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**Environmental Biosciences Program
Fourth Quarter Report
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For

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

By The

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1.0 Introduction

In May 2002, the United States Department of Energy (DOE) signed Assistance Instrument Number DE-FC09-02CH11109 with the Medical University of South Carolina (MUSC) to support the Environmental Biosciences Program (EBP). This funding instrument replaces DOE Assistance Instrument Number DE-FC02-98CH10902.

EBP is an integrated, multidisciplinary scientific 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 health risks.

1.2 Program Expenditures

EBP Expenditure Summary Fourth Quarter

The table below reflects **expenditures** by budgeted category recorded for the period April through June, 2003 and year-to-date, for Cooperative Agreement CH11109. Personnel expenditures reflect approximately \$50,000 that were recorded in this quarter but will be transferred to the new costs centers that begin 6/1/03 to coincide with the five-year renewal. In addition, there remain outstanding encumbrances for operating expenses and F & A; these are not included in the amounts below but total approximately \$130,400.

<u>Budget Category</u>	<u>4th Qrt.</u>	<u>YTD</u>
	(Dollars in thousands)	
Personnel	\$ 293	\$1,341
Supplies	74	159
Travel	03	09
Other	16	23
Subcontract	60	222
Equipment		44
F & A	<u>131</u>	<u>646</u>
Total	\$ 577	\$2,444

The final invoice for the period 5/1/03 – 5/31/03 should be submitted within the next 30-60 days and will reflect any requested carryover funds.

2.0 Program Management and Development Office

The MUSC administration established the Program Management 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
Administrative Specialist:	Percilla Coaxum

3.0 Scientific Research

3.1 Environmental Toxicology Research Projects

3.1.1 <u>Characterization of Species Differences in Trichloroethylene – Induced Peroxisome Proliferation and Hepatocyte Replication</u>
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Project Director:

JoEllyn M. McMillan, Ph.D.

Executive Summary

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

Relevance

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

Objective

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

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

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

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

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

Quarterly Accomplishments

1. A manuscript entitled "Effect of Halogenated Acetates on Hepatocyte Cell Death and Peroxisome Proliferation", J.E. Walgren and J.M. McMillan, is being prepared for submission to Toxicology Letters.
2. A second manuscript entitled "Trichloroacetate and Dichloroacetate are not Complete Mitogens in Hepatocyte Cultures", J.E. Walgren, D.T. Kurtz, and J.M. McMillan, is being modified for submission to Toxicology.

Performance Schedule and Status of Aims

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

3.1.2 <u>Effects of Trichloroethylene Metabolites on Hepatic Cell-Cycle Regulatory Proteins and Transcription Factors</u>
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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. Liver nuclear extracts from wild type and PPARalpha knockout mice treated with Wy-14643, TCE, TCA, and DCA for varying lengths of time were analyzed for their content of C/EBPalpha, C/EBP beta, and AP1 transcription factors.

2. DNA gel shift analysis has been used to determine that DCA and TCA have different effects on the level of AP1 and Stat5b.

Performance Schedule and Status of Aims

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

3.1.3 <u>Cellular and Molecular Actions of the Trichloroethylene Metabolite 1,2-Dichlorovinyl-L-Cystine in Renal Proximal Tubular Cells</u>
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Project Director:

Rick G. Schnellmann, Ph.D.

Executive Summary

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

Relevance

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

extrapolation of rat bioassay data for humans and for the recognition of supersensitive subpopulations of humans, if such exist.

Objective

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

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

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

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

Quarterly Accomplishments

1. We have begun to culture human renal proximal tubular cells (RPTC). Initial studies are being conducted to improve the culture conditions to increase the expression of differentiated functions in these cells. These studies are important because the ability to extrapolate *in vitro* findings to the *in vivo* situation requires cellular models to mimic those cells found *in vivo*.

2. Studies conducted to date have examined the effect of glucose and epidermal growth factor concentrations, and shake versus still culture conditions on the growth and confluency of human RPTC. While the preliminary data revealed that human RPTC require glucose to grow and reach confluency, it does not appear that the glucose concentration makes a difference once the concentration is greater than or equal to 1 mM. The next series of experiments revealed that epidermal growth factor is required for human RPTC growth and confluency. However, it does not appear that the epidermal growth factor concentration makes a difference once the concentration is greater than or equal to 10 ng/ml. The third series of experiments revealed that human RPTC could grow under shake conditions, provided that shaking was initiated 48 hours after cell seeding.
3. Having reduced the number of variables to be tested, the next series of experiments will test the effect of these conditions on the oxidative metabolism of human RPTC.

