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MUSC
MEDICAL UNIVERSITY OF SOUTH CAROLINA

Environmental Biosciences Program
Report for Year 3
July — September 2006

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Principal Investigator

For

Cooperative Agreement DE-FC09-02CH11109

Submitted to the
U. S. Department of Energy

By The

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1.0 Introduction

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

EBP is an integrated, multidisciplinary scientific research program, employing a range of research initiatives to identify, study and resolve environmental health risks. These initiatives are consistent with the MUSC role as a comprehensive state-supported health sciences institution and with the nation's need for new and better approaches to the solution of a complex and expansive array of environment-related health problems.

The intrinsic capabilities of a comprehensive health sciences institution enable MUSC to be a national resource for the scientific investigation of environmental health issues. EBPs success as a nationally prominent research program is due, in part, to its ability to task-organize scientific expertise from multiple disciplines in addressing these complex problems

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

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

1.1 Summary and Significance of Year Two 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.

Risk Assessment

- The adverse health effects of both ionizing and non-ionizing radiation are of concern to DOE and the public. Many important questions about the adverse human health effects of low-dose and low-dose rate radiation exposures remain unanswered – especially with respect to cancer risks. MUSC has developed a comprehensive research program for the study of the effects of low-dose and low-dose rate radiation exposures on human health.

- Population risk studies in areas surrounding DOE sites are of utmost importance to the department and to the citizens who live in these areas. The Savannah River Region Health Information System is a very important national, regional, and DOE resource for the study of population health effects in the area surrounding the Savannah River Site. In conjunction with the Savannah River Region Health Information System, MUSC has developed an extremely powerful Geographical Information System in which databases containing health, environmental, demographic and socioeconomic data can be integrated and analyzed for specific population health risks.

1.2 Program Expenditures

EBP Expenditure Summary

The table below reflects **expenditures** by budgeted category recorded for the period July 2006 through September 2006 and total life-to-date for Cooperative Agreement CH11109.

Budget Category	Current Period (Dollars in thousands)	LTD
Personnel	$ 129	
Supplies	-	
Travel	-	
Other	-	
Subcontract	-	
Equipment	-	
Total Direct Costs	129	$ 6,402
F & A	08	2,406
Total	$ 137	$ 8,809

2.0 Program Management and Development Office

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

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

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

3.0 Scientific Research

3.1 Environmental Toxicology Research Projects

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

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

Executive Summary

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

Relevance

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

Objective

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

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

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

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

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

Quarterly Accomplishments

Assisted David McMillan in re-establishing several experimental systems in his laboratory due to personnel turnover. The experimental systems included: (1) metabolism of chloral hydrate by human hepatocytes and analysis of metabolic products by GC headspace analysis, (2) isolation of genomic DNA from human hepatocytes for ALDH and ADH genotyping, (3) genotyping of ALDH and ADH using genomic DNA from human hepatocytes.

Performance Schedule and Status of Aims

We are continuing studies to determine the anti-apoptotic effect of dihalogenated acetates, including dichloroacetate, on hepatocytes from $B6C3F_1$ mouse, rat and human liver. This data is being prepared for a manuscript submission.

3.1.2 Effects of Trichloroethylene Metabolites on Hepatic Cell-Cycle Regulatory Proteins and Transcription Factors

Project Director: David T. Kurtz, Ph.D.

Executive Summary

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

Relevance

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

Objective

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

Specific Aim 1. To determine the molecular mechanism(s) by which TCE metabolites can serve as priming agents for mitogenesis in rodent hepatocytes and to determine if this effect can occur in human hepatocytes.

Specific Aim 2. To identify the effects of TCE metabolites on signal transduction cascades which may affect cell division in hepatocytes

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

Quarterly Accomplishments

1 We have characterized the effects of DCA and TCA in inducing beta catenin/TCF binding to DNA in rodent liver.

2 We have examined the effects of DCA and TCA in inducing beta catenin nuclear translocation in human hepatoma cells.

Performance Schedule and Status of Aims

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

3.1.3 Human and Rodent Renal Proximal Tubular Cells as Model Systems to Study the Toxicity and Elimination of Trichloroethylene Metabolites

Project Director: Douglas Sweet, Ph.D.

