Environmental Biosciences Program
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Principal Investigator

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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 Second Quarter

The table below reflects expenditures by budgeted category recorded for the period July – September, 2002 and year-to-date, of Cooperative Agreement CH11109. Encumbrances for personnel costs and F & A for the entire budget period are not included in this table but total $1,084,037.

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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.
Grants Coordinator: Nora Futrell, B.S.
Administrative Coordinator: Jill Canaday
3.0 Scientific Research

3.1 Environmental Toxicology Research Projects

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

Project Director: JoEllyn M. McMillan, Ph.D.

Executive Summary

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

Relevance

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

Objective

The hepatocarcinogenicity of TCE is believed to be related to the ability of its metabolites, TCA and DCA, to induce peroxisome proliferative and mitogenic activity in B6C3F1 mice and rats. Humans are considered to be less sensitive, but
their susceptibility to peroxisome proliferation and mitogenesis is largely unknown. The role of PPARα 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 PPARα 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 α (PPARα) in peroxisomal and cell replicative events in rodent and human hepatocytes.

Quarterly Accomplishments

1. We have completed a second, short-term in vivo treatment protocol in female PPARα knockout mice using the compounds TCA, DCA, WY-14,643, and chloral hydrate. The livers were removed and fixed for histological examination to determine hepatocyte replication rates. The remaining liver was frozen for examination of enzyme activities, transcription factor levels and activities and changes in cell signaling molecules.

2. We are continuing to examine the effect of protein kinase A activation on PPARα-dependent peroxisomal and microsomal enzyme induction in rat, B6C3F1 mouse, and human hepatocytes.

Performance Schedule and Status of Aims

1. We will continue to examine the importance of protein kinase A activation in induction of peroxisomal enzymes and other related enzymes in B6C3F1 mouse, rat, and human hepatocytes.

2. We will complete our studies on the comparison of the peroxisome proliferative and cytotoxic activities of halogenated analogs of trichloroacetate and dichloroacetate in cultured hepatocytes and prepare a manuscript for publication.

3. We will complete the processing and staining procedures to determine changes in hepatocyte replication rates in the livers from the treated PPARα knockout mice.
Effects of Trichloroethylene Metabolites on Hepatic Cell-Cycle Regulatory Proteins and Transcription Factors

Executive Summary

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

Relevance

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

Objective

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

Specific Aim 1. To determine the molecular mechanism(s) by which TCE metabolites can serve as priming agents for mitogenesis in rodent hepatocytes and to determine if this effect can occur in human hepatocytes.
Specific Aim 2. To identify the effects of TCE metabolites on signal transduction cascades which may affect cell division in hepatocytes.

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

**Quarterly Accomplishments**

1. We have shown that DCA can enhance cdk4 activity in isolated mouse hepatocytes after 48 hours of treatment.

2. We have shown that DCA leads to an activation of cyclinD/cdk4 association in mouse hepatocytes.

3. Nuclear extracts from PPAR alpha knockout mice which have been treated with TCE, TCA, DCA, and Wyeth-14643 have been found to show several changes which are identical to those seen in wild type mice, indicating that some effects of these drugs are not mediated by the PPAR alpha receptor.

**Performance Schedule and Status of Aims**

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

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

**Project Director:** Rick G. Schnellmann, Ph.D.

**Executive Summary**

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

Relevance

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

Objectives

While DCVC is considered to be the metabolite most likely responsible for TRI nephrotoxicity and nephrocarcinogenicity in rats, only a limited number of studies have addressed the molecular mechanisms underlying these toxicities. We currently have little cellular and molecular information concerning the effects of acute and chronic exposure of renal cells to DCVC. Of importance, while we know that various rodent species differ in susceptibility to TRI-induced renal toxicity and neoplasia, we know little about why they differ; specifically, how the renal cells of these species vary in their cellular response to DCVC. The concentration-dependence of these effects will be crucial in dose extrapolation from the rodent to humans and to the recognition of susceptible human populations, if such exist. The long-term objective of this project is to determine the mechanism(s) by which DCVC and related metabolites injure renal cells and the basis for the relative resistance of less susceptible species. Direct comparisons will be made with human renal cells to provide the mechanistic basis for risk assessment purposes. Experimentally, we will expose mouse-, rat-, rabbit- and human-derived RPTC to the various concentrations of DCVC, acutely and chronically, that cause minimal cell death. The expression, and the time-dependence of the expression, of distinct differentiated and integrated cell functions (e.g. transport, migration, proliferation) and gene expression (using oligonucleotide microarrays) will be determined. The following variables will be examined: DCVC concentration, single DCVC exposure, multiple DCVC exposures and time, and will be related to the expression of specific genes and differentiated functions.
Specific Aim 1. To determine the effect of graded degrees of acute and chronic DCVC exposure on injury and death (necrosis and apoptosis) in mouse, rat, rabbit and human RPTC.