Performance Schedule and Status of Aims

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

3.1.4 <u>Effect of Genetic Variation and of Ethanol on the Formation of Trichloroacetic Acid, a Putative Hepatocarcinogenic Metabolite of TCE</u>
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Project Director:

David McMillan, Ph.D.

Executive Summary

During this 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. Conducted experiments to quantify the formation of TCA and TCE-OH in rat hepatocytes.
2. Conducted experiments to examine the effect of ethanol exposure on the formation of TCA and TCE-OH
3. Conducted experiments to determine the stability of DCA in rat 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. Development and validation of techniques for analysis of TCE and its key metabolites in biological samples and in water. As described in the Final Report, we have made substantial improvements in assays of TCE in liver, lung and kidney, the three primary target organs for TCE-induced carcinogenicity in rodents. These assays will be utilized in studies of presystemic elimination, as well as alcohol and drug interactions with the halocarbon. The most recent area of focus had been development of a gas chromatography (GC)-mass spectrometry (MS) method to analyze dichloroacetic acid (DCA) in tissues. Both DCA and trichloroacetic acid (TCA) are believed to contribute to TCE's ability to cause hepatocellular carcinomas in B6C3F1 mice. DCA is formed in relatively small amounts, exhibits a short biological half-life, and has previously been very difficult to quantify in biological specimens. Experiments are currently being conducted in our laboratories to quantify DCA in animals given cancer bioassay (i.e., high) doses of TCE. Drs. Jeff Fisher and Deborah Keys will utilize these data to aid in development and validation of a DCA PBPK model.
 - a. The aforementioned GC-MS analytical method for TCE in drinking water has a lower limit of detection [i.e., 5 µg/L (ppb)], is more rapid, and gives more reproducible results than existing procedures. A paper describing it and citing the subcontract for support was recently published.
 - b. Brown, S.D., Dixon, A.M., Bruckner, J.V. and Bartlett, M.G. (2003). A validated GC-MS assay for quantitation of trichloroethylene (TCE) from drinking water. *Intern. J. Environ. Anal. Chem.* 83:427-432.
2. Characterize the relationship between blood and liver concentrations of trichloroacetic acid in TCE-dosed rats and mice. Earlier this year we monitored TCA levels in blood, liver and other tissues of rats given a series of oral doses of TCE. The blood TCA levels rose to a greater extent with increase in TCE dose than did liver and kidney TCA levels. The same phenomenon has previously been reported in mice by Dr. Fisher. We repeated some of the experiments during this past quarter, in order to establish that the findings were reproducible. We also closely monitored the time-courses of TCA in rats given 25 mg TCE/kg, so that pharmacokinetic uptake and elimination parameters could be precisely determined. These data have been incorporated into the following manuscript that has just been submitted for publication in *Toxicol. Appl. Pharmacol.*
 - a. Lumpkin, M.L., Muralidhara, S., White, C.A., Fisher, J. W., Dallas, C.E. and Bruckner, J.V. (2003). Species- and dose-dependency of blood and tissue trichloroacetic acid levels as dosimeters of trichloroethylene exposure.

3. The following manuscript describing interspecies differences in plasma protein binding of TCA was recently accepted for publication.
 - a. Lumpkin, M.H., Bruckner, J.V., Campbell, J.L., Dallas, C.E., White, C.A. and Fisher, J.W. (2003). Plasma binding of trichloroacetic acid in mice, rats and humans under cancer bioassay and environmental exposure conditions. *Drug Metab. Dispos.* in press.

Performance Schedule and Status of Aims

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

3.1.6 <u>PBPK Modeling of Toxic Metabolites of Trichloroethylene in Rats, Mice and Humans: Predicting the Health Risks Posed by Low Level Exposure to TCE</u>
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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. Comparison of TCA distribution in mouse, rat and human serum has indicated that the extent of binding to plasma albumin is significantly less in humans than in the two rodent models for TCE and TCE-metabolite hepatocarcinogenicity (mouse and rat).
2. The new parameters for TCA binding and derived distribution have been incorporated into the PBPK model for TCE and TCE-metabolites. These data indicate a significant change in the AUC for TCA and suggest that the risk model based on PBPK-extrapolation from the rodent models to man may require modification.
3. Amy Dixon, a Ph.D. student, has developed and validated a MS method to detect DCA in blood and other tissues at a limit of detection in the low ng/g tissue. Preliminary studies applying this method to tissues obtained from rodents treated acutely with TCE have indicated significant levels of this metabolite in tissues as compared with blood. These data raise the possibility that DCA production in the rodent may have been seriously underestimated.