Executive Summary

A growing body of evidence suggests that trichloroethylene (TCE) exposure induces hepatocellular carcinoma, nephrotoxicity, and nephrocarcinogenicity in rats. Further research has indicated that it is not the parent compound, TCE, but rather several of its metabolites, including trichloroacetic acid (TCA), dichloroacetic acid (DCA), and 1,2-dichlorovinyl-L-cysteine (DCVC) that are the causative agents of the associated renal and hepatic toxicities. The kidney and liver are target organs because they actively remove organic anions from the circulation and are therefore subject to high levels of accumulation of these negatively charged metabolites. In the case of TCE, metabolite formation, particularly at drinking water levels, is extremely rapid and target tissue exposure is largely determined by the relative rates of formation of the metabolites in the liver and their removal from the body by the renal proximal tubule cells (RPTCs). In other words, the peak metabolite concentrations and duration of exposure to the toxins are mostly determined by the kidney's ability to actively remove these substances from the body. Therefore, the mechanisms governing the absorption, distribution, and excretion of these compounds likely play a central role in their associated toxicities.

Two gene families expressed in the kidney and liver that mediate the transport of small organic anions are the Organic Anion Transporter (OAT) family and the Organic Anion Transporting Polypeptide (Oatp) family. Recently, DCVC uptake by rabbit proximal

tubules was demonstrated to be blocked by *p*-aminohippurate (PAH), the prototypical substrate for OATs. This finding suggests the OAT family of transporters is involved in the absorption, distribution, and excretion of TCE metabolites (Oatps do not transport PAH). In further support of this, DCVC inhibited organic anion transport mediated by the rabbit and human orthologs of organic anion transporter 1 (Oat1) expressed in heterologous cell systems.

Variation in the renal elimination rate constants of the ultimate carcinogenic metabolites, TCA and/or DCA, is likely to be a crucial factor in physiology based pharmacokinetic (PBPK) modeling for sensitive populations and in risk assessment based upon such modeling. Current PBPK models used in the assessment of toxicological endpoints for TCE exposure do not incorporate the contribution of active transport mechanisms that impact body compartment distribution and concentration levels, including organ-specific accumulation and/or secretion of TCE and its metabolites. Therefore, the proposed research will quantify the contribution of active transport to the absorption and secretion of the acidic metabolites of TCE (TCA and DCA), as well as the metabolite DCVC, to increase the complexity and accuracy of PBPK models for TCE exposure.

Relevance

It is well known that TCA has a long plasma half-life in rodents and humans and that this is a major contributor to the high "area under the curve" (AUC) for TCA. Restrictive binding to plasma albumen has been suggested as the molecular basis for this long half-life. However, recent studies have indicated that the extent of binding is less than 90% and hence should be non-restrictive for glomerular filtration. An alternative explanation for the lack of rapid renal excretion is glomerular filtration followed by reabsorption from the lumen of the nephron by an active transport process. Thus, renal organic anion transporters may play a significant role in determining the AUC for TCA, and hence be a prime determinant in the dose/response relationship for hepatocellular carcinoma. Knowledge of the rate constants characterizing renal transport would be helpful in validation of PBPK models of TCE hepatocellular carcinoma and essential in the identification of individuals at highest risk.

Knowing which transporters are responsible for mediating the elimination and/or reabsorption of DCVC, TCA, and DCA would aid in the prediction and modeling of potential drug-xenobiotic interactions that might exacerbate or alleviate the toxic effects of these compounds. Understanding the molecular basis for differing sensitivities of exposure between rodents and humans would aid the process of extrapolating rodent bioassay data to humans. This information might also make it possible to include toxicogenetic parameters in risk assessment models to identify individuals/populations predisposed to increased risk of nephrotoxicity and hepatocarcinogenicity after TCE exposure. Expression levels of the identified transporters may even serve as useful biomarkers for the identification of such individuals/populations.

Objective

Preliminary investigations will determine the ability of unlabeled TCA, DCA, and DCVC to inhibit the function of cloned organic anion transporters expressed in *Xenopus* oocytes and transfected cultured mammalian cell lines. Once potential transporters have been identified, their ability to mediate transport of radiolabeled TCA, DCA, and DCVC will be directly examined and the kinetic parameters of transport determined. These observations will then be confirmed in intact tissue systems (*i.e.,* kidney and liver slices) and isolated primary RPTC from mouse, rat, rabbit, and humans. The impact that interindividual variation in transporter expression and function has on susceptibility to TCE induced renal and hepatic toxicity will also be examined.

In order to establish the role active transport of TCA, DCA, and DCVC plays in their associated nephrotoxicity/hepatocarcinogenicity the following specific objectives will be addressed:

Specific Aim 1. The identification of specific transporters controlling the systemic disposition of DCVC, TCA, and DCA in mouse, rat, rabbit, and human RPTC.