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

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

Quarterly Accomplishments

1. This is a new project starting July 1, 2002. Recent epidemiological evidence has focused attention on the capacity of TCE to induce renal neoplasia in humans and has raised the question as to whether TCE-induced renal cancer should be used as basis for the risk assessment of TCE. However, relatively little has been firmly established in regard to the mechanism underlying TCE-induced renal toxicity. While it has long been believed that cysteine conjugates of haloalkenes are the crucial proximate carcinogens in rodents that have been chronically exposed to the parent haloalkenes, the precise pathways by which they are formed, and the factors that affect the activity of these pathways, are yet to be established. The studies getting underway are designed to define the pathways of formation and elimination of TCE-derived cysteine conjugates in rat kidney, and to describe the conditions under which the these cysteine conjugates are converted to their episulphonium-type ultimate carcinogens.

2. The overall experimental approach is to isolate rodent renal proximal tubular cells (RPTC) and to develop the capacity to culture these cells ex vivo. These cultures will then be used to examine the formation and fate of the reactive carcinogenic metabolite(s) derived from TCE. Later comparative studies with human RPTC will place the risk assessment on a firmer mechanistic basis should the kidney tumors become the key toxicity for this purpose. Current activity is centered on the attainment of the facilities to culture isolated RPTC from rodents, and on the procedures to be used to establish that the primary cultures of RPTC retain normal physiological functions at levels similar to that found in vivo.

Performance Schedule and Status of Aims

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

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

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. Gas chromatograph order placed.

2. Initial experiments to characterize CYP and ALD/ALDH gene expression in human liver accomplished.

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. TCA, a mouse liver carcinogen, is a common drinking water contaminant and a major metabolite of solvents such as TCE and perchloroethylene. As many acidic drugs are bound more strongly to human than to rodent plasma, a study was undertaken to determine whether this was the case for TCA and to clarify the mechanistic basis of species differences. Equilibrium dialysis was used to measure \textit{in vitro} binding of a wide range of concentrations of TCA to plasma of humans, rats and mice. TCA concentrations were measured by electron capture gas chromatography. Attempts were made to fit plots of free versus bound TCA concentration to each of 3 binding models. A model that provided for a single, saturable binding process best fit the data. Dissociation constants did not differ significantly from one species to another, but N (number of binding sites per protein molecule) values ranged from 2.80 for humans to 0.13 for mice. Binding capacities for humans, rats and mice were 669, 270 and 26 \(\mu\text{M}\), respectively. The greater plasma protein binding capacity of TCA in humans would be expected to not only increase the residence time of the compound in the bloodstream, but to substantially reduce the proportion of TCA that is available for uptake by the liver and other tissues.

2. A study was conducted to determine whether blood trichloroacetic acid (TCA) levels accurately reflect liver levels in mice and rats given a wide range of oral doses of TCE. TCA peak concentration and area under the concentration versus time curve (AUC) values were determined for blood, liver, kidney, brain, lung
and fat. These values were compared with published data (Abbas and Fisher, 1997) for male B6C3F1 mice dosed similarly with TCE. TCA levels in blood exceeded levels in each tissue in both mice and rats. The blood levels rose to a greater extent with increase in TCE dose than did liver and kidney levels in each species. This dose-dependent partitioning of TCA into the blood apparently results from accumulation of the bound compound there. We found (see 1. above) that the bound fraction of TCA (i.e., that portion not available for hepatic uptake) in human plasma was substantially greater than that in mouse or rat plasma. Thus, the use of free, rather than total blood TCA AUCs would result in a narrower, more precise, and more scientifically rigorous range of human cancer risk estimates from mouse liver bioassay data.

