National Health and Environmental Effects Research Laboratory

PROGRAM UPDATES

HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH

August 1997

This document contains a series of progress reports that describe a subset of the research programs administered by NHEERL. Reports are provided for the following topics:

- *Contaminated Sediments*
- *Tropospheric Ozone*
- *Endocrine Disruptors*
- *Drinking Water*
- *Air Toxics*
- *Indoor Air*
- *Global Climate Change*
- *PM_{10}*
- *Pesticides in the Diets of Infants and Children*

Over the course of the next year, we anticipate additional reports describing the remainder of NHEERL's research programs. The reports are intended to be client-friendly communications that convey, in non-technical terms, the direction and progress of NHEERL research on high-priority environmental issues. The titles of the reports stem from ORD's research planning categories (referred to as "subcomponents" in the FY96-97 budget).

The reports are designed to capture critical features of NHEERL's research in each program area. Contained in the reports are 3 principal sections.

- The first section presents a ***summary*** of the overall research program. This section addresses the regulatory and programmatic issues associated with the program (including a discussion of statutes germane to the research), defines the program goal, discusses scientific uncertainties and research needs relevant to the program, and outlines NHEERL's strategy for realizing research objectives.

- The second section, called ***program highlights***, features key research accomplishments of the fiscal year.

- The third section provides more detailed descriptions of the ***research activities*** within each program area. Discussions are organized to reflect NHEERL's research strategy (in particular, its explicit use of the risk assessment paradigm). Accordingly, the headings that describe the research within each program relate to elements of risk assessment. The descriptions also offer a chronological context to the research. Recent and on-going research efforts are chronicled, and anticipated research directions are projected, rendering a snapshot of the program at a given point in time.

We believe that the material presented here conveys the contributions made by NHEERL in strengthening the scientific foundation for environmental decision-making and trust that these reports will be informative and useful in understanding NHEERL research.

National Health and Environmental Effects Research Laboratory

ECOSYSTEMS PROTECTION: CONTAMINATED SEDIMENTS

HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH

PROGRESS REPORT

August 1997

CONTENTS

INTRODUCTION

PURPOSE

The purpose of this report is to communicate results from the Contaminated Sediments Research Program from EPA's National Health and Environmental Effects Research Laboratory (NHEERL).

CONTENT

The report contains

- a summary of the NHEERL Contaminated Sediments Research Program, which includes an explanation of the regulatory and programmatic context of the program, the program's overall goal, the rationale for research in this area, and the research strategy
- a section which highlights recent key findings (FY95-96 Program Highlights)
- a more detailed description of the NHEERL Contaminated Sediments Research Program, by program area, including a summary of research accomplishments and anticipated progress for the near future

COMMENTS WELCOME

The format of this report is still evolving, and we welcome feedback. Readers with comments or requests for further information are encouraged to contact:

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CONTAMINATED SEDIMENTS RESEARCH PROGRAM SUMMARY

REGULATORY AND PROGRAMMATIC CONTEXT

Numerous environmental statutes authorize EPA to address the health and ecological risks associated with contaminated sediments. Legislation includes the National Environmental Policy Act; the Clean Air Act; the Coastal Zone Management Act; the Federal Insecticide, Fungicide, and Rodenticide Act; the Toxic Substances Control Act; the Marine Protection, Research, and Sanctuaries Act; the Clean Water Act; the Great Lakes Water Quality Agreement; the Comprehensive Emergency Response, Compensation, and Liability Act; the Great Lakes Program Act; and the Water Resources Development Act. EPA research on contaminated sediments is conducted by the laboratories of its Office of Research and Development (ORD); of fundamental concern to ORD is the relationship between sediment contaminants and the viability and sustainability of benthic (bottom-dwelling) ecosystems. NHEERL, which is responsible for effects-based research within ORD, supports the Contaminated Sediments Research Program by developing methods and models for evaluating sediment toxicity and by predicting the effects of sediment contaminants on various components of aquatic ecosystems. This document summarizes NHEERL's research program and highlights some of its recent accomplishments.

PROGRAM GOAL

To determine the nature and magnitude of the toxic effects associated with contaminated sediments.

RATIONALE

According to a recent evaluation of data from the National Sediment Quality Survey, sediment contamination may pose serious risks to aquatic life, wildlife, and human health. Many potentially toxic and persistent contaminants are found in sediments, including metals (such as mercury and lead), organic chemicals (such as PCBs and polycyclic aromatic hydrocarbons, or PAHs), and various pesticides. These contaminants may adversely affect sediment biota and aquatic communities, and they may biomagnify in the food chain, eventually impacting wildlife or human health. There is a critical need for methods and models to detect and predict sediment toxicity and to assess the impact of contaminated sediments on benthic organisms and aquatic ecosystems.

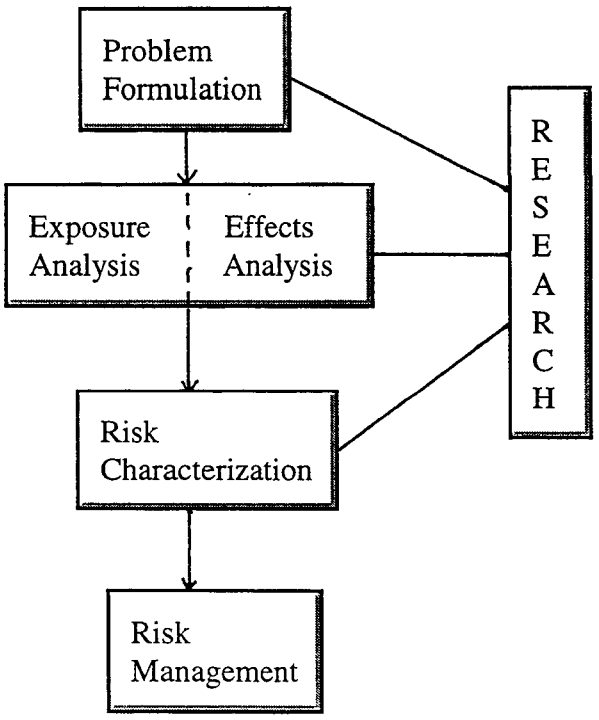
RESEARCH STRATEGY

To ensure that the Agency is equipped with scientific and technical data relevant to the formulation of sound environmental policy, ORD operates a research program founded on the principles of risk assessment. In the area of ecological research, ORD's program follows the framework for ecological risk assessment developed by EPA in 1992 (Fig. 1). The framework is conceptually similar to the human health risk assessment paradigm set forth by the National Academy of Sciences. The steps of the process include problem formulation, analysis (characterization of exposure and effects), and risk characterization. ORD conducts research that can be used in each of these risk assessment steps. NHEERL supports the Agency's risk assessment efforts by developing test methods, predictive models, and scientific data that strengthen regulatory and policy decisions.

In the area of contaminated sediments, NHEERL's objective is to evaluate the impact

of contaminated sediments on the health and sustainability of benthic species, communities, and ecosystems. Two categories of research have been established to meet this objective. In the first category, **PROBLEM FORMULATION**, we are developing methods to detect and describe the ecological hazards associated with contaminated sediments. Our methods include Toxicity Identification Evaluation (TIE) techniques for identifying hazardous contaminants in sediment; toxicity tests for characterizing potential acute and chronic effects; and hazard assessments for determining the sources, causes, and extent of sediment contamination in selected areas. Secondly, we are analyzing **DETERMINANTS OF EFFECT**. This research involves the study of factors that control and/or modify sediment toxicity, such as bioavailability. Our studies of bioavailability are helping us understand the basis for sediment toxicity, which enables us to more accurately define safe levels of contaminants or mixtures of contaminants in sediment. Models are another way in which we examine determinants of effect. Through the development of models that can estimate bioaccumulation and toxicity, we hope to reduce some of the uncertainties associated with risk assessments of contaminated sediments.

FIGURE 1. Elements of ecological risk assessment.



NHEERL CONTAMINATED SEDIMENTS RESEARCH FY95-96 PROGRAM HIGHLIGHTS

PROBLEM FORMULATION (pg. 7)

The goal of this research is to improve our ability to characterize the nature of the hazard associated with contaminated sediments.

- *We demonstrated for the first time that Toxicity Identification Evaluation (TIE) methods can be performed on marine sediment pore waters; these methods showed that PCBs were the cause of acute toxicity in New Bedford Harbor, MA, sediments.*

- *We completed a major Technical Guidance Document describing marine methods for TIEs.*

DETERMINANTS OF EFFECT (pg. 11)

The goal of this research is to better understand the environmental processes that influence sediment toxicity in order to more accurately predict adverse effects.

- *Investigators in our Atlantic, Western, and Mid-Continent Ecology Divisions produced a series of reports appearing in a special issue of* Environmental Toxicology and Chemistry *that provide the scientific basis for an equilibrium partitioning-based approach for predicting metal bioavailability and toxicity in sediments.*

- *In conjunction with the Office of Water, Sediment Quality Criteria (SQC) documents were proposed for two pesticides (endrin and dieldrin) and mixtures of three PAHs (acenaphthene, fluoranthene, and phenanthrene).*

- *We showed that the toxicity of some sediment contaminants (e.g., fluoranthene) increases in the presence of sunlight, and we developed a technique to identify these phototoxic chemicals in contaminated sediments.*

CONTAMINATED SEDIMENTS PROBLEM FORMULATION RESEARCH PROGRAM

In the context of EPA's ecological risk assessment framework, the process of problem formulation includes the identification of assessment endpoints and the relationship between these endpoints and stressors. NHEERL contributes to both of these elements and defines its ***problem formulation research*** as research to demonstrate a causal connection between exposure and effect.

ISSUE

What toxic effects are associated with exposure to contaminated sediments, and what contaminants are responsible for the toxicity?

Exposure to sediment contaminants may adversely affect benthic biota and other aquatic communities. Persistent contaminants can bioaccumulate and enter the food chain, where they have the potential to impact wildlife or human health. Methods are needed to detect toxic contaminants in sediments, to describe their effects on benthic organisms and communities, and to predict their long-lasting impact to aquatic ecosystems.

PROGRAM DESCRIPTION

NHEERL has instituted two lines of research to characterize the toxicity of contaminated sediments. In the first area, we are developing and validating ***TOXICITY ASSESSMENT METHODS*** to identify the contaminants responsible for sediment toxicity and to describe the effects that may result from exposures to contaminated sediments. These methods enable us to establish causal relationships between specific contaminants and toxic response. Another focus of our sediments research is ***ECOLOGICAL HAZARD CHARACTERIZATION***, in which we are conducting field investigations to assess the impact of contaminated sediments on benthic ecosystems. Sediment samples are being collected at various sampling stations and analyzed for temporal and spatial fluctuations in chemistry and toxicity. These data are then related to changes in population dynamics and benthic community composition. This type of analysis permits an initial hazard assessment to be made and provides a means for predicting the effects of sediment contaminants on various components of aquatic ecosystems.