Specific Aim 2. Characterization of mouse, rat, rabbit, and human transporter kinetic constants for DCVC, TCA, and DCA.

Specific Aim 3. Assessment of potential drug-xenobiotic interactions for the mouse, rat, rabbit, and human transporter orthologs and their influence on the toxicity of DCVC, TCA, and DCA.

Specific Aim 4. Incorporation of this information into PBPK models of TCA, DCA, and DCVC distribution for risk assessment purposes.

Quarterly Accomplishments

Murine primary RPTC cultures:

1. We have characterized urate uptake in primary murine RPTCs for use in TCE (TCA and DCA) risk assessment. The urate transporter is also an Slc22a transporter family member and thus a candidate transporter for having potential interactions with TCA and DCA.

2. Experiments determining the toxicity of DCVC to murine RPTCs in culture have been performed to (a) determine the appropriate sublethal toxic dose for exposure prior to mRNA collection for gene chip microarray analysis and (b) complement the data collected previously in the human primary RPTC model.

Human primary RPTC cultures:

3. *In vitro* cytotoxicity studies examining the sensitivity of hRPTCs to sub-chronic exposure to DCVC have been completed. Confluent monolayers of hRPTCs were treated daily with 7 different concentrations of DCVC (0, 0.1, 0.3, 1, 3, 10, and 30 μM) for 10 days to mimic a worker exposure situation. The resultant cytotoxicity was quantitated on days 1, 3, 7 and 10 by MTT assay. Thirty μM DCVC produced greater than 60% toxicity within 3 days, 10 μM produced greater than 75% toxicity within 7 days, and 3 μM produced ~25% toxicity in 7-10 days. The other concentrations were without much effect over the course of the experiment as assayed by MTT activity. However, some mild effects on respiration were noted by day 10 with 1 μM DCVC. Finally, sub-chronic exposure to DCVC produced 50% cytotoxicity in hRPTCs at 7.5 μM after 10 days. Therefore, 0.1 and 1 μM DCVC have been selected as the low dose and high dose non-cytotoxic concentrations to use in experiments aimed at determining the gene expression changes following exposure to sub-lethal doses of DCVC.

4. The *in vitro* cytotoxicity of the glutathione conjugate DCV-GSH was also investigated. Similar to DCVC, DCV-GSH at 30 μM produced ~40% toxicity in 3 days and greater than 60% toxicity within 7 days, and 10 μM DCV-GSH produced ~25% toxicity in 7-10 days. However, all other concentrations of DCV-GSH were without effect.

5. *In vitro* cytotoxicity studies with chloral hydrate (CH) have been conducted. Confluent monolayers of hRPTCs were treated daily with 6 different concentrations of CH (0, 0.03, 0.1, 0.3, 1, and 3 mM) for 10 days. Again, the resultant cytotoxicity was quantitated on days 1, 3, 7 and 10 by MTT assay. Three mM CH produced ~25-30% toxicity within 1 day and increased to 50-60% within 7-10 days. Other concentrations of CH were without any significant consistent effect. Thus, while CH was cytotoxic, it required 1,000-fold higher doses than DCVC (1-3 mM over 10 days).

6. The metabolism of CH (1 mM) to TCE and TCA by hRPTCs in culture has been observed at 1, 2, 7, and 10 days. The percent of CH converted to TCE varied between ~30-60% on each day and to TCA varied from ~2-4%. Thus, hRPTCs can metabolize CH to TCE and TCA.

Performance Schedule and Status of Aims

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

3.1.4 Effect of Genetic Variation and of Ethanol on the Formation of Trichloroacetic Acid, a Putative Hepatocarcinogenic Metabolite of TCE

Project Director: David McMillan, Ph.D.

Executive Summary

During this quarter we are continuing to collect and perform studies on chloral hydrate metabolism using human hepatocyte cultures. We have begun to observe some variability in the formation of TCA and TCE-OH, though more human samples will be required to determine the extent of the variability and the relationship to genotype. We are in the process of determining how many samples it will take to determine variability using power calculations using the known variability in the metabolism of ethanol. We also continue to detect the formation of DCA in human samples, and it appears that formation of this metabolite is real and not an artifact. We plan confirm its identity using GC/MS analysis in the next quarter. An abstract to the Society of Toxicology meeting on these data have been prepared.