3. Two manuscripts (one each for 1. and 2.) are nearing completion for submission for publication (1. to Drug Metabolism and Disposition and 2. to Toxicology and Applied Pharmacology).

Performance Schedule and Status of Aims

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

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

Project Director: Jeffery W. Fisher, Ph.D.

Executive Summary

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

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

Relevance

The scientific issues related to determining the health risks posed by low levels of TCE in the environment are relevant to many other solvents found in water supplies. If sound science and extrapolation methodology can be demonstrated for this chemical, then other chemicals can be evaluated in a similar manner. This could lead to a potential saving of multiple millions of dollars in unnecessary clean-up costs.

Objectives

1. Harmonize current PBPK models used by the US EPA into one PBPK model for TCE and metabolites. Incorporate newly published and unpublished data in humans and rodents. New data sets include published and unpublished rat data on first pass metabolism of TCE from the laboratory of Dr. Jim Bruckner at the University of Georgia, published human and unpublished rat data on glutathione conjugation of TCE [(S-(1,2-Dichlorovinyl) Glutathione (DCVG)] obtained by Dr. Larry Lash at Wayne State University, and published Epidemiology studies performed in Europe, where urinary excretion of TCA was quantified.

2. Conduct laboratory studies to refine PBPK model predicted dose metrics in laboratory animal and humans that will be used in the formulation of the final product of this project, namely a TCE human health risk assessment. Determine the stoichiometric yield of DCVG for relevant doses of TCE in rats. Information on DCVG will provide data to develop the DCVG pathway in a PBPK model for TCE and to offer plausible dose-metrics that can be associated with the risk of kidney cancer in humans. Colleagues and I have time course data for DCVG in humans exposed to TCE vapors [Lash, LH, DA Putt, WT Brashear, R Abbas, J Parker and JW Fisher. 1999. Identification of S-(1,2-Dichlorovinyl) Glutathione in the Blood of Human Volunteers Exposed to Trichloroethylene. J. Toxicol. Environ. Health Part A, 56, 1-21].
3. Conduct laboratory studies to evaluate how much dichloroacetic acid (DCA) is formed metabolically from TCE. This minor metabolite remains an important risk assessment issue because of its carcinogenic potency and the requirement that the US EPA account for cumulative risks. DCA is the number one by-product from chlorination of water. Thus, to account for the health risks poised by TCE in drinking water, the health risks from exposure to DCA itself must be quantified and accounted for in the health risk assessment of TCE.

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

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

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

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

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

Quarterly Accomplishments

1. Deborah A. Keys was hired and started working on August 22, 2002. Drs. Jeff Fisher, Jim Bruckner and Deborah Keys traveled to the Medical University of South Carolina (MUSC) in Charleston, SC on August 27th -August 29th to meet with MUSC personnel for a project briefing.

2. TCE Rat PBPK Model (Drs. Keys, Fisher and Bruckner):

   a. Dr. Bruckner provided unpublished kinetic datasets of TCE blood and several organs and tissues in rats following various routes of exposure (inhalation, intra-arterial, oral). These datasets are currently being examined and compared to PBPK model predictions. These datasets represent doses and inhaled concentrations at lower levels than presently in the literature. ACSL (Advanced Continuous Simulation Language) is being used for model simulations. S-Plus, a statistical package is being used to run graphing scripts to produce publication quality graphics of model comparisons to measured data.
b. An abstract titled, “Low-dose Validation of a Physiologically Based Pharmacokinetic Model for Trichloroethylene in the Rat” was submitted to the Society of Toxicology Annual Meeting to be held in Salt Lake City, March 9-13, 2003.

3. DCA PBPK Model (Drs. Keys and Fisher):
   a. Dichloroacetic acid (DCA) is a minor metabolite of TCE that remains an important risk assessment issue because of a) its carcinogenic potency and b) its background exposure levels as the number one by-product from chlorination of water.
   b. Dr. Fisher provided unpublished DCA kinetic drinking water studies in mice and rats. These datasets are currently being compiled for use in a PBPK model for DCA.

