Michigan State University
1995-1996 Research Progress

Health Hazards from Groundwater Contamination

P42 ES04911

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Project 1A: Ecology, Physiology, Molecular Genetics and Evolution of Microorganisms that Degrade Aromatic Xenobiotic Pollutants (Olsen)

Trichloroethylene (TCE), a suspected cancer causing agent and a USEPA priority pollutant, is the most commonly reported volatile organic contaminant of groundwater at hazardous waste sites. The degradation of TCE and other solvents such as benzene and toluene by bacteria commonly found in groundwater environments has been extensively studied, yet the relationship between the rate of degradation and the physiological characteristics of individual strains of bacteria is largely unknown. Moreover, the influence of environmental conditions, particularly the availability of oxygen, nutrient materials and other chemicals found in the environment, has received little attention.

To study the effects of these environmental conditions, the ability of a variety of bacterial strains isolated from toxic waste sites to degrade TCE was compared under laboratory and simulated environmental conditions. Results from these studies showed that the potential for naturally occurring bacteria to degrade toxic compounds such as TCE may be significantly enhanced by the addition to soils and aquifers of fluids containing harmless chemicals (such as nitrate salts, vinegar and minerals). These additives were selected based on their ability to stimulate that portion of the native bacterial populations that are functioning under the prevailing environmental conditions. Moreover, these studies suggest that the use of treatments which stimulate relatively inactive bacteria can stop naturally occurring TCE degradation. Information gained from these studies can be used to select optimal subsurface treatment conditions for rapid and efficient degradation of solvent contaminated Superfund sites.

Project 1B: Ecology and Diversity of BTEX Degrading Bacteria in Bioreactors and Aquifers (Tiedje)

BTEX (benzene, toluene, ethylbenzene and xylenes) is one of the two most common groups of potentially hazardous groundwater pollutants. Government agencies have established cleanup standards, in some cases as low as 1 ppb, for benzene in groundwater because of its carcinogenic potential. It is not clear, however, how effectively low concentrations of BTEX can be degraded by bacteria in the environment and whether the current stringent regulatory targets can be met and, if so, the resources required.

Researchers have isolated 16 different bacterial strains from contaminated sites which degrade benzene when it is present in low concentrations under oxygen-rich conditions. Examination of the

speed and effectiveness of benzene degradation by each strain indicated that at least two strains had optimal activity characteristics for removal of low concentrations of benzene while other strains had somewhat less than optimal activity. In addition, it was possible to demonstrate that, under conditions of adequate oxygen, bacterial removal of benzene is sufficiently effective to meet current regulatory standards provided the required bacterial strains are present in or added to the contaminated site.

Project 1C: Kinetic, Ecological and Genetic Factors Affecting Bioaugmentation of Carbon Tetrachloride-Contaminated Sites with *Pseudomonas sp.* Strain KC (Criddle)

Carbon tetrachloride is a suspected human carcinogen that is often present in groundwaters because of its widespread past use as a fumigant, dry cleaning agent, and solvent. Frequently, microorganisms naturally present in groundwater environments can transform carbon tetrachloride. However, this transformation is slow and a common byproduct is chloroform which is also toxic. Pseudomonas sp. strain KC is a naturally occurring bacterium that rapidly transforms carbon tetrachloride to carbon dioxide and other harmless end products, without producing chloroform. Over the past year, a team of researchers from Michigan State University and University of Michigan carried out a field experiment with this organism at Schoolcraft, Michigan. The organism was injected into a small test section of the carbon tetrachloride-contaminated aquifer along with appropriate amounts of nutrients to maintain the bacteria. Carbon tetrachloride concentrations declined within the test section and it was found that the organism colonized portions of the test section where growth conditions were favorable. These results indicate this bacterium has promise for use in site remediation.

To apply the degradation process to site remediation at sites with various contaminant concentrations, computer modeling tools are needed, but such tools require an accurate understanding of the growth rate of the bacterium and its carbon tetrachloride transformation kinetics. A computerized model based on information generated from laboratory experiments has been developed. This model provides reasonable predictions of carbon tetrachloride degradation in laboratory-scale systems and is expected to be a useful tool in the design of a field remediation system.