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

During this quarter we have continued to evaluate cryogenically-preserved human hepatocytes for chloral hydrate disposition and ADH/ALDH genotype. We now have the ability to determine all of the relevant single nucleotide polymorphisms for the two enzyme systems. The results of this work are contained in a manuscript that has been accepted for publication in Environmental Health Perspectives.

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. One of our two original Specific Aims was to establish the influence (or lack thereof) of ethanol on the metabolic activation of trichloroethylene (TCE), including alteration of the ratio of its metabolites trichloroacetic acid (TCA) (a mouse carcinogen) and trichloroethanol (TCOH). Experiments during the initial year of the project established that ethanol did indeed enhance the metabolism of relatively high doses of TCE to TCA and TCOH. We hypothesized that induction of the hepatic cytochrome P450 (CYP) isozyme 2E1 (i.e., CYP2E1) would enhance the metabolism of high, but not low, environmentally-encountered doses of TCE. Upon consideration of our results and experimental design, it became evident that ethanol was not only inducing CYP2E1, but altering alcohol and aldehyde dehydrogenases (ADH and ALDH), two enzymes responsible for conversion of the intermediate TCE metabolite, chloral hydrate, to TCOH and TCA. Therefore, we chose to use pyridazine (PZ) rather than ethanol for the next phase of the project. PZ is a potent CYP2E1 inducer, but has relatively modest effects on ADH and ALDH.

2. In my third quarterly report for the second year of the project (Dec. 2004 – February 2005), I described two (2) important findings that were presented at the 2005 Society of Toxicology national meeting: (1) PZ pretreatment resulted in a dose-dependent increase in the rate of TCE elimination, a substantial decrease in blood TCA levels and a modest increase in TCOH levels in TCE-dosed rats. These alterations were pronounced in animals given 200 mg TCE/kg orally, but barely manifest at 10 mg TCE/kg, the lowest dosage administered. These results support the aforementioned Hypothesis about a lack of influence of CYP2E1 induction on low TCE doses. A gas chromatography-mass spectrometry (GC-MS) method has just been developed that will allow us to continue these experiments with much lower (i.e., environmentally-relevant) TCE exposures; (2) The marked reduction in blood TCA levels (noted above) in CYP2E1-induced animals implies that liver cancer risks may be lower under such conditions.

3. The primary focus of our work, during the reporting period of the project, has been on clarifying the mechanistic basis for the substantial decrease in blood TCA concentrations in PZ-induced rats.

4. The existence and potential contributions of several interaction mechanisms have been investigated. These experiments have yielded some interesting findings and are still ongoing.

5. The results of one study indicate that PZ pretreatment of rats results in a significant increase in the rate of clearance of TCA from the bloodstream. Half-lives (t½) of TCA in uninduced rats given 50 mg TCA/kg iv in two experiments were found to be ~800 and 930 minutes. The t½ of TCA is much longer (3,300 minutes) when it is formed as a metabolite in TCE-dosed animals. This indicates that TCA is a rate-limited metabolite of TCE. PZ-induced rats that received 50 mg TCA/kg iv exhibited a TCA t½ of ~240 minutes (versus the 800- and 930-minute t½s in uninduced rats). This phenomenon may have resulted from: increased metabolism of TCA to dichloroacetic acid (DCA) and/or other metabolites; induction/activation of organic anion transporters in the kidneys; and/or displacement of TCA from plasma binding sites by PZ. Equilibrium dialysis experiments have shown that PZ's ability to displace TCA from rat plasma proteins is quite limited. The highest PZ concentrations displaced only 20% of bound TCA. This alone cannot account for the pronounced increase in TCA clearance *in vivo*. Experiments are currently underway to assess the influence of PZ on urinary elimination of TCA.

6. A study is also being conducted to learn whether PZ pretreatment influences: metabolism of CH to TCA versus TCOH; and conversion of TCOH to TCA. In the latter case, rats were given 50 mg TCOH/kg iv, and blood TCA profiles monitored for a period of hours. TCA concentrations in blood were substantially lower over time in PZ-induced rats than in uninduced rats. This may be due to decreased metabolism of TCOH to TCA and/or increased urinary excretion of TCA. *In vitro* experiments are planned to determine whether the former occurs.

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. Human Dichloroacetic acid PBPK model: A model structure and metabolic descriptions of DCA were patterned after work in our laboratory with the development of a PBPK model for DCA in rats and mice. The Michaelis-Menten affinity constant for GSTz (Km) and enzyme degradation rate (Kde) in the model were fixed. The initial maximum velocity of GSTz for metabolism of DCA (Vmaxc), the non-metabolism loss rate (Kfc), inhibition rate (kd) and oral absorption rates were estimated through fitting DCA blood kinetic data sets from different human clinic studies.