PROGRAM PROGRESS

TOXICITY ASSESSMENT METHODS

Identifying the cause of sediment toxicity. One way to identify and localize toxic contaminants in sediment is through the use of Toxicity Identification Evaluation (TIE) methodologies. TIE procedures employ a toxicity-based chemical fractionation scheme that segregates and identifies contaminants responsible for acute toxicity. Since 1992, scientists in our Atlantic Ecology Division (AED) have been developing and validating TIE procedures for the marine environment. During that time, we have published numerous scientific papers and technical support documents describing the application of TIE methodologies to contaminated sediments. Considerable progress was made in this area in FY96. For the first time, we developed TIE techniques that enabled us to assess marine sediment pore waters. Using these methods, we analyzed contaminated sediments in New Bedford Harbor, MA, and discovered that PCBs were the cause of acute toxicity in these sediments. Our marine TIE methods were recently released by EPA in the form of

a Technical Guidance Document.

We also are conducting research to field validate TIE methods for freshwater sediments. Scientists in our Mid-Continent Ecology Division (MED) used these methods during FY96 to demonstrate differences in the cause of toxicity in surficial and deep river sediments. This finding could prove important when determining possible sediment remediation strategies. The development of TIE procedures for use in solid-phase sediment testing is technically more challenging. Progress was made in this area during FY96 using metal chelating agents, zeolite, and organic sorbents to differentiate among sources of acute toxicity.

TIE procedures were coupled with analyses of quantitative structure-activity relationships (QSAR) during FY96 to identify phototoxic chemicals in sediments contaminated by mixtures of polycyclic hydrocarbons. The detection of these compounds is significant given our findings that some contaminants in sediments can be photoactivated, resulting in increased sediment toxicity (see discussion under **DETERMINANTS OF EFFECT, Mechanistic Research**).

Sediment toxicity tests. The development and evaluation of toxicity tests that characterize acute and chronic effects caused by contaminated sediment is another important component of this program area. During FY95-96, our evaluations of acute toxicity tests led to a better understanding of the behavior of test endpoints and helped bound our confidence in predictions of ecological effect. Several examples can be cited. An evaluation of the relative sensitivities of several species of amphipods provided information valuable for conducting interspecies extrapolations, an assessment of the statistical performance of the amphipod mortality test provided a point of departure for determining ecologically relevant reponse from a population perspective, a study of the relative sensitivity of freshwater invertebrates to 10 contaminants helped validate current toxicity test methods, and an examination of the effects of storage duration and temperature on the acute toxicity of whole sediments led to recommendations for sediment storage conditions.

In addition to the above-mentioned acute toxicity tests, we also developed and standardized several chronic toxicity tests during FY95-96. A method for evaluating the chronic toxicity of contaminated freshwater sediments was developed which uses an aquatic midge to assess reproductive effects in invertebrates, and a bioassay to detect potential toxic and teratogenic effects caused by contaminated estuarine sediments was designed that uses grass shrimp embryos as the test species. The latter assay has shown exceptional performance in tests of individual contaminants as well as whole sediment samples and pore waters. Currently, we are using the assay at a Superfund site in Charleston Harbor, SC (in collaboration with the National Oceanic and Atmospheric Administration, or NOAA) to assess possible reproductive and developmental effects. Initial results indicate delayed spawning at impacted sites, but no terata have been observed.

Most species recommended for sediment toxicity testing are not indigenous to the area of concern, and their relevance in sediment hazard assessments for that region is unknown. For this reason, we are conducting research to examine the sensitivity of selected species to contaminants found in Gulf of Mexico sediments. The objective of the project is to determine whether indigenous species are more appropriate for hazard assessment than conventional test species, and thus better predictors of impact. In FY95, scientists in our Gulf Ecology Division (GED) began evaluating a variety of test species common to southeastern estuaries, including amphipods, bivalves, and aquatic plants.

These species are being examined for their reponse to whole sediment and pore water exposures. During FY96 we reported that the most sensitive test organism was an indigenous species of mollusc; it was considerably more sensitive than standard test species (such as the amphipod) used in sediment toxicity tests. We also successfully cultured aquatic plants in the laboratory, and in F97 we intend to expose these plants to contaminated sediments to determine relative species sensitivities and toxic endpoints. Our objective in future years is to continue developing toxicity test methods for organisms not represented by existing methodology. These studies will focus on more subtle responses, such as behavior (e.g., burrowing activity, avoidance).

ECOLOGICAL HAZARD CHARACTERIZATION

Field studies. This research is designed to assess the overall condition of benthic ecosystems by correlating data on sediment chemistry, toxicity, and community composition. During FY96, scientists in our Western Ecology Division (WED) investigated the ecological effects of DDT contamination on the macrobenthic community in sediments at a Superfund site in Richmond Harbor, CA. Our chemical measurements revealed that sediment concentrations of DDT were as high as 100,000 times background. Our analyses of community composition showed that taxonomic composition and density changed along the DDT pollution gradient; specifically, as DDT increased, amphipod density and the Infaunal Index decreased. The dose-response relationships determined in this study have permitted accurate predictions of DDT effects on macrobenthic communities.

Comprehensive assessments of sediment quality are underway in GED. These multi-year studies are designed to determine the sources, causes, and extent of toxic impacts in Gulf sediments using a hazard assessment approach. Our analytical tools include acute and chronic sediment toxicity tests, field biomonitoring, chemical analyses of sediments (including measurements of heavy metals, pesticides, and organics), determinations of biocontaminant levels in sediment species (tissue analyses), and assessments of benthic community dynamics. Areas of study include the northern Gulf of Mexico region and South Florida. Sampling stations were set up at various locations beginning in FY94. Water, sediment, and biota samples were collected and analyzed in FY95, and from this information we were able to produce baseline information on contaminant levels. Our data revealed considerable differences in the concentrations of contaminants in sediments collected from the same location at different times, indicating that the ability of sediment assessment guidelines to predict toxic effects was dependent on when the samples were collected. In contrast, results from the acute and chronic toxicity tests were consistent over time, suggesting that toxicity tests may be a more reliable index of sediment quality than chemical analyses. In FY97 and beyond, we will evaluate the efficacy of selected sediment criteria to protect benthic organisms at the population, community, and ecosystem levels. Sensitive species will be identified to determine their effectiveness as bioindicators of habitat quality, and a protocol for conducting hazard assessments of contaminated sediments will be developed.

Effects on microbial community dynamics. GED is studying the structure/function of communities of sedimentary microorganisms in an effort to better understand the effects of sediment contaminants on microbial ecology. During FY96, we used molecular techniques to resolve the community structure of bacteria in a sediment contaminated with mercury. We showed that the bacteria could be stratified into distinct populations according to sediment depth. Our results suggest that

microorganisms form distinct communities whose localization in the sediment may be influenced by contaminants. A specific group of bacteria was identified in the layers of sediment exhibiting high rates of mercury methylation; it is likely that these bacteria are responsible for the transformation of mercury. During FY97, we will begin to examine the effects of sediment contaminants on bacterial communities associated with the rhizosphere (root zone) of submerged aquatic plants. This research will help determine whether contaminants alter the microbial ecology of the rhizosphere, leading to plant stress and die-off. In future years, we plan to identify sensitive communities that could serve as indicators of pollutant stress.

CONTAMINATED SEDIMENTS DETERMINANTS OF EFFECT RESEARCH PROGRAM

NHEERL defines ***determinants of effect research*** as research to identify and describe the basis for the health and/or environmental effects caused by exposure to environmental stressors or chemical contaminants.

ISSUE

What factors affect the toxicity of sediment contaminants, and how can we account for these factors when establishing safe levels of contaminants in sediments?

The toxicity of sediments may be influenced by any number of physical or chemical determinants (such as pH) and by biological processes (such as contaminant bioaccumulation). These factors affect risk and must be considered when defining acceptable thresholds for sediment contamination. In many cases, the mechanisms by which these factors regulate toxicity are not clearly understood. Such information would help explain the basis for toxicity, leading to more scientifically defensible sediment quality criteria, and would assist efforts to improve models that estimate toxicity.

PROGRAM DESCRIPTION

The primary objective of this research program is to develop methods and models that advance our understanding of the environmental processes influencing sediment toxicity. Such information confers biological plausibility to toxicity estimates and places us in a better position to define realistic benchmarks for sediment contamination. Two research areas form our program. ***MECHANISTIC RESEARCH*** is being conducted to examine the factors that control or modify sediment toxicity. A significant research effort is devoted to analyzing the biological availability of sediment contaminants. A mechanistic understanding of bioavailability is essential to accurately predict contaminant uptake--and hence toxicity--from concentrations measured in the environment. We also are developing ***MODELS*** that will enable us to predict the uptake and bioaccumulation of sediment contaminants. These mathematical models enhance our ability to predict toxicity, which, in turn, strengthens ecological risk assessments.

PROGRAM PROGRESS

MECHANISTIC RESEARCH

Bioavailability. Research on the bioavailability of sediment contaminants has been a fundamental component of NHEERL's CONTAMINATED SEDIMENTS RESEARCH PROGRAM for 10 years. During that period, we have produced numerous scientific papers, technical support documents, and Sediment Quality Criteria (SQC) documents that define acceptable thresholds for sediment contaminants. Of particular concern are *metals* and *organic compounds.*

METALS: NHEERL scientists are responsible for major advances over the last five years in the use of the equilibrium partitioning (EqP) approach for assessing the ecological risk of metals in sediments. Our studies have shown conclusively that acid volatile sulfide (AVS) is a controlling factor in the partitioning of metals between sediment and water: AVS binds to metals, effectively sequestering them so they are not bioavailable. Consequently, AVS concentrations can be used to predict bioavailability and toxicity. In 1994, we presented the technical basis for using AVS in predictions of bioavailability to the EPA Science Advisory

Board. Their review concluded that the EqP approach using AVS analyses was a more reliable and predictable tool than any other available.

Since 1994, we have continued to define the state-of-the-science in the area of the EqP approach. In the December 1996 issue of *Environmental Toxicology and Chemistry*, a series of 16 research reports was published by investigators in AED, WED, and MED. These papers describe critical research supporting the concept of an EqP approach, and they provide the scientific basis for using EqP to predict metal bioavailability and toxicity in sediments. Results from this important area of research, involving both laboratory and field studies, are being used to assist in efforts to derive sediment quality criteria (SQC) for metals. During FY97, in conjunction with the Office of Water, we intend to publish SQC documents for cadmium, copper, lead, nickel, and zinc. Plans for future research include an evaluation of seasonal variations in AVS ratios and their relationship to trends in sediment toxicity.

ORGANICS: NHEERL research has led to advances in the use of EqP for setting sound SQC for non-ionic organic contaminants found in sediments. Our studies have resulted in improved methods for predicting the biological availability of organic compounds, such as PCBs and pesticides, in sediments and sediment pore waters. For example, in FY95, we developed an EqP technique that involves the isolation of colloidal and freely dissolved PCBs in marine sediments, and in FY96, we used this methodology to determine the distribution of PCBs in New Bedford Harbor sediments. This project demonstrated the importance of the colloidal phase when evaluating contaminated marine sediments.