4. TCA PBPK Model. (Drs. Keys and Fisher):
   a. Trichloroacetic acid (TCA) is considered a principle metabolite responsible for TCE-induced liver cancer in mice.
   b. Dr. Fisher provided unpublished TCA kinetic drinking water studies in mice and rats. These datasets are currently being compiled for use in PBPK models.

Performance Schedule and Status of Aims

Neither the performance status nor the status of aims has changed

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<th>3.1.7 Trichloroethylene Exposure and Host Genetic Factors in Autoimmune Diseases</th>
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<td>Project Director: Janardan P. Pandey, Ph.D.</td>
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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, anticientromere, 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 P450IIIIE1, 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. Ms. Lori Hudson, the graduate student working on the project, submitted an abstract entitled “CTLA-4 gene polymorphisms in systemic lupus erythematosus: a highly significant role for a determinant in the promoter region” for presentation on MUSC Student Research Day, November 1, 2002.

2. Continued to investigate the role of cytokine genes in the etiopathogenesis of other autoimmune diseases. Our ongoing collaboration with Christian A. Vedeler, M.D., Ph.D., University of Bergen, Norway, resulted in the following publication:

3. As part of our collaborative project with Dr. Glinda Cooper, Epidemiology Branch, NIEHS, we genotyped 546 SLE patients and controls for four CTLA-4 determinants.

4. Genotyped 239 SSc patients and controls for two new CTLA-4 determinants (-1722 and -1661).

5. During the previous quarter, we planned the following:
   a. Characterization of the remaining SSc patients and controls for the new CTLA-4 genes.
   b. Submit manuscripts describing 1) the role of CTLA-4 genes in SSc pathogenesis 2) the role of CTLA-4 genes in SLE pathogenesis.
   c. Characterization of myositis patients and controls from NIEHS for GM and KM genes.
   d. Determine the role of polymorphic genes encoding transforming growth factor beta-1—a cytokine involved in the production and degradation of extracellular matrix—in susceptibility to scleroderma.
Performance Schedule and Status of Aims

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

### 3.1.8 Immunological Effects of Trichloroethylene Exposure

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

**Executive Summary**

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

**Relevance**

There is growing concern about the effects of in utero exposure to environmental toxins. It is of obvious importance to determine if in utero exposure to common environmental toxins has effects on the developing immune system. These experiments in normal and lupus prone mice will provide insight into potential affects on the immune system that can then be assayed in humans exposed in utero to TCE. We will also hope to develop potential assays for TCE exposure using the now available anti-TCE adduct polyclonal sera.

**Objective**

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

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

**Quarterly Accomplishments**

1. B6C3F1 pups were born and have been tested at 3 and 8 weeks of age. Endpoints assessed included splenic and thymic weights and cellularity, natural killer cell (NK) activity, antibody plaque forming cell (PFC) response, lymphocyte proliferation, and T-cell immunophenotypes. At 21 days of age, alterations were evident. Body weight and length were significantly decreased by the 10,000 ppb treatment. NK cell activity and T- and B-cell proliferation were not altered. IgM antibody responses to sRBC challenge were suppressed in both male and female pups by 10,000 ppb TCE and by 1000 ppb TCE in the male pups only. Few alterations were apparent in thymic T-cell populations, whereas there was a distinct decrease in splenic CD4+CD8- T-cells resulting in a concomitant decrease in the CD4+:CD8+ ratio. At 56-days of age, the most striking effect was noted with increased NK cell activity in both treatment groups. Currently studies are being conducted to verify the PFC response and T-cell immunophenotypes in the adult mice.