Another accomplishment of this project was the determination of the species for Pseudomonas sp. strain KC. Using DNA-based technology it was found that the strain KC matched *Pseudomonas stutzeri*, an important finding because there are no known human pathogens in this species indicating its potential safety for use in environmental clean-up. These results represent important contributions in the development of a more rapid and effective bioremediation process for cleaning subsurface sites contaminated with carbon tetrachloride.

Project 2: Modified Clays for Environmental Remediation (Boyd)

Novel pollutant adsorbents for retention of various pollutants in subsurface environments have been prepared by the grafting of chelating (metal-holding) functionalities to the framework of clay-like materials. The physical properties of the new materials were characterized prior to testing for their ability to remove toxic heavy metals such as mercury (Hg) from aqueous systems. One such material (denoted as MP-HMS) was found to quantitatively retain inorganic mercury to an adsorption capacity of 1.5 mmol Hg/g (or 310 mg/g), making it the mercury adsorbent with the highest loading capacity known. Moreover, the maximum amount of adsorbed mercury was

equivalent to the molar quantity of thiol groups in MP-HMS indicating a very high efficiency in binding the metal.

This new class of adsorbents shows great promise for use in the remediation of metal-contaminated water systems. Current research is investigating the physical properties of the new adsorbents, as well as ways of leaching out the bound metals from the loaded adsorbent to regenerate the material for further use. In the long term, efforts will be made to modify the physical and chemical nature of the adsorbents to promote the selective adsorption of metals other than mercury (e.g., lead and cadmium).

Project 3A: Impact of Heterogeneities on the Entrapment, Mass Transfer, and Biodegradation of Organic Contaminants in the Subsurface (Abriola)

The release of hazardous, water-insoluble organic liquids into the subsurface environment has resulted in the widespread contamination of soil and aquifer formations in the United States. Through the action of retentive forces a portion of the organic contaminants will remain trapped within the structure of these underground formations. This entrapped organic liquid serves as a persistent source of contamination to soil and ground water, hampering clean-up efforts at contaminated sites. The physics and chemistry of organic liquid entrapment and mass transfer are, at present, not completely understood. This is especially true for heterogeneous aquifer systems or for multi-component contaminant mixtures.

In this project, laboratory soil column experiments have been undertaken to explore the volatilization of residual organic liquid pollutants, such as tetrachloroethylene (TCE), in the sandy soil zone above the groundwater table. The removal by evaporation of entrapped organic liquids over extended periods of time has been studied. Results indicate that previously developed prediction methods are applicable over the entire organic liquid removal period. Volatilization removal experiments were also conducted for two-component mixtures. Analysis of the experimental results has confirmed that predictive methods developed to describe single component volatilization will also provide good predictions of the volatilization of the two-component organic liquid mixtures. Thus, this work shows that volatilization rates of entrapped material in natural sands may be predicted from easily determined flow and soil parameters.

A related investigation has shown that after the removal/recovery of approximately 99% of the residual organic contaminant, concentrations ceased to decline rapidly and low contaminant levels persisted for extended periods. This persistence was shown to result from slow transfer from soils, most likely the result of organic/solid interactions. To quantify this behavior, experiments to measure solid-gas transfer under different conditions are currently being performed. The increased understanding of the persistence and recovery of organic liquid contaminants in natural environments will help to predict the effectiveness and costs of clean-up of organic solvent contaminants in soil/aquifer systems.

Project 3B: The Bioavailability, Dissolution and Sorption of Insoluble Mixtures (NAPLs) in Subsurface Systems (Weber)

It has proven difficult to predict the length of time for the degradation of environmental pollutants by microorganisms present in contaminated soil. This has been especially troublesome for

mixtures of pollutants present in the subsurface in the form of dense liquids having low solubility in water. These nonaqueous phase liquids (NAPLs) contaminating soil are often incompletely characterized mixtures of similar classes of organic compounds. Most coal tar and creosote NAPLs, for example, comprise a broad array of polynuclear aromatic compounds (PAHs), some of which are carcinogenic. Knowledge of the rates of individual PAH dissolution from such multi-component mixtures to water is crucial for predicting the environmental impact of NAPL contamination, and the effectiveness of water based clean-up technologies.

