using Geographic Information System Technology

Project Director:

Daniel Lackland, Dr.P.H.

Executive Summary

We have developed the infrastructure resources and 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 overlay comparisons.

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

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

Existing Databases

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

Objectives

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

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. The Project work resulted in the acceptance of the following manuscript:
 - a. Silverstein M.D., Nietert P.J., Ye X., Lackland D.T. Racial disparities in the incidence of lung cancer: The Savannah River Region Health Information System Cancer Registry, 1991-1995. Journal of Health Care for the Poor and Underserved. 14:23-33,2003.
2. The health status of the population residing in the Savannah River Site region has continued to be assessed using data from SRRHIS, as well as mortality and morbidity rates from the SC Office of Research and Statistics and the SC Department of Health and Environmental Control.
3. The geographic location of the former Savannah River Site workers was plotted and used as a resource for recruitment into the Savannah River Site Former Workers Study.
4. The health status of the former Savannah River Site workers is being analyzed with respect to the overall population health status in the Savannah River Site region. Specific plans for the analysis of mortality records of deceased former Savannah River Site workers have also been developed. The death rates of former Savannah River Site workers will be analyzed in comparison to population death rates for the Savannah River Site region.

Performance Schedule and Status of Aims

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