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- α , TNF- β , IL-1 β , 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. We investigated the role of IL-10 gene polymorphism in 98 African American (55 SSc patients and 43 controls) and 275 Caucasian (135 SSc patients and 140 controls) subjects. IL-10 is a pleiotropic anti-inflammatory cytokine. Patients with scleroderma have elevated levels of IL-10, and elevated levels are associated with thicker skin and pulmonary fibrosis, which are poor prognostic factors for the disease. A single nucleotide polymorphism (SNP) in the IL-10 promoter at position –1082 has been associated with inter-individual differences in IL-10 levels: the G allele at this locus was associated with higher IL-10 production. These observations provided the rationale for investigating if allelic variation at this locus is a risk factor for the development of scleroderma. We found that in Caucasians the frequency of GG homozygotes was significantly higher in patients than that in controls (30 vs 17.9%, $P = 0.01$; OR = 2.0). The following report is the first, to our knowledge, implicating IL-10 gene polymorphism in susceptibility to scleroderma:
 - a. Hudson LL, Rocca R, Pandey JP. Interleukin-10 gene polymorphism in systemic sclerosis. *Ann Rheum Dis* 2003; Submitted.

2. In collaboration with Dr. Karin Manger, University of Erlangen-Nuremberg, Erlangen, Germany, we have investigated the role of SNPs encoding CTLA-4—a competitive antagonist of CD28 and a negative costimulatory molecule primarily expressed on activated T cells—in susceptibility to SLE in German subjects. The distribution of genotypes in exon 1, which have been previously associated with the CTLA-4 expression, was significantly different in patients from that in controls: Compared to controls, the frequency of GG homozygotes was higher (24.5 vs. 13.5%, $P = 0.02$; OR = 2.0) and the frequency of AG heterozygotes was lower (36.3 vs. 50.6%, $P = 0.02$; OR = 0.55) in patients. These results, presented in the following manuscript, suggest that allelic variation at the exon 1 +49 locus of the CTLA-4 gene contributes to the risk of developing SLE:
 - a. Hudson LL, Kalden JR, Manger K, Pandey JP. A functional polymorphism of the CTLA-4 gene is associated with systemic lupus erythematosus. *Hum Immunol* 2003; Submitted.
3. The most recent results of our ongoing collaboration with Dr. YW Song, Seoul National University College of Medicine, Seoul, Korea, are presented in the following manuscript:
 - a. Lee EB, Lee YJ, Shin DH, Choi YM, Pandey JP, Song YW. Immunoglobulin allotypes and Fc γ RIIA 176F/V polymorphisms in Korean patients with SLE. *Ann Rheum Dis* 2003; Submitted.
4. We have identified a 66-kDa epitope, which appears to be a target for anti endothelial cell autoantibodies in SSc patients. Experiments to further characterize this epitope are in progress. The endothelial cell damage is considered to be the primary event in the pathogenesis of scleroderma.
5. MUSC's Institutional Animal Care and Use Committee (IACUC) has approved our vertebrate animal review application for a murine study to investigate the mechanism underlying the association between TCE exposure and susceptibility to scleroderma.
6. During the previous quarter, we planned the following:
 - a. Characterization of SLE patients and controls from the Carolina Lupus Study for several IL-10 genes.
 - b. Submit a manuscript describing the interactive effects of CTLA-4 and GM genes in susceptibility to SLE.
 - c. Initiate murine experiments to investigate the mechanism underlying the association between TCE exposure and susceptibility to scleroderma.
 - 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 affects on the immune system that can then be assayed in humans exposed *in utero* to TCE. We will also hope to develop potential assays for TCE exposure using the now available anti-TCE adduct polyclonal sera.

Objective

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

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

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

Quarterly Accomplishments

1. Tissues have been obtained from 8 week old B6C3F1 mice that had been treated with TCE (1,000 ppb and 10,000 ppb). Autoantibody levels in the serum of these animals are in the process of being analyzed.
2. Preliminary data indicate that exposure to TCE *in utero* can induce aberration in the development of immune competency in this rodent model.