The present investigation represents an exhaustive evaluation of (I) equilibrium partitioning and dissolution in water of PAHs in liquid mixtures and, (ii) the processes by which PAHs in such mixtures become available for degradation by bacteria. Using toluene and eight PAH compounds (most of which exist as solids under ambient conditions) NAPLs were prepared to serve as models for environmental contaminant mixtures such as coal tars and creosotes. In laboratory experiments with NAPLs of known composition, each compound was found to partition into water at a rate consistent with its individual physical properties and the composition of the NAPL. Using this knowledge of the dissolution rates for the different PAHs, studies are underway to determine if microorganisms can take up PAHs in the NAPL phase as well as those dissolved in the aqueous phase. Parallel experiments are also being conducted to investigate the bacterial degradation of PAHs adhering to a number of different types of soils.

Information from such studies will provide a better understanding of the limiting factors in biodegradation of soil and aquifer pollutants. This can be used to make time and cost estimates for clean-up of Superfund sites that are more accurate than those currently available.

Project 4A: Mechanisms and Consequences of Neutrophil Activation by Hazardous Chemicals (Ganey, Roth)

These studies have demonstrated that PCBs and two different pesticides, lindane and dieldrin, stimulate neutrophils, white blood cells, to make reactive oxygen species (ROS) and secrete enzymes that degrade protein. These neutrophil functions are important for defense of the body against pathogens such as invading bacteria. Activation of neutrophil functions by environmental chemicals raises the possibility that PCBs, lindane and dieldrin may alter the ability of organisms to mount an appropriate defense response. Accordingly, the mechanisms by which these environmental chemicals stimulate neutrophils to produce a response are being investigated.

Phospholipase A2 (PLA2) is an enzyme within all cells that frees arachidonic acid from cellular membranes. Arachidonic acid serves two general functions within cells: 1) as a signal to turn on several important cell functions; and 2) as a precursor of prostaglandins which support other cell functions. In neutrophils, arachidonic acid causes generation of ROS. Recent results from this project suggest that PCBs stimulate neutrophils by activating PLA2, which liberates arachidonic acid, causing production of ROS. Lindane and dieldrin also activate PLA2, and this appears to be involved in the mechanism by which they stimulate neutrophils.

Since PLA2 is contained within all cells, and because PCBs and pesticides have biological actions in a number of cell types and tissues, it is also important to determine whether these environmental chemicals activate PLA2 in other cell types. Results of related studies suggest that PCBs activate PLA2 in rat in sulinoma cells. In addition it was found that lindane activates PLA2 in a rat epithelial cell line. These results suggest that activation of PLA2 and events subsequent to

liberation of arachidonic acid may be involved in toxic effects of PCBs and other environmentally persistent organochlorine pollutants. This information can be used to refine estimates of the health risk from mixtures of environmental pollutants that can stimulate a particular enzyme that is present in a variety of cells and tissues in the body.

Project 4C: Hepatotoxicant-Induced Immune Suppression Through TGF- Release (Kaminski)

There is a well established causal relationship between the onset of liver injury resulting from chemical exposure or disease and a decrease in the functioning of the immune system. This relationship is supported by clinical and animal studies. The objective of this project is to characterize the mechanism by which interactions between the liver and immune system produce altered immune status following liver injury produced by environmental chemicals. The importance of elucidating this mechanism is that many environmental toxicants, especially the large class of chlorinated compounds which constitute the majority of persistent environmental pollutants, have been well established to target the liver. More recent examination of a number of these compounds has also demonstrated that many alter immune function. Below is a brief description of the most significant observations to date in this project.

The primary mechanism responsible for decreased immune competence following liver injury is through the production by the liver of a regulatory factor (transforming growth factor 1; TGF-1) which controls liver repair. Although this factor is beneficial to liver repair, it concomitantly exerts negative influences on various cellular components of the immune system. Specifically, the release of TGF-1 into the blood stream inhibits a population of T-cells which control the generation of antibody responses. The significance of this reduced capacity to mount antibody responses is that this is the primary mechanism by which the host is able to defend against a wide variety of opportunistic pathogens, most notably bacteria. The specific deficit within this population of T-cells targeted by TGF-1 is that they are unable to produce specific growth factors that are essential for the development of B-lymphocytes into antibody secreting cells. As part of characterizing of this mechanism, steps have been taken to identify specific genes within T-cells that are adversely regulated by exposure to TGF-1. In summary, results from these studies support the hypothesis that the release of TGF-1 from the liver following liver injury is a general mechanism for immune suppression following exposure to liver toxicants.