Our research has resulted in the development of SQC documents for numerous organic contaminants. In FY96, we helped produce SQC documents for the pesticides dieldrin and endrin and mixtures of three PAHs: acenaphthene, phenanthrene, and fluoranthene.

Biological processes involved in the bioavailability of organic contaminants, such as uptake and assimilation by benthic organisms, are also important considerations in assessments of ecological risk. In FY95 we found that infaunal and epibenthic organisms accumulate similar concentrations of non-ionic organic chemicals regardless of feeding habit. This has important implications because it demonstrates that SQC must be derived to protect all benthic species, not just infaunal deposit-feeding species. We also observed that metabolic alterations of PCBs by marine organisms resulted in changes in the pattern and abundance of PCB congeners in the organisms (as well as in the water and sediment). During FY96, we found similarities in the uptake of nonplanar and coplanar PCB congeners in blue mussels. This finding opens up the possibility of substituting the more easily measured and less costly nonplanar PCB analytes for the more difficult and costly coplanar compounds in PCB risk assessments. In future years, we plan to address contaminant bioavailability by constructing bacterial indicator strains that respond to pollutants by producing light that can be readily measured.

Water quality characteristics. During FY95, we conducted extensive research to determine the influence of water chemistry (pH and hardness) on the acute toxicity of ammonia, a common sediment contaminant. The studies were conducted using freshwater benthic invertebrates (amphipods). Our results showed that, unlike responses previously reported for other organisms, ammonia toxicity was not affected by pH in soft water.

In FY96, we demonstrated that humic acids and total organic carbon affect pH-

dependent sorption of contaminants on estuarine sediments, and these interactions, in turn, affect toxicity.

Ultraviolet (UV) radiation. During FY95-96, scientists in MED and WED showed that the toxicity of some sediment pollutants (specifically, PAHs) increased in the presence of sunlight. Toxicity was demonstrated in both freshwater and marine invertebrates, and it increased in direct proportion to the intensity and energy of the light. Our data suggest that sediment toxicity tests conducted on chemicals prone to photoactivation may underestimate potential toxicity by several orders of magnitude. Consequently, predictions of sediment toxicity (and subsequent estimates of risk) should take into consideration the enhanced effects of phytotoxic contaminants. These findings have verified predictions made by our QSAR models. During FY96, we expanded research in this area by devising a technique whereby phototoxic chemicals in sediments could be identified through a combination of chemical fractionation and QSAR modeling (previously discussed under **TOXICITY ASSESSMENT METHODS**).

MODELS

During FY95-96, scientists in WED developed a bioaccumulation model of pollutant uptake for neutral organic compounds. This model, which is applicable to organisms that ingest sediment particles, differs from the established method for calculating Biota-Sediment Bioaccumulation Factor (BSAF) values in that it estimates the internal dose to the organism and assumes that equilibrium between tissue residues and gut contents is the most important factor governing bioaccumulation. We applied this new model in predictions of PCB bioaccumulation and found that our BSAFs were less variable across sediment types than those predicted by the standard model, and they were in better agreement with established BSAF maximal values.

In FY95, we developed and tested an innovative method for predicting the toxicity of a mixture of PAHs in sediments. Called the "$\sum$PAH Model," it predicts the probability of survivorship of sensitive species exposed to mixtures of PAHs. Comparisons of model predictions with observed toxicity in field-collected PAH-contaminated sediments showed an 86% correspondence in results. This research enhances our ability to predict interactions of multiple contaminants in sediment, leading to more realistic estimates of risk.

FY95-96 BIBLIOGRAPHY

Ankley GT (1996) Laboratory versus field measurement endpoints: a contaminated sediment perspective, in: *Risk Assessment Considerations for Contaminated Sediments*, SETAC Pellston Series.

Ankley GT (1996) Evaluation of metal/acid volatile sulfide relationships in the prediction of metal bioaccumulation by benthic macroinvertebrates, *Environ. Toxicol. Chem.* 15:2138-2146.

Ankley GT, SA Collyard, PD Monson, PA Kosian (1994) Influence of ultraviolet light on the toxicity of sediments contaminated with polycyclic aromatic hydrocarbons, *Environ. Toxicol. Chem.* 13:1791-1796.

Ankley GT, MK Schubauer-Berigan (1994) Comparison of the techniques for the isolation of pore water for sediment toxicity testing, *Arch. Environ. Contamin.* 27:507-512.

Ankley GT, MK Schubauer-Berigan (1995) Background and overview of current sediment toxicity identification evaluation procedures, *J. Aquat. Ecosystem Health* 4:133-149.

Ankley GT, MK Schubauer-Berigan, P Monson (1995) Influence of pH and hardness on toxicity of ammonia to the amphipod *Hyalella azteca*, *Can. J. Fish Aqat. Sci.* 52:2078-2083.

Ankley GT, DM DiToro, DJ Hansen, WJ Berry (1996) Assessing the ecological risk of metals in sediments (Editorial), *Environ. Toxicol. Chem.* 15:2053-2055.

Ankley GT, DM DiToro, DJ Hansen, WJ Berry (1996) Technical basis and proposal for deriving sediment quality criteria for metals, *Environ. Toxicol. Chem.* 15:2056-2066.

Ankley GT, RJ Erickson, GL Phipps, VR Marttson, PA Kosian, BR Sheedy, J Cox (1995) Effects of light intensity on the photoxicity of fluoranthene to a benthic macroinvertebrate, *Environ. Sci. Technol.* 29:2828-2833.

Ankley GT, K Liber, DJ Call, T Markee, T Canfield, CG Ingersoll, LA Carnahan (1996) A field investigation of the relationship between zinc and acid volatile sulfide concentrations in freshwater sediments, *J. Aquatic Ecosyst. Health,* in press.

Ankley GT, OG Mekenyan, P Kosian, DR Mount, P Monson, DJ Call (1996) Identification of phototoxic polycyclic hydrocarbons in sediments through sample fractionation and QSAR analysis, *SAR QSAR Environ. Res.*, in press.

Ankley GT, MK Schubauer-Berigan, J Dierkes (1996) Application of toxicity identification evaluation techniques to pore water from Buffalo River sediments, *J. Gt. Lakes Res.*, in press.

Benoit DA, PK Sibley, PS Juenemann, GT Ankley (1996) *Chironomus tentans* life cycle test: design and evaluation for use in assessing toxicity of contaminated sediments, *Environ. Toxicol. Chem.*, in press.

Bergen BJ, WG Nelson, RJ Pruell (1996) Comparison of nonplanar and coplanar PCB congener

partitioning in seawater and bioaccumulation in blue mussels *(Mytilus edulis), Environ. Toxicol. Chem.*15:1517-1523.

Berry WJ, DJ Hansen, JD Mahony, DL Robson, DM DiToro, BP Shipley, B Rogers, JM Corbin, WS Boothman (1996) Predicting the toxicity of metal-spiked laboratory sediments using acid-volatile sulfide and interstitial water normalizations, *Environ. Toxicol. Chem.*15:2067-2079.

Boese BL, M Winsor, H Lee, S Echols, J Pelletier, R Randall (1995) PCB congeners and hexachlorobenzene biota-sediment bioaccumulation factors for *Macoman asuta* exposed to sediments with different total organic carbon contents, *Environ. Toxicol. Chem.*, 14:303-310.

Boese BL, JO Lamberson, RC Swartz, RJ Ozretich (1996) Photoinduced toxicity of fluoranthene to seven marine benthic crustaceans, *Arch. Environ. Contamin. Toxicol.*, in press.

Boese BL, H Lee, DT Specht, J Pelletier, R Randall (1996) Evaluation of PCB and hexachlorobenzene biota-sediment bioaccumulation factors based on ingested sediment in a deposit-feeding clam, *Environ. Toxicol. Chem.*, 15:1584-1589.

Burgess RM, GE Morrison (1995) Clams as a species for sublethal sediment toxicity studies, *Quintessence*, 1:48.

Burgess RM, WA Brown, RA McKinney, JG Quinn (1996) Enrichment of marine sediment colloids with polychlorinated biphenyls (PCBs): Effects of PCB solubility and chlorination, *Environ. Sci. Technol,* 30:1923-1932.

Burgess RM, KT Ho, GE Morrison, G Champman, PL Denton (1996) Marine Toxicity Identification Evaluation (TIE) Phase I Guidance Document, EPA/600/R-96/054.

Burkhard LP, BR Sheedy (1995) Evaluation of screening procedures for bioconcentratable organic chemicals in effluents and sediments, *Environ. Toxicol. Chem.*, 14:697-711.

Burton G, TJ Norberg-King, CG Ingersoll, GT Ankley, PV Winger, J Kubitz, J Lazorchak, ME Smith, E Greer, F Dwyer, DJ Call, KE Day, P Kennedy, M Stinson (1996) Interlaboratory study of precision: *Hyalella azteca, Chironomus tentans* freshwater sediment toxicity assays, *Environ. Toxicol. Chem.*, 15:1335-1343.

Cotter AM, P Kosian, RA Hoke, GT Ankley (1996) Extraction and analysis of low levels of DDT, DDE and DDD in small volumes of sediment pore water, *Chemosphere*, 33: 1341-1354.

Devereaux R, MR Winfrey, J Winfrey, DA Stahl (1996) Depth profile of sulfate-reducing bacterial ribosomal RNA and mercury methylation in an estuarine sediment, *Microbiol. Ecol.*, 20:23-31.

DeWitt T, R Swartz, D Hansen, D McGovern, W Berry (1996) Bioavailability and chronic toxicity of cadmium in sediment to the estuarine amphipod *Leptocheirus plumulosus, Environ. Toxicol. Chem.*15:2102-2112.

DiToro D, CS Zarba, DJ Hansen, WJ Berry, RC Swartz, CE Cowan, SP Pavlou, HE Allen, NA Thomas, PR Paquin (1995) Equilibrium partitioning in setting sediment quality criteria, *Quintessence-Excellence in Environ. Contamin. Toxicol.* 1:45.

Ferraro SP, FA Cole (1996) Effects of ∑DDT sediment contamination on macrobenthic community structure and composition in San Francisco Bay, *Mar. Biol.*, in press.

Gundersen J. WG MacIntyre, RC Hale (1996) pH-dependent sorption of chlorinated guaicols on estuarine sediments: the effects of humic acids and TOC, *Environ. Sci. Technol.*, in press.

Hansen DJ, WJ Berry, J Mahony, DM DiToro, GT Ankley, ZQ Ma (1996) Predicting the toxicity of metal-contaminated field sediments using interstitial concentrations of metals and acid-volatile sulfide normalizations, *Environ. Toxicol. Chem.* 15:2080-2094.