Performance Schedule and Status of Aims

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

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

Project Director:

Stanley Hoffman, Ph.D.

Executive Summary

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

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

Relevance

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

Objectives

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

Quarterly Accomplishments

Congenital heart defects involve developmental miscues in the division of the heart into chambers and its connection to the rest of the circulatory system. These processes are mediated by endocardial cells that undergo epithelial-mesenchymal transformation (EMT), i.e. they lose cell:cell adhesion, migrate into the previously acellular basement membrane known as the cardiac jelly that separates the myocardium and endocardium, and there differentiate into the endocardial cushion tissue (ECT), a progenitor of cardiac valves and septa. The goal of this project is to determine the effect of TCE on the development of the ECT. In previous quarters, we found that TCE inhibits the migration of endocardial cells into the cardiac jelly. This quarter was dedicated to two additional activities: i) determining the actual TCE concentrations in the culture medium over the length of the culture period, and ii) in-depth analysis of morphological data from stage 14 chick whole embryos cultured with 25-250 ppm TCE.

1. Aliquots of culture medium were removed from culture dishes incubated at 37°C over the 22 hours of embryo exposure. TCE levels were determined by gas chromatography in collaboration with Dr. David McMillan, Department of Pharmacology, MUSC. Results showed an initial rapid loss of TCE (60% loss/hr for the first two hours) for all test concentrations, followed by a slow and sustained loss (6% per hour) thereafter. These losses of TCE from the medium occurred both in cultures containing a chick embryo and in mock cultures. In our experiments, at least 75 minutes pass between the time when TCE-containing medium is placed into a culture dish and the time when an embryo is placed in the medium. Therefore, in reality embryos are exposed to a TCE concentration that is at most 30 % of the concentration originally placed in the culture dish and that is continually decreasing. Based on these observations, we are performing experiments designed to determine whether the inhibitory effects of TCE on endocardial cell migration are mediated by the initial high TCE concentration encountered by the cells or by the longer-term exposure of the cells to relatively low concentrations of TCE.
2. Cell counts have been collected for superior vs. inferior atrioventricular canal cushions for all doses of TCE. Results show a dose-dependent inhibition of mesenchyme formation in the inferior cushion at all concentrations, while only higher concentrations of TCE affect the superior cushion. This is in good agreement with the kinetics of EMT in these cushions in vivo, i.e. cells appear first in the inferior cushion, then in the superior cushion 8-10 hours later.

Performance Schedule and Status of Aims:

We consider it to be of the utmost importance to focus in the upcoming quarter on testing the hypothesis that TCE metabolites inhibit cushion mesenchyme formation at much lower concentrations than are required when using TCE itself.

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

Project Director:

Alice Boylan, M.D.

Co-Director:

Besim Ogretmen, Ph.D.

Executive Summary

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

genes (e.g., bcl-2, bcl-x, bax and p53) in normal and/or cancerous human lung epithelial cells that are resistant to these agents.

Relevance

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

Objectives

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

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

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

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

Quarterly Accomplishments

Our previous studies showed that chronic long-term exposure of human lung cancer cells (A549) to asbestos and cigarette smoke in combination results in the development of resistance to apoptosis via increased levels of anti-apoptotic proteins (such as Bcl-2). To confirm these results, we isolated more A549 cell populations, which were exposed to these two agents in combination. Then, the total proteins were extracted and these extracts are about to be ready to examine the expression of anti-apoptotic protein levels

by Western blotting. We expect that these new population of cells which were chronically exposed to increasing concentrations of asbestos and cigarette smoke will express higher anti-apoptotic protein(s) and higher resistance to apoptotic cell death.

In addition, we started developing our mouse model system which allows to develop spontaneous lung cancers in response to doxocyclin induction. When dox is removed, cancer cells go under apoptosis. To test our hypothesis *in vivo*, mice will be exposed to asbestos and cigarette smoke after dox is removed. We predict that exposure to these agents will cause selection or development of population of cancer cells which express resistance to apoptosis. These exciting *in vivo* studies are underway, and will provide important information on the validity of anti-apoptotic proteins as biomarkers for environmental exposure.

Performance Schedule and Status of Aims

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

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

Lucille London, Ph.D.