Project 5A: Effects of PCBs on Uterine Function (Loch-Caruso)

The culminating event of a successful pregnancy is the safe delivery of a healthy infant. This process is carried out by the uterus. During pregnancy, the uterus provides a nurturing environment for the developing infant. The uterus is also a muscular organ, and the development of strong, repetitive, oscillating contractions of the uterus characterizes normal labor leading to birth of the infant. Precise regulation of uterine contractions is needed to prevent premature labor and to ensure the development of effective labor at the end of gestation. The aim of this research is to understand whether environmental contaminants commonly found in Superfund sites pose a risk to pregnant women and their offspring by altering uterine muscular functions involved in labor. The research currently focuses on polychlorinated biphenyls (PCBs), a family of chemicals related by their chemical structure. Although now banned from use, these chemicals are prevalent environmental contaminants due to their wide-spread use, limited environmental degradation, and bioaccumulation.

Studies with laboratory animals, wildlife, and human populations suggest that PCBs alter the length of gestation and modify labor. This laboratory has found that PCBs exert a myriad of effects on uterine contraction in rats. When PCBs are applied to isolated segments of rat uterus, uterine contractions may be stimulated, inhibited, or unaffected, depending on the structure of the particular PCB present in the organ bath. Because humans are typically exposed to mixtures of PCBs, studies were performed to examine whether PCB mixtures modify uterine contraction. In organ bath experiments, PCB mixtures stimulated rat uterine contractions. These findings may have implications for remediation strategies, providing insight into possible risks to pregnancy from PCB exposure. Additional experiments are examining responses to PCBs in appropriate cellular models. In preliminary experiments, it has been found that PCB mixtures modify cell functions that would be expected to improve muscular contraction, suggesting possible mechanisms for the previously observed PCB stimulation of uterine contraction.

Project 5B: Toxic Chemical Influences on *In Vivo* and *In Vitro* Reproduction (Dukelow)

Human exposure to mixtures of PCBs is a common occurrence. During the past year it has been found that PCBs can alter the fertilization process in mice by altering normal egg development and the development of the embryo after fertilization has occurred. No effect on sperm was observed at levels of PCBs shown to alter fertilization. The PCBs also caused an increased number of abnormal embryos and increased incidence of death of the embryos. These effects occurred at PCB exposures slightly higher to those which may occur in humans.

In other experiments PCBs were also found to alter sexual behavior and brain function in rats. Animals exposed right after birth showed decreased reproductive behavior two months later. There were also changes in the brain regions which are known to control sexual behavior. The results support the hypothesis that PCB exposure of animals can alter reproduction and development in animals.

Project 5C: Development of Novel Bioassay/Biomarker Systems for Detection of Estrogen Agonists in Complex Mixtures (Giesy)

PCBs are industrial chemical mixtures that have a variety of biological actions and are found as pollutants in the environment. In laboratory animals and wildlife they produce toxicity including cancer, immune suppression, reproductive disorders and alterations in the central nervous system. They are removed very slowly from the environment and from animals by conversion to hydroxylated products which may possess hormone-like activity, particularly estrogenic and/or antiestrogenic activity. If this is the case these hydroxylated PCB conversion products may contribute to the reproductive toxicity observed from environmental PCB exposure.

The estrogen-like and antiestrogen activity of a series of hydroxylated PCBs was determined using human breast cancer cells (MCF-7) which respond in an easily measurable fashion to chemicals possessing estrogen activity. The results indicate that none of the common hydroxylated products of PCBs exhibit significant estrogen or antiestrogenic activity. This indicates that the ability of PCBs to produce reproductive toxicity in wildlife and laboratory animals is not due to disruption of the estrogen system, a system that plays an important role in reproductive function in animals. The reproductive changes produced by environmental exposures to PCBs and their hydroxylated conversion products may be due to other hormones such as those associated with the thyroid. These studies contribute to the increasing body of knowledge of the effects of environmental PCBs

on endocrine systems.

Project 6A: Modulation of Gap Junctional Intercellular Communication as a Biomarker for Epigenetic Toxicants after Remediation (Trosko)

A normal function of most organs of the body is that adjacent cells within an organ communicate with one another. This activity is called gap junctional intercellular communication (GJIC). Interruption of GJIC between normal mammalian cells is being evaluated as a biomarker for detecting chemicals which can cause a variety of toxic effects in living systems. Current studies are directed toward understanding the basis for alteration of GJIC by specific chemicals and determining whether chemicals produced by degradation at Superfund sites affect GJIC.