Hansen DJ, JD Mahony, WJ Berry, S Benyi, J Corbin, SD Pratt, DM DiToro, MB Able (1996) Chronic effects of cadmium in sediments on colonization by benthic marine organisms: an evaluation of the role of interstitial cadmium and acid-volatile sulfide in biological availability, *Environ. Toxicol. Chem.* 15:2126-2137.

Hassan S, AW Garrison, HE Allen, D DiToro, GT Ankley (1996) Estimation of partition coefficients of five trace metals in sandy sediments and application to the sediment quality criteria, *Environ. Toxicol. Chem.* 15:2198-2208.

Ho K, R McKiney, A Kuhn, M Pelletier, R Burgess (1997) Identification of acute toxicity in New Bedford Harbor sediments, *Environ. Toxicol. Chem.*, 16:551-558.

Hoke RA, GT Ankley, JF Peters (1995) Use of freshwater sediment quality database in evaluation of sediment quality criteria based on equilibrium partitioning and screening level concentrations, *Environ. Toxicol. Chem.* 14:451-459.

Hoke RA, PA Kosian, GT Ankley, AM Cotter, FM VanderMeiden,GL Phipps, EJ Durhan (1995) Check studies with *Hyalella azteca* and *Chironomus tentans* in support of the development of a sediment quality criterion for dieldrin, *Environ. Toxicol. Chem.* 14:435-450.

Hoke RA, GT Ankley, PA Kosian, AM Cotter, FM VanderMeiden,GL Phipps, CW West, J Cox (1996) Equilibrium partitioning as the basis for an integrated laboratory and field assessment of the impacts of DDT, DDE, and DDD in sediments, *Ecotoxicol.*, in press.

Ingersoll CG, GT Ankley, DA Benoit, EL Brunson, GA Burton, FJ Dwyer, RA Hoke, PF Landrum, TK Norberg-King, PV Winger (1995) Toxicity and bioaccumulation of sediment-associated contaminants using freshwater invertebrates: a review of methods and applications, *Environ. Toxicol. Chem.* 14:1885-1894.

Ingersoll CG, GT Ankley, R Baudo, A Burton, W Lick, S Louma, D MacDonald, T Reynoldson, K Solomon, R Swartz, W Warren-Hicks (1996) Methodological uncertainty in conducting risk assessment with contaminated sediments, in: *Risk Assessment Considerations for Contaminated Sediments*, SETAC Pellston Series, Chapter 17.

Kosian PA, RA Hoke, GT Ankley, FM VanderMeiden (1995) Determination of dieldrin binding to dissolved organic material in sediment pore water using a reverse phase technique, *Environ. Toxicol. Chem.* 14:445-450.

Lake JL, R McKinney, CA Lake, FA Osterman, J Heltshe (1995) Comparison of patterns of polychlorinated biphenyl congeners in water, sediment, and indigenous organisms from New

Bedford Harbor, MA, *Arch. Environ. Contamin. Toxicol.* 29:207-220.

Leonard EN, VR Mattson, GT Ankley (1995) Horizon-specific oxidation of acid volatile sulfide in relation to the toxicity of cadmium spiked into a freshwater sediment, *Arch. Environ. Contamin. Toxicol.* 28:78-84.

Leonard EN, GT Ankley, RA Hoke (1996) Evaluation of metals in marine and freshwater surficial sediments from the Environmental Monitoring and Assessment Program relative to proposed sediment quality criteria for metals, *Environ. Toxicol. Chem.* 15:2221-2232.

Leonard EN, AM Cotter, GT Ankley (1996) Modified diffusion method for analysis of acid volatile sulfides and simulaneously extracted metals in freshwater sediment, *Environ. Toxicol. Chem.* 15:1479-1481.

Liber K, DJ Call, T Markee, KL Schmude, MD Balcer, FW Whiteman, GT Ankley (1996) Effects of acid volatile sulfide on zinc bioavailability and toxicity to benthic macroinvertebrates: a spiked field experiment, *Environ. Toxicol. Chem.* 15:2113-2125.

Liber K, DJ Call, TD Dawson, FW Whiteman, TM Dillon (1996) Effects of *Chirononus tentans* larval growth retardation on adult emergence and ovipositing success: implications for interpreting freshwater sediment bioassays, *Hydrobiologia* 323:155-167.

Masunaga S, S Susarla, JL Gunderson, Y Yonezawa (1996) Pathway and rate of chlorophenol transformation in anaerobic sediment, *Environ. Sci. Technol.* 30:1253-1260.

Mekeyan OG, GT Sankley, GD Veith, DJ Call (1995) QSARs for photoinduced toxicity of aromatic hydrocarbons, *SAR QSAR Environ. Res.* 4:139-145.

Monson PD, GT Ankley, PA Kosian (1995) Phytotoxic response of *Lumbriculus variegatus* to sediments contaminated by polycyclic aromatic hydrocarbons, *Environ. Toxicol. Chem.* 14:891-894.

Mount DR (1996) Use of metal chelating agents to differentiate among sources of acute aquatic toxicity, *Environ. Toxicol. Chem.*, in press.

Oremland, RS, LG Miller, P Dowdle, T Connell, T Barkay (1995) Methylmercury oxidative degradation potentials in contaminated and pristine sediments of the Carson River, Nevada, *Appl. Environ. Microbiol.* 61:2745-2753.

Pesch CE, DJ Hansen, WS Boothman, WJ Berry, JD Mahony (1995) The role of acid- volatile sulfide in determining bioavailability of cadmium and nickel from contaminated sediments: experiments with *Neanthes arenaceodentata*, *Environ. Toxicol. Chem.* 14:129-141.

Peterson GS, GT Ankley, EN Leonard (1996) Effect of bioturbation on metal-sulfide oxidation in surficial freshwater sediments, *Environ. Toxicol. Chem.* 15:1147-2155.

Phipps GL, VR Mattson, GT Ankley (1995) Relative sensitivity of three freshwater benthic invertebrates to ten contaminants, *Arch. Environ. Contam. Toxicol.* 28:281-286.

Schlekat CE, KJ Scott, RC Swartz, B Albrecht, L Antrim, K Doe, S Douglas, J Ferretti, DJ Hansen,

DW Moore, C Mueller, A Tang (1995) Interlaboratory comparison of a 10-day sediment toxicity test method using *Ampelisca abdita, Eohaustorius estuarius* and *Leptocheirus plumulosus, Environ. Toxicol. Chem.* 14:2163-2174.

Schubauer-Berigan MK, PD Monson, CW West, GT Ankley (1995) Influence of pH on the toxicity of ammonia to *Chironomus tentans* and *Lumbriculus variegatus, Environ. Toxicol. Chem.* 14:713-718.

Schuytema GS, AV Nebeker, MA Cairns (1995) A comparison of recirculating, static, and elutrite aquatic sediment bioassay procedures, *Bull. Environ. Contamin. Toxicol.* 56:742-749.

Schuytema GS, AV Nebeker, TW Stutzman (1996) Salinity tolerance of *Daphnia magna* and potential use for estuarine sediment toxicity tests, *Arch. Environ. Contamin. Toxicol.,* in press.

Sibley PK, GT Ankley, AM Cotter, EN Leonard (1996) Predicting chronic toxicity of sediments spiked with zinc: and evaluation of the acid volatile sulfide model using a life cycle test with the midge (*Chironomus tentans*), *Environ. Toxicol. Chem.*, in press.

Sibley PK, DA Benoit, GT Ankley (1996) The significance of growth in *Chironomus tentans* sediment toxicity tests: relationship to reproduction and demographic endpoints, *Environ. Toxicol. Chem.*, in press.

Solomon K, G Ankley, R Bauso, A Burton, C Ingersoll, W Lick, S Luoma, D MacDonald, T Reynoldson, R Swartz, W Warren-Hicks (1996) Methodological uncertainty in conducting sediment ecolgical risk assessments with contaminated sediments, in: *Ecological Risk Assessments of Contaminated Sediments*, GT Biddinger, T Dillon, C Ingersoll (Eds.), SETAC Special Publication Series, in press.

Swartz R, D Schults, R Ozretich, J Lamberson, F Cole, T DeWitt, M Redmond, S Ferraro (1995) ∑PAH: A model to predict the toxicity of polynuclear aromatic hydrocarbon mixtures in field-collected sediments, *Environ. Toxicol. Chem.* 14:1977-1987.

Swartz RC, DM DiToro (1996) Sediments as complex mixtures: an overview of methods to assess ecotoxicological significance, in: *Ecological Risk Assessments of Contaminated Sediments*, GT Biddinger, T Dillon, C Ingersoll (Eds.), SETAC Special Publication Series, in press.

Thursby G, Heltshe, JK Scott (1996) A statistical performance assessment of the 10-day solid-phase toxicity test using the marine amphipod *Ampelisca abdita, Environ. Toxicol. Chem.*, in press.

Tracey G, D Hansen, (1996) Use of biota-sediment accumulation factors to assess similarity of non-ionic organic chemical exposure to benthically-coupled organisms of differing trophic mode, *Arch. Environ. Contamin. Toxicol.* 30:467-475.

Veith GD, OG Mekenyan, GT Ankley, DJ Call (1995) A QSAR analysis of substituent effects on the photoinduced acute toxicity of PAHs, *Chemosphere* 30:2129-2142.

Weber DE, CL McKenney, MA MacGregor, DM Celestial (1996) Use of artificial sediments in a comparative toxicity study with larvae and postlarvae of the grass shrimp, *Palaemonetes pugio, Environ. Pollut.* 93:129-133.

Whiteman FW, GT Ankley, DJ Call, PM Cook, D Rau (1996) Evaluation of interstitial water and a route of exposure for ammonia in sediment tests with benthic macroinvertebrates, *Environ. Toxicol. Chem.* 15:794-801.

National Health and Environmental Effects Research Laboratory

CRITERIA AIR POLLUTANTS: TROPOSPHERIC OZONE

HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH

ANNUAL REPORT

JULY, 1997

CONTENTS

INTRODUCTION

PURPOSE

The purpose of this report is to communicate results from the Tropospheric Ozone Research Program of EPA's National Health and Environmental Effects Research Laboratory (NHEERL).

CONTENT

The report contains

- a summary of the NHEERL Tropospheric Ozone Research Program, including an explanation of its regulatory and programmatic context, the overall program goal, the rationale for the program, and the research strategy
- a section which highlights recent key findings (FY95-96 Program Highlights)
- a more detailed description of the NHEERL Tropospheric Ozone Research Program, by program area, including a summary of research accomplishments and anticipated progress for the near future

COMMENTS WELCOME

The format of this report is still evolving, and we welcome feedback. Readers with comments or requests for further information are encouraged to contact:

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TROPOSPHERIC OZONE RESEARCH PROGRAM SUMMARY

REGULATORY AND PROGRAMMATIC CONTEXT

EPA is required by the Clean Air Act (CAA) to prescribe National Ambient Air Quality Standards (NAAQS) that protect public health and the environment from the harmful effects of tropospheric ozone (O_3). The standards must be reviewed on a regular basis and revised as appropriate. In July 1997, the EPA promulgated new NAAQS for ozone in which the level of the standard was reduced from 0.12 ppm to 0.08 ppm, and the averaging time increased from 1 hour to 8 hours. Scientific support for revision of the standards and their implementation is provided by the Agency's Office of Research and Development (ORD). NHEERL, a research arm of ORD, investigates the effects of O_3 on human health and ecosystem vitality. The objective of NHEERL's research is to reduce uncertainties in ozone risk assessment, thereby strengthening the scientific foundation for future O_3 NAAQS decisions and attainment strategies. NHEERL studies were critical to the decision to establish a new primary standard (human health based) and to the decision to retain with confidence the existent secondary standard (welfare based). This document summarizes NHEERL's research program and highlights some of its recent accomplishments.