   Several published kinetic studies exist for DCA. Additionally, we are using new unpublished low dose pharmacokinetic data collected with 8 males and 8 females under an EPA grant at Battelle NW. These individuals were given a single iv dose (0.3 mg/kg) followed by a 2 mg/kg oral dose at the beginning of the study and then repeated14 days later. The subjects drank 0.02 mg/kg DCA for 14 consecutive days between the two doses.

   The human DCA kinetic model suggests the polymorphic forms of GSTz and oral absorption rates influence the kinetics of DCA. Our simulations also suggest that DCA is degraded by another unknown metabolic pathway as proposed in our DCA modeling with rats and mice.

2. Two trichloroacetic acid (TCA) modeling papers are nearly finished for submission to journals. One paper describes the influence of serum protein binding on the dosimetry of TCA in liver of mice and humans. The other paper evaluates the pharmacokinetic evidence that TCA is the primary metabolite responsible for liver tumors in mice.

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.

3.2 Environmental Epidemiology and Risk Assessment Projects

3.2.1 Low Dose Radiation: Toxicological Models of Cancer Risk

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

Executive Summary

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

The important questions to be answered are 1) whether or not non-cancer effects such as cardiovascular disease (CVD) are effected by low doses of radiation. 2) What is the increase in risk for a equivalent dose of alpha or neutron compared to gamma or x-ray? 3) Is the risk of chronic radiation exposure the same as that of acute exposure of both high LET (alpha, neutron) and low LET (gamma, beta, x-ray) exposures. 4) Are cancer and non-cancer effects present at low doses of radiation (neutron, alpha and gamma)?

Relevance

By comparing the two stage clonal expansion models for cancer with the *in vivo* experimental data, 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. Further, the effects of neutron and alpha exposure at low-doses is of importance to radiation workers.

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 risk models will be used for analysis and compared with the results from traditional methods of 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. Additionally non-cancer effects such as cardiovascular disease will be estimated at low doses of radiation.

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 with both gamma or neutron exposures.

Specific Aim 2 To use the data from new studies at the Bologna Institute of Oncology (Italy) on gamma exposed Spague-Dawley rats. This data will provide for the estimation of low-dose effects of gamma exposures. These studies involve both acute and chronic exposures.

Specific Aim 3 To use this data from the Harwell Laboratory (U.K.), which involves alpha and beta radiation by inhalation and injection exposures to mice. These studies provide an accurate comparison of the cancer effects of alpha exposure to that of beta.

Quarterly Accomplishments

Aim 1- The mouse data from Argonne labs was analyzed for gamma and neutron effects. This involved both acute and fractionated exposures and a manuscript is being revised which evaluated the effects of radiation on CVD. A manuscript on leukemia/lymphoma and also solid tumors using biologically based risk models has been accepted. Currently effects on life-shortening are being analyzed and also the radiation effects on various types of hematopoietic cancers.

Aim 2 - Gamma exposed rats have been analyzed from a very large study recently completed in Italy. The data provides estimates of experimental low dose effects at 0.1 Sv from acute exposures. Manuscripts are being prepared. A second large study involving chronic exposures has been completed and the histopathology is being evaluated in preparation of analysis

Aim 3 - Dr. Hoel and Dr. Priest have completed the analysis of the beta and alpha exposed mouse studies from Harwell. Lung cancer was the endpoint and the RBE between alpha and beta turned out to be much smaller than what is believed by the radiation regulators. The analysis has mostly been completed on a second Harwell study involving alpha and beta exposures from radionuclide injections in mice. These should further our understanding of RBE values for various cancer sites. A paper reporting these results has very recently been accepted.

Computing methods for the two stage clonal expansion model are near completion. This will allow us to apply the analysis to the available animal data (Argonne, Harwell, Bologna) for improved cancer risk estimates.

Publication

Baker GS, Nakamura T, Hoel DG. Comparison of two models of cancer risk estimation: a statistical analysis. Eur. J. Oncol., Vol 11, n.3, pp. 000-000 2006 (in press)

Performance Schedule and Status of Aims

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

3.2.2 Low Dose Radiation: Epidemiological Risk Models

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

Executive Summary

The data used for estimating health risk from low LET radiation (e.g. x-ray, gamma) has been obtained from the A-bomb survivor cohort. This group, along with some cohorts of high dose medically exposed individual's makes up our source of information. Two important issues are of current concern: 1) Does the risk of cancer follow a linear dose-response at low-doses?, 2) Are individuals exposed at older ages (i.e. greater than 45 years) more susceptible to developing cancer than expected?, 3) What are the non cancer radiation effects?