Two major sets of results emerged last year. The first was to establish a structure-function relationship for the effects of polycyclic aromatic hydrocarbons (PAHs) on GJIC. It was found that PAHs with bay regions formed by either a benzene ring such as phenanthrene or methyl group such as 1- or 9-methyl anthracene inhibited GJIC. Anthracene and 1-methyl anthracene, which possess no bay regions, did not inhibit GJIC. The second was determining the feasibility of using an ozone-oxidation remediation system in the detoxification of PAHs as assessed by GJIC-bioassays. In one study, the products of the ozonation of pyrene were tested for effects on GJIC. At low ozone dosages, the product mixtures were shown to contain primarily phenanthrenes with carboxylic and aldehyde functional groups and were toxic to GJIC. At higher ozone dosages, the mixtures' predominant compounds were shown to be biphenyls containing aldehyde and carboxylic acid groups and were not toxic to GJIC. This type of information can be used to select optimal conditions to destroy the potential toxic activity of PAHs present at contaminated sites.

Project 6B: Molecular Signals of Epigenetic Interactions of Superfund Chemicals (Madhukar)

A hallmark of cancer cells is the acquisition of unregulated growth potential that ultimately leads to the development of clinical cancers. Cancer cells undergo a series of biochemical changes to reach this stage. Many chemicals in the environment are known or suspected to promote this process and such chemicals are termed cancer promoters. The action of such chemicals appears to involve interference with critical biochemical pathways that are necessary for regulation of cell growth. This research is directed to identify how environmental pollutants such as polychlorinated biphenyls (PCBs), as commercial mixtures or individual components (congeners) might cause cancers.

To study this phenomenon researchers have used cell cultures to examine whether PCBs are affecting a key enzyme system termed protein kinase C which could perturb growth regulation in cancer initiated cells. A disturbance of the expression of genes critical for cell growth could cause the induction of a precancerous lesion that ultimately becomes a tumor. The results indicated that the PCB mixture Aroclor-1254 and certain PCB congeners can activate protein kinase C in rat liver cells. The effects of these chemicals on this enzyme appear to be long-term and involve the expression of another protein termed AP-1 in the cells. Together protein kinase C and AP-1 protein appear to be important in cancer cell growth and these studies show that Aroclor-1254 and certain pure PCBs cause this system to be activated. Thus this research has identified an important pathway through which PCBs might be inducing cancer cell growth. This information provides a more rational basis for cancer risk assessment applied to PCBs. In addition it serves as a base for strategies to interrupt the PCB-induced carcinogenic process.

Outreach Core (Kamrin)

One of the most difficult problems in communicating with citizens and communities about the health risks related to local Superfund sites is to provide information that is comprehensive, comprehensible and trusted. Both the government and responsible parties are perceived as providing information that is tainted because they have a stake in particular solutions to the problems related to the Superfund site.

Journalists, on the other hand, have more public trust and also have a ready and continuous channel for reaching most members of the public. However, reporters usually do not have the background or the training to analyze statements about health risks made by various parties and thus are reluctant to do in depth assessments of the situation at the Superfund site.

To improve the situation, the MSU Institute for Environmental Toxicology joined together with the MSU School of Journalism to develop a Summer Institute for Environmental Journalists that was held on June 5-8, 1996. This event provided 25 journalists from the Great Lakes region with the skills and information to better understand the toxicology and environmental fate of Superfund site contaminants, the roles of Federal and state agencies in managing risks from these compounds, methods of accessing current information and regulations using Internet, and ways of utilizing scientific expertise most effectively.

Participants heard from academic scientists from MSU and the University of Michigan; government scientists from the Great Lakes Environmental Research Laboratory and EPA Region V; and environmental journalists from regional and national publications such as USA Today and the Chicago Tribune.

This Summer Institute, the first of its kind, is an important first step in a major effort being mounted at MSU to educate and train environmental journalists. It was very well received by the participants and will be a model for future programming in this area. The Summer Institute and future efforts are expected to result in more accurate and unbiased reporting of environmental risks, including those at Superfund sites.