PROGRAM GOAL

To develop scientifically sound and defensible data on the health and ecological effects of O_3 for use in criteria development.

RATIONALE

Ozone, a major component of smog, is a reactive gas that can irritate the lungs and damage agricultural crops and forest ecosystems. While the acute health effects of O_3 are fairly well documented (e.g., its tendency to exacerbate asthma), very little is known about the effects of chronic O_3 exposure on humans or the role of ozone in respiratory disease. From an ecological standpoint, there remain unresolved questions regarding ozone's impact on tree growth and its contribution to reported declines in forest productivity. For EPA to promulgate a standard that adequately protects both human health and the environment, critical areas of uncertainty surrounding O_3 toxicity must be resolved.

RESEARCH STRATEGY

To ensure that the Agency is equipped with scientific and technical data relevant to the formulation of sound environmental policy, ORD operates a research program founded on principles of risk assessment. In the area of health effects, the risk assessment paradigm of the National Academy of Sciences (NAS) provides the research context. This paradigm consists of 4 fundamental steps (hazard identification, dose-response assessment, exposure assessment, and risk characterization) that support risk management decisions. In the area of ecological effects, ORD's research program follows the framework for ecological risk assessment developed by EPA in 1992, consisting of problem formulation, analysis (characterization of exposure and effects), and risk characterization. NHEERL's research in tropospheric ozone emphasizes two areas of risk assessment:

- ✦ hazard identification (or problem formulation), in which we are developing methods that enable us to detect and characterize human health and ecological hazards associated with O_3, and
- ✦ the assessment of dose-response, in which we are studying the events that link exposure and effects; these data form the

basis for predictive models used to quantify risk.

In the area of health effects, the objective of our program is to elucidate the role of O_3 in the development and exacerbation of respiratory disease. Two fundamental questions need to be resolved: 1) Do exposures to O_3 lead to an increase in acute respiratory illness in sensitive populations? and 2) Do long-term exposures lead to the development of chronic lung disease, such as asthma or chronic obstructive lung disease? To answer these questions, we are performing epidemiology studies and clinical research in humans, and toxicological assessments of O_3 morbidity and mortality in experimental animals. In the area of **EPIDEMIOLOGY**, we are characterizing the acute and chronic effects associated with ambient exposures to O_3 in urban environments, and we are identifying susceptible subpopulations who may be especially vulnerable to those effects. We are studying **DETERMINANTS OF EFFECT** to better understand the biological basis for injury and disease. Included is research on O_3 dosimetry and mechanisms of toxicity, the results of which are used to help us develop biologically plausible models of human response to O_3. In the area of **ECOLOGICAL EFFECTS**, we are examining the effects of O_3 on trees, including direct effects on growth and indirect effects below ground (on the roots and soil). These effects are being evaluated in the context of individual tree characteristics, changing exposure dynamics, and multiple stressors. We are using these experimental data to construct risk models that can estimate large-scale forest effects over time.

NHEERL TROPOSPHERIC OZONE RESEARCH FY95-96 PROGRAM HIGHLIGHTS

- *In July 1997, the EPA promulgated new National Ambient Air Quality Standards for Tropospheric Ozone to protect public health and welfare. Our research results were included in the Air Quality Criteria Document for Ozone and Related Photochemical Oxidants, which we helped to develop. This document provided the scientific basis for decisions on the primary and secondary NAAQS for O_3, decisions with multi-billion dollar implications for public health and the economy.*

EPIDEMIOLOGY (pg 8)

The goal of this research is to characterize the human health effects associated with O_3 exposures and to identify subpopulations who may be especially susceptible to O_3.

- *Collaborating with epidemiologists from Harvard University and Mexico City, we demonstrated that exposure to O_3 is associated with decrements in lung function in children. A similar response was demonstrated in controlled clinical studies at our exposure facility in Chapel Hill, NC, suggesting that data from clinical studies can be used to predict O_3 response in children.*

- *We demonstrated for the first time that asthmatics are more susceptible to O_3 than healthy individuals. Furthermore, we showed that asthmatics experience a different kind of inflammatory response (eosinophil-driven) than non-asthmatics (neutrophil-driven), indicating that there may be objective ways to measure differences in O_3 sensitivity between individuals.*

- *In cooperation with researchers at Loma Linda University, we found that O_3 exposure is significantly related to the development of asthma in males but not in females.*

- *We found that age plays an important role in O_3 sensitivity: responsiveness to O_3 is greatest in young adults and decreases with age.*

DETERMINANTS OF EFFECT (pg 11)

The goal of this research is to describe the dose distribution of O_3 in the lungs and the mode of action for O_3 toxicity.

- *We formulated an artificial lung lining fluid that simulates human lung lining fluid; it is being used to improve dosimetry estimates and to elucidate the toxic mechanisms of O_3 damage.*

- *We showed that O_3 induces lung injury and inflammation as early as one hour following exposure. Some inflammatory mediators are elevated at this early time point, while others do not appear until 18 hours later, suggesting mediators have different kinetics of appearance in the lung.*

- *We demonstrated that a common anti-inflammatory drug (Ibuprofen*) reduced decrements in lung function caused by O_3 but failed to reduce underlying lung injury and inflammation. This is the first study to show that O_3-induced changes in lung function can be dissociated from lung damage and inflammation in humans.*

- *Our findings in O_3-exposed mice and in human lung cells suggest that Vitamin C confers a protective anti-oxidant effect against O_3. In other studies in Guinea pigs, we showed that a combination of Vitamin C deficiency, O_3 exposure, and infectious challenge results in hypersusceptibility.*

- *We developed a cross-species, biologically based dose-response model for ozone toxicity based on concentration x time (CxT) relationships that assists criteria development.*

- *We constructed a mathematical model capable of accurately predicting the proportion of individuals expected to respond adversely to O_3 as a function of O_3 dose and concentration.*

ECOLOGICAL EFFECTS ON VEGETATION (pg 15)

The goal of this research is to quantify the effects of acute and chronic O_3 exposures on trees and forests.

- *Ozone was shown to have profound effects on the rhizosphere (the root/soil complex) of tree seedlings, making them more susceptible to nutrient and moisture stress and affecting carbon movement and sequestration. These results suggest that O_3 may magnify the effects of global climate change.*

- *In a study of 11 important tree species, we demonstrated that ponderosa pine, black cherry, and aspen are the most sensitive species to O_3, while douglas fir, virginia pine, and red maple are relatively insensitive.*

- *We showed that plants are more apt to take up O_3 during the daylight hours and that there is a greater effect on plant growth during episodic exposures to higher concentrations of O_3. These results were used in the NAAQS review process to support decisions on setting a secondary NAAQS for O_3.*

* Mention of trade names is not an endorsement or recommendation for use.

TROPOSPHERIC OZONE EPIDEMIOLOGY RESEARCH PROGRAM

NHEERL defines ***epidemiology research*** as research to identify and describe the human health risks posed by exposure to environmental contaminants.

ISSUE

Does ambient exposure to O_3 lead to an increase in acute and/or chronic respiratory effects? Do individuals differ in their responsiveness to O_3?

Millions of individuals are exposed to levels of tropospheric O_3 that exceed the national ambient air quality standard. Although a number of health effects (e.g., respiratory ailments, asthma) have been documented or are suspected to occur as a result of these exposures, very little is known about the magnitude of the public health burden or the long-term effects of exposure to high levels of O_3. The issue is complicated by susceptibility. Some individuals may be at greater risk than the general population to the adverse effects of air pollutants, and protecting these individuals is an explicit requirement of many environmental laws. It is important to identify and investigate sensitive subpopulations in order to support decisions on the form and level of the National Ambient Air Quality Standard for tropospheric ozone.

PROGRAM DESCRIPTION

The purpose of the Epidemiology Research Program is to characterize the public health burden associated with ambient O_3 exposure. The program is supported in large part by the Ozone Epidemiology Initiative, which is designed to relate acute and chronic O_3 exposures with adverse health outcomes, such as asthma or chronic obstructive lung disease. The Initiative received first-year funding in FY92, and many of the projects are now coming to fruition. Among our investigative tools are questionnaires, health databases, measurements of lung function, and autopsy samples (lung tissue). One important facet of the research is the identification of ***ACUTE AND CHRONIC HEALTH EFFECTS*** caused by O_3. Another focus of our research is the identification and characterization of ***SENSITIVE SUB-POPULATIONS***, such as asthmatics and children, who may be more susceptible than the general population to the adverse effects of O_3. Our findings are leading to a more confident evaluation of the need for revisions to the NAAQS for O_3.

PROGRAM PROGRESS

ACUTE AND CHRONIC HEALTH EFFECTS

NHEERL, through an FY92 Cooperative Agreement with Loma Linda University, is studying the respiratory health of California Seventh Day Adventists (non-smokers) exposed to ambient O_3. The project, originally initiated in the 1960s, is a prospective epidemiology study. Respiratory health questionnaires were administered periodically over a 15-year period to a cohort of approximately 3000 individuals. Ozone exposures were estimated from measurements taken at various monitoring stations, and these estimates were then related to the respiratory health findings. A subset of participants underwent lung function testing in FY93, which was intended to provide a physiological basis for the findings from the questionnaire. By FY96, we were able to report that O_3 was significantly related to the development of asthma in males, but not in females. We have postulated that the gender difference may be the result of different exposure patterns; for example, males spend more time outdoors in summer

than females. As a corollary to this study, we are examining the relationship between long-term exposure to O_3 and mortality. During FY96, data collection on mortality was completed, and we will begin analysis of the data in FY97.

In collaboration with the University of California at San Francisco, we are determining the relationship between short-term O_3 exposure and morbidity and mortality. We are using the California Kaiser Permanente database, which offers a population of 10 million from which to draw information. A pilot study was conducted during FY92, in which data on medical care (e.g., hospital admissions, physician visits, emergency room visits) for acute respiratory conditions were obtained. A final assessment of the pilot study was made in FY95, and a three-year Cooperative Agreement was executed to determine the relationship between O_3 exposure and medical care usage. During FY96, air pollution (O_3) data were collected and assembled. An analysis of mortality data is now underway. In FY97, hospitalization data will be collected and analyzed, and we will begin to plot trends in hospital admissions over time, relating these trends with O_3 levels.