2. An abstract was submitted October 1 to the Society of Toxicology meeting and will be published in Toxicologist 2003.

**Performance Schedule and Status of Aims**

The project is on schedule and no changes have been made in the status of aims.
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 supersusceptibility. 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. Most congenital hearts defects involve developmental miscues in the division of the heart into chambers and the connection of the heart to the rest of the circulatory system. These processes are mediated by endocardial cells that undergo EMT, migrate into the previously acellular basement membrane known as the cardiac jelly that separates the myocardium and endocardium, and then differentiate into the endocardial cushion tissue. Therefore, to allow us to analyze how TCE may produce heart defects, this quarter we have compared methods for the morphological assessment of early cushion tissue morphogenesis. These methods will allow us to evaluate the volume of the cardiac jelly, the number of cells that have seeded into the cardiac jelly, and will provide us with 3D renderings of the early heart. The method of choice must provide faithful preservation of native morphology, resolution of individual cells, not counting the same cell twice, and efficiency of data acquisition. Three protocols were
evaluated: paraffin-embedded thin (6 µm) serial sections (epifluorescent microscopy), polyacrylamide-embedded thick (200 µm) sections (laser confocal microscopy), and whole mount-cleared embryos (laser confocal microscopy). Each of these methods has different benefits and limitations; however, comparative studies to date suggest that the whole mount approach gives optimal results in the minimal amount of time. The main limitation of the whole mount approach is that signal intensity is markedly reduced in the deeper optical sections. However, this problem can be overcome by flipping the embryo over and repeating the analysis from the backside and by using a new confocal microscopy with more powerful lasers that has just been installed in our department.

2. In the current quarter, we have also continued to optimize our ability to culture whole chick embryos. In particular, stage 14 embryos can be cultured for 24 hours with greater than 90% viability. We are now ready to apply our optimized morphological methods to these embryos.

Performance Schedule and Status of Aims

As detailed in our previous quarterly report experiments under Objective 1 are likely to take a year or more. We have cleared the first major set of goals towards that objective. Work in the upcoming quarter will be focused on a comparison of control and TCE-treated embryos (both in culture and in ovo). In addition, we will initiate experiments to determine if TCE metabolites are effective at inhibiting or delaying cushion mesenchyme formation in the collagen gel culture model.

| 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

4. A series of cytotoxicity assays were performed using anticancer drugs with different mechanisms of action. We found that smoke and asbestos resistant A549 cell line have 2-4 fold increased resistance to -cisplatinum, taxol, and doxorubicin.

5. We also confirmed that these cells over-express anti-apoptotic Bcl-2 protein, while they contain down-regulated pro-apoptotic Bax protein using Western analysis.
6. However, Western analysis did not confirm increased expression of three interesting genes found to be upregulated in the asbestos and cigarette smoke resistant cell line by microarray analysis (HSP-70, Niemann Pick Disease Protein, Caveolin-2). To strengthen our studies, we have identified a collaborator who has agreed to provide us with other immortalized airway epithelial cell lines to use for development of additional cell lines with resistant to asbestos and cigarette smoke. Once developed the cell lines will be evaluated to determine if the changes in expression of genes involved in the apoptotic pathway are observed.

7. To assess the potential use of anti-apoptotic and pro-apoptotic genes previously identified as having altered expression in our cell lines as biomarkers in patients exposed to both carcinogens, we have begun obtaining bronchoscopic biopsies of lung cancers and adjacent “normal” airway epithelium. These specimens will be evaluated using RT-PCR due to their small size. The findings from these studies will be correlated with occupational and smoking histories.

8. We have now established the transgeneic mouse colonies that express murine K-Ras4b\(^{G12D}\) under the control of doxycycline in type II pneumocytes. Mice homozygous for the lung promoter have been crossed with those homozygous for K-ras are now pregnant. The first experiment will entail dosing of doxycycline at day 3 postpartum, the time of maximal cell proliferation in the lung. If an increase in number of tumors is found (supporting a role for specific cell-cycle proteins in tumorogenesis) we will then proceed with asbestos-exposure experiments. Cells in the lung will be synchronized in various points of the cell cycle prior to asbestos exposure. By this means we hope to tease apart the important proteins tumorogenesis which may serve as early markers of disease or risk.

**Performance Schedule and Status of Aims**

The project is on schedule as proposed, and there are no changes in the status of aims with the exception of the addition of the K-ras murine lung cancer model. We believe the addition of this model significantly strengthens our aims.