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 A-bomb survivor data, the investigators seek to refine our understanding of the mathematical relationship between health outcomes (cancer and non 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 cancer and non-cancer effects 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. A comprehensive and focused analysis of epidemiological data from Japanese A-bomb survivors will greatly increased our understanding of the true epidemiological relationship between cancer and non-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 in order to increase our understanding of the cancer and non-cancer risks related to low-dose radiation exposure.

Specific Aim 2. The DOE worker data from CEDER (DOE's data repository) will be used to evaluate the effect of exposures at older ages and reported increased 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 Accomplishments

The analysis of CVD risk in A-bomb survivors has been assessed. Next we will evaluate the CVD risk in the analysis of animal studies as it projects to human risk.

Performance Schedule and Status of Aims

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

3.2.3 Health Risks of Low Dose Plutonium Exposure

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

Executive Summary

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

Relevance

The processing and storage of plutonium requires a quantitive understanding of the health risks of plutonium, particularly in the low-dose range. Furthermore, DOE workers who

may be exposed to plutonium should be monitored with a state-of-the-art medical surveillance program that includes the use of validated biomarkers.

Objectives

1. The general problem we are considering is the evaluation and protection of the health of DOE workers in their handling of plutonium at the SRS and other DOE facilities. The project will develop risk models of the health effects, including non cancer effects, of low dose plutonium exposures and subsequently the design of an appropriate medical surveillance system.

2. The first step is a quantitative evaluation of the human and animal data so that we have good productive risk models.

3. We will develop a medical and environmental surveillance system which includes the use of urine analyses for the measurement of internal plutonium levels.

Specific Aim 1 To develop human risk models for cancer and non-cancer effects of plutonium exposures.

Specific Aim 2. To develop a medical surveillance system for DOE workers. This includes methods for the medical and environmental surveillance of the workers

Quarterly Accomplishments

Current data analyses are restricted to beagle dogs exposed via inhalation to plutonium-238 and plutonium-239. The analysis includes evaluating the feasibility of pooling data across the two isotopes of plutonium and the two laboratories (ITRI and PNNL) that conducted the research including methods to reconcile the exposure and outcome where needed, describing methods of internal dose estimation that may be applied to human exposures, generation of estimates of internal dose and incidence of cancers and other diseases, including pulmonary fibrosis. The experiment time-dependent exposure models for pu-238 and pu-239 are completed and current risk modeling is underway for pulmonary fibrosis. A manuscript is being drafted.

Performance Schedule and Status of Aims

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

3.2.4 Population Risk Studies Using Geographic Information System Technology

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

Executive Summary

We have developed the infrastructure, resources and technical expertise necessary to conduct epidemiological assessments of population health risks using a geographical information system (GIS). 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 analyzed with respect to various aspects of population characteristics, demographic data and environmental exposures.

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 MUSC Environmental Biosciences Program 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. Information from these databases is being used in conjunction with SRRHIS data for the epidemiological assessment of population health risks in the vicinity of the Savannah River Site. The MUSC Environmental Biosciences Program 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 conduct several epidemiology risk assessments of populations in the vicinity of the Savannah River Site (SRS) 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 in order to provide more accurate and comprehensive population risk assessment.