SENSITIVE SUBPOPULATIONS

Children. Scientists in our Human Studies Division (HSD) are collaborating with investigators from Harvard University and researchers in Mexico City on a series of studies designed to document the effects of O_3 on respiratory disease in children. Pilot studies were conducted during FY91-92 in Mexico City, an area of high O_3 concentrations. Two schools were selected for the study: one located in an area of high O_3 levels and one in an area of moderate exposure levels. Pulmonary function tests were administered to schoolchildren from both regions. Upon analysis of the data, we reported in FY95 that exposure to O_3 was associated with decrements in lung function in the children. The magnitude of response was similar to the responses we observed in young adults exposed to equivalent levels of O_3 in clinical studies conducted by HSD. These results suggest that clinical data can be used to predict the responses of children living in areas of high ambient levels of O_3.

The relationship between ambient O_3 exposures and absenteeism from school due to respiratory-related causes was examined in these same Mexico City school districts during FY93-94. Our findings, reported in FY96, indicated that children in the "high ozone" region suffered acute respiratory illness severe enough to result in absenteeism. Currently, we are examining the effect of O_3 on lung growth in these schoolchildren and on emergency room visits for respiratory complaints. Although results are still being analyzed, preliminary findings suggest that high O_3 levels (and particulate matter exposure) account for a significant increase in emergency room visits for pneumonia. These findings support those from another EPA-sponsored study conducted in 1992 in the U.S., which also showed an increase in pneumonia-related emergency visits associated with O_3 exposures.

Through a Cooperative Agreement with the University of Southern California (USC), NHEERL and the California Air Resources Board are co-funding an epidemiologic investigation designed to identify the health effects of long-term exposure to O_3 (and other pollutants) in children. The project was initiated in FY92 and involves approximately 4000 children living in twelve Southern California communities. During Phase I of the study (FY92-94), communities were selected based on air pollution characteristics and population demographics. During Phase II (FY93-96), children were enrolled from each of the 12 communities, and baseline data on health status, medical history, demographics, etc. were gathered. Additional children were enrolled during

Phase III (FY95-97) to compensate for children who might withdraw from the study, and questionnaires were updated during this period. Pulmonary function tests are being performed annually over a seven-year period (1993 - 1999). A longitudinal study is now underway in which the children will be followed for up to seven years to define which pollutants and what concentrations are associated with changes in health status. Respiratory illness surveillance will be achieved by monitoring absenteeism from school and conducting telephone interviews with parents.

Through another Cooperative Agreement with USC, we are comparing lung tissues from young, accidental-death victims who lived in an area of high-level O_3 exposures (Los Angeles) to lung tissues from individuals who lived in an area of low-level O_3 exposure (Miami). The autopsy samples are being examined for evidence of disease and damage. The presence of pathological lung lesions, which may be a marker of early chronic lung disease, is being used as a measure of damage. During FY94-96, the lungs of 75 individuals in each area were collected and studied. Results are currently being analyzed.

Asthmatics. The sensitivity of asthmatics to O_3 is a question that has produced conflicting answers in the past, but clinical studies conducted during FY95-96 by HSD helped clarify the issue. Our scientists determined that allergic asthmatics (dust-mite sensitive) exposed to 0.1 ppm O_3 do not experience decrements in lung function, nor do their lower airways appear to be sensitized to a subsequent allergen challenge. However, higher levels of O_3 (0.16 ppm) did cause decrements in lung function, and at this concentration, the asthmatics were indeed more susceptible to the effects of O_3 than normal, healthy individuals. We also showed that subsequent challenge by dust-mite allergen resulted in increased airway reactivity, suggesting that exposure to O_3 may contribute to asthma morbidity. Bronchoscopies were performed on asthmatics exposed to O_3, and lung fluids were recovered for analysis. Our findings show that asthmatics experience a different kind of inflammatory response (eosinophilic-driven) than nonasthmatics (neutrophil-driven), indicating a biological difference in the way asthmatics respond to O_3. These results support the contention that asthmatics are more susceptible to O_3 than healthy individuals, both in terms of direct and indirect response (i.e., when they come in contact with an allergen following O_3 exposure). Our findings also offer biological plausibility for epidemiology findings that indicate a relationship between ambient O_3 exposure and asthma attacks, emergency room visits, etc.

Other Variables. In clinical research conducted during FY93-94, we measured spirometric responses to O_3 and demonstrated that neither gender nor race affected responsiveness at low concentrations for exposures of moderate duration. During FY95, we further demonstrated that neither socioeconomic status nor hormonal status (i.e., stage of menstrual cycle) altered pulmonary response to O_3. In FY96, we reported that there was no evidence of an effect of body size on response to O_3. However, we did find that age was a factor in O_3 sensitivity: responsiveness was greatest in young adults and decreased with increasing age.

These findings in humans have been corroborated by our studies in rats conducted during FY96, which suggest that younger rats are more sensitive to O_3 exposure than older animals. This age factor is important in light of the Clean Air Act requirement to protect the most sensitive individuals in the population.

TROPOSPHERIC OZONE DETERMINANTS OF EFFECT RESEARCH PROGRAM

NHEERL defines ***determinants of effect research*** as research to identify and describe the bases for the effects caused by exposure to environmental stressors or chemical contaminants.

ISSUE

How does O_3 produce its toxic effects?
The relationship between O_3 exposure, dose to the target tissue, and effect (lung injury/disease) has not been adequately explained. Accurate estimations of target dose, which are based on regional deposition and cellular uptake, are important because the extent of lung damage depends on where and how much O_3 is deposited in the lung. In addition, it is important to understand the mechanisms by which O_3 produces its toxic effects because such information would help explain the biological basis for disease initiation and progression, thereby leading to a more biologically defensible standard for O_3.

PROGRAM DESCRIPTION

The primary emphasis of this research program is to improve our ability to estimate O_3 dose and effect in target tissues. Our ***DOSIMETRY*** research focuses on obtaining accurate estimates of dose to specific target tissues in humans. While clinical studies are the preferable and prevailing approach for examining O_3 dosimetry, we also conduct laboratory studies *(in vitro* and *in vivo)* to describe cellular uptake and regional deposition. These data are then extrapolated to humans. In addition, we are conducting studies to elucidate the biochemical ***MECHANISMS*** by which O_3 causes lung damage and inflammation. Understanding how O_3 damages lung cells is critical for developing biologically plausible risk models and for explaining individual variations in susceptibility to O_3. Using the information we generate on dosimetry and mechanisms of toxicity, we are developing ***RISK MODELS*** to predict human response to O_3.

PROGRAM PROGRESS

DOSIMETRY

Historically,O_3 dosimetry has been difficult to study because there have been few approaches for tracing the deposition of this rapidly decaying chemical once it comes in contact with biological tissue. Scientists in our Experimental Toxicology Division have advanced the science in this area by developing a system that uses a non-radiolabelled isotope (^{18}O) to trace O_3 deposition and estimate target dose. During FY94-95, we used this method to compare the dose of O_3 that reaches the respiratory tract of humans and laboratory animals (rats). Following exposure to ^{18}O-labelled ozone, we collected lung cells and fluids by bronchoalveolar lavage (BAL), measured the amount of recovered ^{18}O, and calculated O_3 dose. Effects measurements (molecular and cellular responses) were made on both sets of fluids to determine whether there was a correspondence between dose and effect. We found that human cells incorporated 4 to 5 times the O_3 dose of rat cells, and that effects were more marked in humans. In FY96, we used ^{18}O-labelled O_3 to test the effect of ventilatory parameters (tidal volume and respiratory rate) on site-specific dose. We found that changes in ventilation produce only small variations in site-specific doses in rodents. However, mucociliary clearance does affect site-specific dose. Our findings are enabling us to more accurately estimate cellular O_3 dose and response, to

analyze species differences in dosimetry, and to examine the influence of other factors on dose distribution.

Chemical reactions that take place in the thin mucus layer of the lung may mediate the dose (and effect) of inhaled air pollutants. For this reason, we formulated an artificial lung lining fluid in FY94 that duplicates the contents of human lung fluid. In FY95, we measured the incorporation of O_3 into this fluid; results were comparable to those obtained with broncheoalveolar and nasal lavage fluids collected from rodents. These findings indicate that our test system may represent a simple, but effective, approach for studying O_3 dosimetry. In the future, this method will be used to elucidate mechanisms of toxic effect.

MECHANISMS OF TOXICITY

In an effort to better understand how O_3 causes lung damage and inflammation, we are analyzing the mode of action for mediators of cellular response and inflammation (e.g., prostaglandins and cytokines). In FY95, scientists in HSD showed that lung epithelial cells are the primary target of O_3. Ozone causes the epithelial cells to increase production of prostaglandins. The release of these prostaglandins sets up a cascade of events leading to lung function decrements and inflammation. During FY96, we examined some possible biochemical pathways that might help explain the link between prostaglandin production and lung damage/ inflammation. We found that O_3 increases prostaglandin production by inhibiting arachidonic acid esterification. This leads to increased availability of arachidonic acid in the lung, which metabolizes to produce the prostaglandins that mediate inflammation.

We also are examining the mechanisms involved in cytokine mediation of O_3-induced response. During FY95, we used a sampling technique called nasal lavage to examine O_3-induced cytokines in humans. We demonstrated that asthmatic children have elevated baseline levels of inflammatory cytokines in their upper respiratory passages, which may be related to their increased sensitivity to inhaled compounds. In FY96, we reported that the cytokine interleukin-6 is an essential mediator of cellular adaptive response to O_3.

Additional research by scientists in HSD has suggested that different mediators of inflammation have different kinetics of appearance. We examined lung fluid for the presence of inflammatory mediators at different time points following O_3 exposure, and we examined the speed with which lung injury and inflammation was induced. We demonstrated . that lung injury and inflammation occur within one hour of O_3 exposure, and that while some inflammatory mediators were elevated during this period, others did not appear until 18 hours later. This study will enhance our ability to correlate mediators and inflammatory response with rapid changes in lung function.

We are exploring the possibility that there may be ways to protect individuals from the harmful effects of O_3. We have attempted to determine whether an over-the-counter anti-inflammatory drug (Ibuprofen) can protect individuals from the effects of O_3 by reducing or eliminating lung inflammation. In FY96, we reported that while Ibuprofen blunts decrements in lung function caused by O_3, it does not reduce underlying lung injury or inflammation. However, levels of some inflammatory mediators were lowered by Ibuprofen, which may help us understand the mechanism by which O_3 reduces lung function. This is the first study to demonstrate that O_3-induced changes in lung function can be dissociated from lung damage and inflammation in humans.