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<th>3.1.11</th>
<th>Immunotoxicological Assessment of Non-degraded and Biodegraded PCB Mixtures</th>
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<td><strong>Project Director:</strong></td>
<td>Lucille London, Ph.D.</td>
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**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 our analysis of surface Ig expression in 70Z/3 cells stimulated with LPS in the presence of either Aroclors or individual PCB congeners. Exposure of 70Z/3 cells to LPS in the presence of either Aroclor 1242 (mixture), 2,2-PCB (ortho), 4,4-PCB (coplanar), or 2,4,4-(mono-ortho coplanar) PCB did not inhibit the induction of surface Ig expression.

2. Although surface Ig expression in LPS-induced 70Z/3 cells was not inhibited by either Aroclors or individual congeners, we did observe an effect on cell division. At higher PCB exposures (above 10 ppm) the total number of live cells recovered was decreased although the viability (live versus dead cells) was unaffected. This trend was observed with Aroclor 1242 as well the individual congeners listed above. This suggests that PCBs do not effect 70Z/3 viability but they do inhibit 70Z/3 proliferation.

3. Submitted an abstract to the Society of Environmental Toxicology and Chemistry Meeting which will be held in November 2002.

4. Preliminary date on the examination of PCB induced apoptosis in LPS-induced spleen cells is promising. At high PCB concentration (Aroclor 1242 at 20 ppm) significant induction of DNA fragmentation was observed. We have begun experiments to evaluate apoptosis related genes via PCR analysis. Preliminary data suggest that LPS stimulation of spleen cells induces the anti-apoptotic gene BCL-xl which does not appear to be inhibited in the presence of low concentrations of PCBs. These are very preliminary data.


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

Peter Baker went to Toronto, Canada, September 29, 2002 to gather data from the Cancer Registry Ontario.

Performance Schedule and Status of Aims

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

3.2.2 Low Dose Radiation: Toxicological Models of Cancer Risk

Project Director: David G. Hoel, Ph.D.

Executive Summary

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

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

Relevance

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

Objective

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

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

Quarterly Accomplishments


Performance Schedule and Status of Aims

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

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<th>3.2.3 Low Dose Radiation: Epidemiological Risk Models</th>
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<td><strong>Project Director:</strong> David G. Hoel, Ph.D.</td>
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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


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. The following presentations, with published abstracts, were made at professional meetings.


2. The following manuscripts are being prepared:

b. 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. (This paper is based on a Master’s level research project completed August 2002.)

3. Data required for a standardized mortality study has been obtained from ALPA. Application has been made to the NDI, and we are waiting for approval.

Performance Schedule and Status of Aims

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

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<tr>
<th>3.2.5 Health Risks of Low Dose Plutonium Exposure</th>
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<td>Project Director: David G. Hoel, Ph.D.</td>
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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 Accomplishments

1. Work has begun on collecting experimental data from rats and dogs exposed to inhaled plutonium. This information, which will complete the basis of an early computer model of cancer risk, must be identified.

2. Graduate student, Lui Jiang, has identified some of the many sources and is in the process of entering the data into a computer program for use in the environmental risk modeling effort.

Performance Schedule and Status of Aims

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

3.2.6 Population Risk Studies Using Geographic Information System Technology

Project Director: Daniel Lackland, Dr.P.H.

Executive Summary

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

Savannah River Region Health Information System (SRRHIS)
The geographic cancer registry incorporates 25 counties around the Savannah River Site. Cancer incidence data obtained in a high quality manner is an essential component of epidemiological investigations.
A direct link to this resource has been established in which cancer cases are geographically identified and incorporated in the data analysis. SRRHIS provides the cancer-related component of the assessment system. Cancer incidence and mortality rates are associated with various aspects of population.

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

2. Other progress includes:
   a. Five summer students were trained and worked on GIS related projects:
      i. Megan Singleton – Spelman College
      ii. Chris Yako – Vanderbilt University
      iii. John Knapp – Porter-Gaude High School
      iv. Kristen Newson – SC Governor’s School for Science and Mathematics
      v. Liwen Jin – SC Governor’s School for Science and Mathematics

3. Sub-analysis of population cancer risks in the vicinity of SRS continues.

4. Data pertaining to health risks of former SRS production workers is being processed for GIS analysis.

5. Plans are being developed to monitor population health risks related to plutonium transportation and disposition.

Performance Schedule and Status of Aims

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