Specific Aims

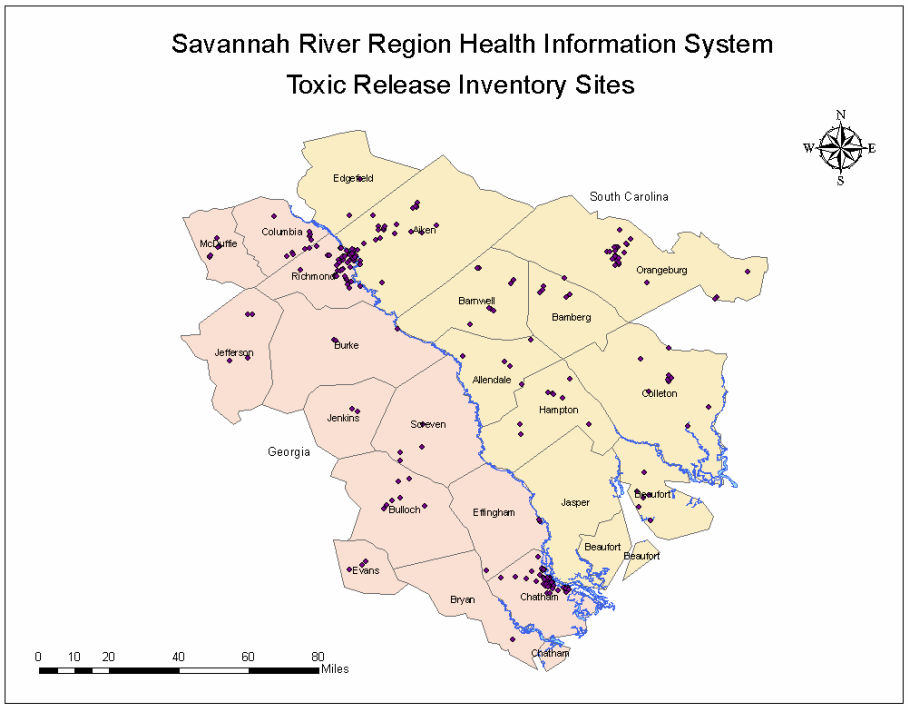
Specific Aim 1. To continue to develop and enhance the Geographic Information System as a tool for the conduct of population risk studies.

Specific Aim 2. To continue the analysis of population cancer risks in the vicinity of the Savannah River Site (SRS).

Specific Aim 3. To assess population health risks in relation to specific environmental hazards at SRS and other DOE sites.

Quarterly Accomplishments

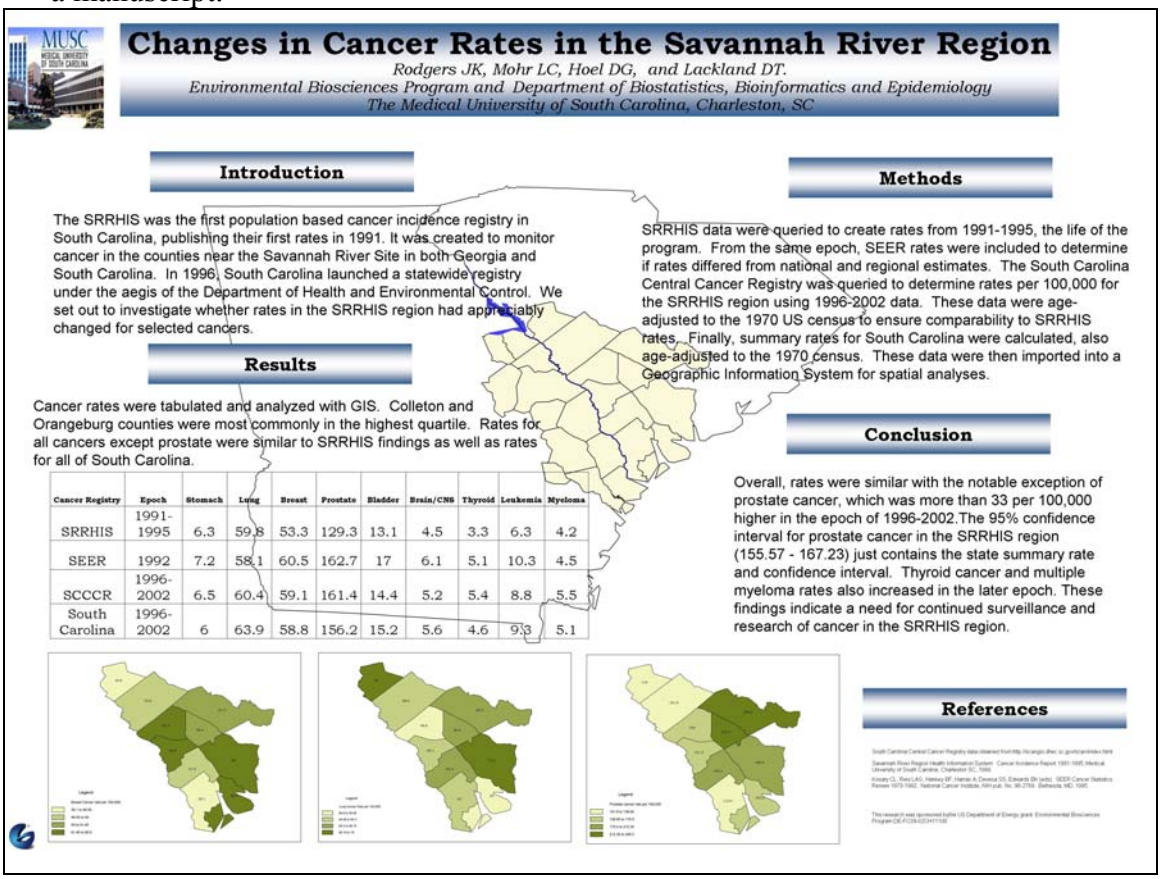
1. The Toxic Release Inventory has been initially analyzed, and these data as related to TCE sources will be plotted with particular emphasis to the Savannah River Region. The system will incorporate data from the US Geological Survey and EPA Toxic Release Inventory with a GIS application. As an example of analyses currently underway, the following map depicts the distribution of all TRI registered sites in the SRRHIS region. Incorporating contamination measurements from various sources will lead to better understanding the environmental state of the region. These results will be the focus of an ecological assessment of TCE sites and a primary focus on various cancers and cancer rates in the Savannah River Region. The data base is structured such that other conditions can be mapped with these source points.