Finally, we are conducting research to determine the mechanistic basis for adaptive responses to O_3. Ozone, an oxidant, is known to impart tolerance to itself with

continued exposure, but the reasons for this are unclear. In FY96 we found that mice repeatedly exposed to O_3 exhibit an increase in the anti-oxidant Vitamin C (ascorbic acid) in their lung lining fluid, suggesting that adaptation to O_3 is associated with the protective anti-oxidant effects of Vitamin C. We also have shown that anti-oxidants confer protection to human lung cells treated *in vitro* to oxidants. Our findings are currently being examined more fully in human clinical studies.

RISK MODELS

Models capable of predicting human response to O_3 are critical to setting an O_3 standard. Our scientists have made significant advances in this area by developing mathematical and biologically-based models from observations made in humans and experimental animals. In FY95 scientists in our Experimental Toxicology Division developed a biologically based dose-response (BBDR) model of O_3 toxicity by combining spirometric data obtained from humans with information on lung permeability in animals. This model, based on concentration X time (C x T) relationships, has demonstrated remarkable homology of response between humans and animals over a range of exposure concentrations and durations.

Also during FY95, scientists in HSD constructed a mathematical model capable of utilizing data on multiple variables related to O_3 exposure and response. Because the model is dynamic, different exposure scenarios and individual factors (e.g., age, changing patterns of exercise, body size) may be considered. We developed new statistical methods that permitted us to use clinical data collected since the 1980s at our human exposure facility in Chapel Hill. These data, which represent lung function measurements (decrements in FEV_1) collected from 485 individuals, were integrated into our model to predict the proportion of individuals expected to respond adversely to O_3 as a function of O_3 concentration and duration. The predictions of our model were in good agreement with observed human response. Specifically, we showed that exposure to 0.12 ppm O_3 for 6.6 hours resulted in a 10% decrement in lung function in 47% of individuals. Our results, which have assisted criteria development, led us to conclude that O_3-induced changes in lung function (FEV_1) can be accurately described as a sigmoid-shaped function of exposure rate and duration of exposure.

Our studies on ozone prior to FY94 suggested that O_3 impairs host defense systems in the lungs of experimental animals. Ozone, it was shown, affects immune defenses, rendering the animal more susceptible to lung infections caused by bacteria (specifically, Streptococcus infection). We went on to show that the increase in susceptibility was caused by a depression of the pulmonary macrophage defense system, which allowed the Streptococcus bacteria to form protective capsules, thus increasing their virulence. During FY94, we expanded this research effort to include humans. Using a combination of *in vitro* and *in vivo* studies, we compared pulmonary macrophage activity in humans and mice. Our data demonstrated comparable sensitivity between the species, suggesting that humans, like mice, may be at increased risk of bacterial infection when exposed to O_3. From our data, we published a qualitative extrapolation model in FY95 to predict response in humans; a quantitative model is now under development that will enable the Air Program Office to make better use of animal data in assessing human risk.

In a related study, we are examining the role of diet in altered susceptibility. Epidemiology evidence has suggested a relationship between low consumption of dietary anti-oxidants (particularly Vitamin C), exposure to air pollution, and respiratory infection and asthma. Furthermore, as discussed in the

previous section, our own laboratory data suggest that Vitamin C confers a protective effect against O_3. We are developing an animal model to help explain this association. During FY95-96, we induced Vitamin C deficiency in Guinea pigs and exposed these Guinea pigs to O_3 followed by an infectious challenge to Streptococcus bacteria. Preliminary findings indicate that a combination of diet (Vitamin C deficiency), O_3 exposure, and infectious challenge results in hypersusceptibility.

We are expanding this research to examine the effects of O_3 on viral infections. Surprisingly, our *in vitro* models suggested in FY96 that lung cells pre-exposed to O_3 were actually more resistant--rather than less resistant--to subsequent viral infection. This may be due to the inflammatory mediators induced by O_3, which impair viral replication. We plan to continue our studies in this area to explain this phenomenon.

TROPOSPHERIC OZONE ECOLOGICAL EFFECTS RESEARCH PROGRAM

NHEERL defines ***ecological effects research*** as research that leads to a better understanding of the response of ecosystems (and their component parts) to anthropogenic stressors.

ISSUE

What are the effects of O_3 on trees and forests?

Tropospheric ozone is the most widespread air pollutant affecting vegetation in the U.S. However, its effects on trees and forest systems are not clearly understood. Although it is known that the primary, or more direct, site of O_3 action is in the leaves (which affects plant growth), little is known about the indirect effects of O_3 (those affecting the root system and soil biota). Studies of plant growth generally have involved less complex levels of biological organization, such as seedlings or individual tree species; the results from these studies are subsequently used to make inferences on the effect of O_3 on mature trees or mixed forests. To accurately characterize the risk of O_3 to trees and forests, additional information is needed to describe the direct and indirect effects of O_3 in the context of changing exposure dynamics and other environmental stressors, and models are needed that can simulate long-term growth effects in forest systems.

PROGRAM DESCRIPTION

The purpose of this research is to quantify the effects of acute and chronic O_3 exposure on tree species at the single-tree and community level, and to extrapolate those effects to large-scale forest responses. To accomplish our objective, we have developed a two-pronged approach that takes into consideration different levels of biological complexity. We are studying the ***EFFECTS OF OZONE ON TREES***, including the nature and extent of O_3 damage above-ground (direct effects) and below-ground (indirect effects). Effects are being evaluated in the context of individual tree characteristics, such as age, size, and species; changing exposure dynamics, such as concentration, frequency, and duration of exposure; and exposure to other stressors, such as temperature changes or drought. The experimental data are then used to inform the modeling component of our program, in which we are predicting the ***EFFECTS OF OZONE ON FORESTS***. Our models characterize forest behavior by combining physiological data from individual trees or species with information on O_3 exposure and regional environmental conditions. Results from this research program are forming the biological basis for the secondary standard for O_3.

PROGRAM PROGRESS

EFFECTS OF OZONE ON TREES

Indirect Effects. This research is designed to characterize below-ground responses of trees to O_3, which includes effects on root physiology and growth, rhizosphere processes (activity in the root zone of the soil), and soil biota. In research conducted during FY95-96 by scientists in our Western Ecology Division (WED), O_3 was found to have profound effects on the rhizosphere. It reduced root growth and mycorrhizal activity (the symbiotic associations of fungi and roots), and it altered the movement of carbohydrates in plants. We have shown that the consequences of such changes are substantial. Reduced root growth makes the seedlings more susceptible to other stressors, such as drought and nutrient deficiencies. Changes in mycorrhizal activity

affect carbon movement and sequestration, yielding a less favorable carbon balance. These results suggest that O_3 stress may magnify the effects of global climate change. By adversely affecting the rhizosphere, O_3 impacts on forest ecosystems may be more widespread than previously estimated based on conventional measurements of foliage damage.

During FY95-96, we found that when plants are exposed to O_3, root exudation increases, which increases levels of soil organic matter, providing substrate for rhizosphere organisms. Consistent with this observation, we demonstrated that bacterial and fungal biomass also increase in the soil of plants exposed to O_3, and the respiration of soil organisms is altered. The extent to which O_3 disrupts soil biota is of importance because changes within the soil food web may affect the long-term health and productivity of forested systems. In FY97, we will study shifts in soil microbial populations caused by O_3 and the impact of these shifts on soil properties.

Direct Effects. This research area has two major components: 1) quantifying the response of different tree species to changing O_3 concentrations, and 2) defining the role of exposure dynamics, both temporal and seasonal, on biological response to O_3.

1) *Response of tree species*: During FY94-95, we demonstrated the effects of O_3 on the growth of 11 important tree species. Scientists in WED grew seedlings in open-top chambers, keeping variables such as water and temperature constant while varying ozone concentrations. The experiments were conducted over a two-year period, and various measures of growth response--such as gas exchange, water use, and changes in biomass partitioning--were made. In FY96, we assembled the data geographically using a Geographic Information System (GIS)-based approach. From these data, we were able to estimate biomass losses as a function of changing O_3 exposures and to rank species according to their sensitivity to O_3. We found that ponderosa pine, black cherry, and aspen are the most sensitive to O_3, while douglas fir, virginia pine, and red maple are relatively insensitive. These exposure-response data are critical for predicting the potential risks of O_3 to individual species and to different geographic regions.

2) *Exposure dynamics*: Because exposures to O_3 may be episodic or chronic (depending on such factors as geographic region, season, etc.), we are conducting research that considers the effects of changes in O_3 concentration, duration, and frequency. Research conducted by WED prior to FY94 demonstrated that growth response in trees and grasses differs according to O_3 exposure regimen, even when the overall seasonal O_3 concentration remains constant. Ozone uptake was shown to be a function of temporal distribution and frequency of O_3 occurrence. These early studies also indicated that changes in growth do not always occur during the season of exposure, but may be exhibited the following year. This "carry-over" of response indicates that the effects of O_3 on growth may be cumulative. More recently, we have associated this effect with reduced storage of carbohydrates.

During FY95, we found that plants are more likely to take up O_3 during the daylight hours. We also showed that there is a greater effect on plant growth with episodic exposures to high concentrations of O_3. These results were recently used in the Criteria Document to support setting a secondary NAAQS for O_3. During FY96 we showed that because O_3 uptake is a function of temporal distribution and frequency of O_3 occurrence, exposure in different regions of the country can be expected to have differing effects on plant response. In future studies, we plan to identify the exposure components that are most influential in long-term, multiple-year exposures, we will examine how these

exposure components affect the rate of O_3 uptake, and we will determine how this information can be incorporated into an index allowing extrapolation of effects both spatially and temporally.

EFFECTS OF OZONE ON FORESTS

During FY95, spatial analysis techniques were used to develop a method for characterizing the risk of tropospheric O_3 to forests in the U.S. We developed a Geographic Information System (GIS)-based framework that predicts forest behavior by linking exposure-response data with information on O_3 exposures, regional environmental conditions, and species distribution. Findings from our experimental studies of the effects of O_3 on tree species are being incorporated into a series of models that can simulate long-term growth effects on a regional scale. Using a model called TREGRO, we predicted the long-term effects of O_3 on tree growth based on our experimental data that describes the physiological reponse of leaves, seeds, and seedlings. We then integrated the TREGRO simulated growth rate into a model called ZELIG that simulates the response of a stand or community of trees to O_3 over time. This GIS-based approach quantifies the impact of current ozone air quality and has been used in benefits analyses for the Office of Air Quality Planning and Standards in the review of the O_3 NAAQS. Due to reductions in this program, FY96 was the final year to study the impact of O_3 on forest systems.

National Health and Environmental Effects Research Laboratory

ENDOCRINE DISRUPTORS

HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH

ANNUAL REPORT

NOVEMBER, 1996

CONTENTS

INTRODUCTION

PURPOSE

The purpose of this report is to communicate results from the Endocrine Disruptors Research Program of EPA's National Health and Environmental Effects Research Laboratory (NHEERL).