Executive Summary

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

of specific PCB mixtures change after bioremediation in the laboratory will help determine the potential toxicity associated with PCB contamination in the environment.

Relevance

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

Objectives

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

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 continued to evaluate whether the observed inhibition of LPS-induced splenocyte proliferation is a result of an induction of apoptosis? As previously disclosed both DNA fragmentation assays and induction of specific genes as measurements of apoptosis did not reveal any significant differences between control and PCB treated groups. To further investigate this lack of proliferation in PCB treated spleens, we evaluated cell cycle progression induced by LPS in the presence or absence of PCBs. We found a significant reduction in the number of cells which were able to enter the S phases of the cell cycle in the presence of

PCB mixtures (Aroclors & para and meta dechlorinated Aroclors) as well as in the presence of ortho substituted individual PCB congeners, whereas meta and para substituted individual PCB congeners did not block entry of LPS stimulated cells into the S phase of the cell cycle. These results suggest that ortho substituted PCB congeners and PCB mixtures block the cell cycle at the G1/S interphase.

2. To begin to investigate the mechanism behind this cell cycle arrest, we began to investigate the expression of cell cycle regulatory proteins that function at the restriction point of G1, including D-type cyclins and cdks. In b cells cyclin D2 is a key player in mediating cell cycle progression after mitotic signals such as LPS, and B cell receptor crosslinking. We found that ortho-substituted individual PCB congeners, PCB mixtures (Aroclors & para and meta dechlorinated Aroclors (ortho enriched mixtures)) inhibit Cyclin D2 expression. Likewise, meta and para substituted individual congeners do not inhibit the expression of Cyclin D2. These results suggest that LPS induced spleen cells exposed to PCB mixtures or ortho substituted PCBs are blocked in the G1 phase of the cell cycle due to the lack of expression/induction of cyclin D2. Whether this is a direct or indirect effect of PCB exposure on Cyclin D2 expression remains to be determined.

Dissertation:

Smithwick, Laura Ashley: Effect of Polychlorinated Biphenyl Transformation Processes on the Immunological Responses in Murine Splenocytes. Successful defense and completion of Ms. Smithwick's Ph.D. dissertation took place on June 19, 2003.

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 previously reported that we received from the Ontario Cancer Registry the cases of childhood leukemia. We just received the small area census data and are beginning the data analysis. We plan to complete the work on Specific Aims 1-3 in the Fall of 2003.

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 have used 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 examined 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. The analysis suggests that the bystander effect does not apply to the mouse lung at the neutron doses we used.

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 has begun. The appropriate worker databases are being reviewed for analysis.
2. The manuscript Baker GS, Hoel DG, "Corrections in the Atomic Bomb Data to Examine Low Dose Risk has been accepted for publication by 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. Accepted manuscript
 - 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.
2. Accepted abstract
 - 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. Submitted manuscript
 - a. Padgett SL, Nicholas JS, et al. Skin cancer among commercial airline pilots: rates, predictors, and potential impact. Aviation, Space and Environmental Medicine.
4. Progress on mortality study: Application to NDI approved, additional state forms completed, data being prepared for submission to NDI.

This project has achieved its specific objectives and was brought to a successful conclusion in May 2003.

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 study has been presented to her dissertation committee. 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? These questions will be addressed in her research.

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 overly comparisons.

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

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

Existing Databases

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

Objectives

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

Specific Aims

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

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

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

Quarterly Accomplishments

1. GIS Resource - Updated GIS ArcView software was successfully installed. Satellite positioning equipment was successfully tested. Staff and investigators successfully completed online training. Investigators attended the GIS conferences in San Diego and New Orleans.
2. Six summer students completed GIS training and successfully completed a population assessment of environmental exposures:
Christina Walters – Slippery Rock University
Rowena Devera – College of Charleston
Jyris Sims – Louisiana State University
Lissette Alvarado – University of Puerto Rico
Erica Dean – SC Governor’s School for Science and Mathematics
Mallory Hudson – SC Governor’s School for Science and Mathematics

3. Sub-contracts and agreements were successfully implemented with the SC Department of Health and Environmental Control and the SC Office of Research and Statistics for geo-coded health and exposure data.
4. National Death Index search was implemented for former SRS workers. This process will yield the identification of underlying cause of death for deceased workers.

Performance Schedule and Status of Aims

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