2. A comparison system incorporating the Savannah River Health Information System (SRRHIS) and data from the SC Central Cancer Registry and the Georgia Central Cancer Registry was completed. Changes in incidence of cancer in the Savannah River Region were assessed along with an assessment of cancers proximal to the Savannah River Site. The design of this project involves the sharing of protected health data between these two registries to synthesize cancer data and calculate updated SRRHIS region rates. With this information we will conduct spatial and epidemiological analyses and compare updated and historical results.

The cancer rates in the Savannah River Region are being updated for the time period1996-2000 to assess significant changes in cancer incidence from the 1990-1995 Savannah River Region Health Information System report. This comparison has identified changes in SMRs and cancer incidence. This work entitled,

"Changes in the Cancer Trends and Rates in the Savannah River Region" was presented at the 2nd North American Congress of Epidemiology, June 21-24, 2006 in Seattle, Washington. For the next quarter, these results will be written as a manuscript.



Changes in Cancer Rates in the Savannah River Region

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Introduction

The SRRHIS was the first population based cancer incidence registry in South Carolina, publishing their first rates in 1991. It was created to monitor cancer in the counties near the Savannah River Site in both Georgia and South Carolina. In 1996, South Carolina launched a statewide registry under the aegis of the Department of Health and Environmental Control. We set out to investigate whether rates in the SRRHIS region had appreciably changed for selected cancers.

Methods

SRRHIS data were queried to create rates from 1991-1995, the life of the program. From the same epoch, SEER rates were included to determine if rates differed from national and regional estimates. The South Carolina Central Cancer Registry was queried to determine rates per 100,000 for the SRRHIS region using 1996-2002 data. These data were age-adjusted to the 1970 US census to ensure comparability to SRRHIS rates. Finally, summary rates for South Carolina were calculated, also age-adjusted to the 1970 census. These data were then imported into a Geographic Information System for spatial analyses.

Results

Cancer rates were tabulated and analyzed with GIS. Colleton and Orangeburg counties were most commonly in the highest quartile. Rates for all cancers except prostate were similar to SRRHIS findings as well as rates for all of South Carolina.

Cancer Registry	Epoch	Stomach	Lung	Breast	Prostate	Bladder	Brain/CNS	Thyroid	Leukemia	Myeloma
SRRHIS	1991-1995	6.3	59.8	53.3	129.3	13.1	4.5	3.3	6.3	4.2
SEER	1992	7.2	58.1	60.5	162.7	17	6.1	5.1	10.3	4.5
SCCCR	1996-2002	6.5	60.4	59.1	161.4	14.4	5.2	5.4	8.8	5.5
South Carolina	1996-2002	6	63.9	58.8	156.2	15.2	5.6	4.6	9.3	5.1

Conclusion

Overall, rates were similar with the notable exception of prostate cancer, which was more than 33 per 100,000 higher in the epoch of 1996-2002.The 95% confidence interval for prostate cancer in the SRRHIS region (155.57 - 167.23) just contains the state summary rate and confidence interval. Thyroid cancer and multiple myeloma rates also increased in the later epoch. These findings indicate a need for continued surveillance and research of cancer in the SRRHIS region.

References

3. Planned manuscripts and abstracts include: 1] an assessment of population disease rates and the disease rates of former workers; 2] an assessment of environmental exposures and cancer rates; 3] the geographic comparisons of adverse outcomes and the availability of primary medical care; 4] cancer rates and the location of cancer prevention services 5] cardiovascular disease among retires SRS workers; 6] cancer rates for former SRS workers.

Performance Schedule and Status of Aims

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