CONTENT

The report contains

- a summary of the NHEERL Endocrine Disruptors Research Program, including an explanation of its regulatory and programmatic context, the overall goal, the rationale for the program, and the research strategy
- a section that highlights recent key findings (FY95-96 Program Highlights)
- a description of the NHEERL Endocrine Disruptors Research Program, by program area, including a summary of research accomplishments and anticipated progress for the near future

COMMENTS WELCOME

The format of this report is still evolving, and we welcome feedback. Readers with comments or requests for further information are encouraged to contact:

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ENDOCRINE DISRUPTORS RESEARCH PROGRAM SUMMARY

REGULATORY AND PROGRAMMATIC CONTEXT

Several pieces of legislation sanction EPA to protect human health and the environment from the potentially harmful effects of endocrine disruptors. These laws include the Toxic Substances Control Act, which mandates EPA to evaluate the toxicity of new and existing chemicals; the Federal Insecticide, Fungicide, and Rodenticide Act, which regulates pesticides; and the newly enacted Food Quality Protection Act of 1996 and the Safe Drinking Water Act Amendments of 1996, both of which authorize a screening program for endocrine effects. To assist the Agency in meeting its requirements in these areas, EPA's Office of Research and Development (ORD) maintains an Endocrine Disruptors Research Program that assesses the risks posed by chemicals affecting the endocrine system. NHEERL supports this program by developing methods to identify endocrine disrupting chemicals, by evaluating the potential effects of these chemicals on human health and the environment, by producing models that improve quantitative risk assessment, and by providing chemical-specific data on contaminants of unknown toxicity.

PROGRAM GOAL

To determine the nature and magnitude of the health and ecological effects associated with exposure to endocrine disruptors.

RATIONALE

A growing body of scientific evidence suggests that domestic animals and wildlife have suffered adverse consequences from exposures to environmental chemicals that disrupt endocrine function. These chemicals, collectively called endocrine disrupting chemicals (EDCs), exert their toxicity by mimicking or interfering with the actions of hormones. Most of the effects associated with EDCs, such as reproductive dysfunction and sexual abnormalities, have been observed in wildlife populations receiving relatively high levels of exposure. Whether similar, albeit more subtle, effects are occurring in humans is unclear. Reports of declining sperm production in humans over the last four decades--as well as increases in rates of cancers that may have an endocrine-related basis (breast, prostate, testicular)--have led to speculation about environmentally mediated endocrine disruption in humans. These observations, coupled with available data from laboratory studies, have generated a climate of concern surrounding the potential long-term consequences of exposure to endocrine disruptors.

RESEARCH STRATEGY

To ensure that the Agency is equipped with scientific and technical data relevant to the formulation of sound environmental policy, ORD operates a research program founded on principles of risk assessment. Research in the area of health effects is guided by the risk assessment paradigm of the National Academy of Sciences (NAS), which outlines 4 steps in risk assessment: hazard identification, dose-response assessment, exposure assessment, and risk characterization. Research on ecological effects follows the framework for ecological risk assessment developed by EPA in 1992, comprised of problem formulation, analysis (characterization of exposure and effects), and risk characterization.

NHEERL's research programs adhere to these risk-based strategies. Emphasis is

placed on two areas of risk assessment:

- hazard identification, or problem formulation, in which research focuses on the development and utilization of methods to identify human health and ecological hazards, and
- the characterization of dose-response, which seeks to explain events linking exposure to effects; these events form the basis of the predictive models used to quantify risk.

NHEERL has designed its Endocrine Disruptors Research Program to address the uncertainties surrounding the health and ecological effects of endocrine disruptors. The program can be sectioned into two categories. In the first category, our efforts are focused on **HAZARD IDENTIFICATION.** We are developing test methods--including screening assays, toxicity tests, and biomarkers--to identify potential EDCs and to characterize endocrine-mediated toxic effects. Secondly, we are constructing **PREDICTIVE MODELS** that more accurately estimate risks to humans and wildlife posed by endocrine disruptors. To support the development of these risk models, we are conducting research that describes the uptake and metabolism of EDCs, the fundamental processes involved in endocrine regulation, and the toxicity of mixtures of endocrine disrupting chemicals.

NHEERL ENDOCRINE DISRUPTORS RESEARCH FY95-96 PROGRAM HIGHLIGHTS

▸ *We organized and sponsored two international workshops involving all stakeholders that identified EDC-related research needs in areas of human health and ecological effects.*

▸ *We helped draft the ORD Research Plan for Endocrine Disruptors, which provides strategic direction to Agency research efforts in endocrine disruption.*

HAZARD IDENTIFICATION (pg 7)

The goal of this research is to develop and validate cost-effective test methods for identifying human health and ecological hazards associated with endocrine disrupting chemicals.

▸ *We have uncovered significant new data that challenge the prevailing scientific opinion regarding endocrine disruption. In research that has received widespread national and international recognition, we found that in addition to chemicals that mimic estrogens, there are anti-androgenic chemicals present in the environment.*

▸ *We have identified a sperm protein in rodents, which is also common to humans, that is highly correlated with fertility. Because this protein may represent a suitable biomarker for fertility, there is significant commercial interest, and it is the subject of a recent EPA patent application.*

▸ *A novel assay was developed to detect the effects of EDCs on early development in fish. The approach permits correlations to be made between EDC exposure, P450 enzyme induction, and transgenerational health effects.*

PREDICTIVE MODELS (pg 12)

The goals of this research are to better understand the factors that influence dose-response relationships and to use this information to develop reliable risk models.

▸ *We found that p,p'-DDE, the major metabolite of DDT, binds to the androgen receptor, thereby blocking the action of endogenous androgens in the body. This important discovery represents a new mode of action for endocrine disruptors.*

▸ *We showed that the persistent hearing loss caused by developmental exposure of rodents to PCBs is associated with reduced levels of circulating thyroid hormones, which may help explain the endocrine-related mechanism of action for this neurotoxic effect.*

▸ *Exposing rats to dioxin during development was found to reduce their core temperature by decreasing the homeostatic set point, suggesting that fundamental metabolic processes may be altered by endocrine disruptors.*

ENDOCRINE DISRUPTORS HAZARD IDENTIFICATION RESEARCH PROGRAM

NHEERL defines ***hazard identification research*** as research to characterize the association between environmental exposure and adverse effect.

ISSUE

What chemicals interact with the endocrine system? What effects are caused by these chemicals?

Disruption of endocrine function provokes a cascade of reactions involving many organ systems. Response is often subtle and complex, making it difficult to establish a direct link between EDC exposure and an adverse effect. Existing toxicity tests for endocrine disruption, such as those used in product registration or for monitoring environmental samples, are limited in their ability to detect key reproductive, developmental, and immunological effects. New and improved tests are needed that are capable of detecting a broad range of endocrine-mediated responses.

PROGRAM DESCRIPTION

NHEERL's efforts to characterize and predict the hazards associated with exposure to endocrine disrupting chemicals are focused on two research areas. In the first area, we are developing and validating ***SCREENING AND TOXICITY TESTS*** for EDCs. Short-term *in vitro* and *in vivo* assays, as well as first tier computer models, are being developed to screen putative EDCs for endocrine disrupting activity and to evaluate the types of toxic effect that may be produced in humans and wildlife. We are concentrating our efforts on endpoints relevant to endocrine disruption (e.g., estrogenicity, anti-androgenicity, Ah-receptor binding, and anti-thyroidal activity) and on effects most likely to be produced (such as reproductive and developmental toxicity). We also are developing ***BIOMARKERS*** to serve as early indicators of response specific to EDC exposure. At present, our focus is on biomarkers of reproductive dysfunction in aquatic wildlife and in humans.

PROGRAM PROGRESS

SCREENING AND TOXICITY TESTS

Structure-Activity Relationships (SAR). NHEERL is using a combination of computational chemistry, molecular modeling, and toxicity test outcomes to study the interactions that occur between key structural features of a chemical and its biological target. During FY95, we made significant progress in modeling quantitative structure-activity relationships (QSAR) for compounds that have the ability to bind to steroid hormone receptors. Using advanced computer techniques, scientists in our Experimental Toxicology Division developed a preliminary three-dimensional QSAR model that predicts the endocrine disrupting potential for polychlorinated hydroxybiphenyls. Our model, which estimates estrogen receptor binding affinities, was among the first to offer evidence of a structural basis for estrogenic activity for this class of compound. During FY96, we expanded our efforts to include a more diverse set of chemicals and additional toxic endpoints. For example, using *in vitro* androgen receptor (AR) affinity data, we have developed a QSAR model based on AR ligands. Our goal is to describe the structural parameters that underlie binding to the androgen receptor.

In other SAR studies, we are examining a subclass of PCBs (ortho-substituted congeners) that do not bind to the aryl-hydrocarbon (Ah) receptor. These chemicals have been reported to cause neurological effects, and we are attempting to link key neurochemical events with structural features of these chemicals. Such a linkage could provide a strategy for predicting the endocrine disruptive potency of these and other congeners. Utilizing a number of neurochemical measures from cell culture systems, we determined during FY95 that these PCB congeners perturb calcium homeostasis, a balance critical to the functional integrity of the nervous system. These findings are important because they suggest that a neurochemical measure may be used in structure-activity modeling for EDCs. We are now conducting studies to further characterize this effect in an effort to identify a receptor target for neurotoxic endocrine disruptors.

Our Mid-Continent Ecology Division is modeling a mechanistically-based QSAR that addresses the conformational flexibility of molecules. During FY95-96, we used results from mammalian toxicity tests to model the binding of halogenated aromatic compounds to the Ah receptor and the binding of PCBs and related compounds to the estrogen receptor. In the future, we will focus on modeling post-transcriptional and post-translational events using data from representative aquatic species; cross-species comparisons will be made to determine how well mammalian tests predict perturbations of receptor-based processes in aquatic species.

Reproductive and Developmental Toxicity Tests. During FY95, a landmark paper published by scientists in NHEERL's Reproductive Toxicology Division challenged the prevailing scientific notion of endocrine disruption. Our scientists found that some environmental chemicals, including *p,p'*-DDE (the major metabolite of DDT), are potent anti-androgens and not environmental estrogens as previously thought. Exposure of rats to these chemicals demasculinized male offspring such that they displayed--among other effects--undescended testes and retained nipples. Exposure also delayed the onset of puberty. These effects are consistent with androgen receptor binding. Combined with our observations of altered sex differentiation in male rats exposed to a fungicide whose metabolites inhibit binding of the androgen response elements on DNA, it became apparent that anti-androgens exist in the environment and that they can cause developmental effects. We recently demonstrated that these chemicals also bind to the human androgen receptor. With the publication of these important findings, the scientific community immediately enlarged its research focus to include anti-androgens in its assessments of endocrine disruption.

In FY95, we reported that a number of adverse reproductive effects, such as delayed puberty, abnormalities of the vagina, and difficulties in mating, occur in the female offspring of rats and hamsters exposed to an endocrine disruptor (dioxin) during pregnancy. Male offspring exhibited reduced accessory sex gland weight and reduced sperm counts. Upon further analysis, we have found evidence to suggest that the primary target for damage in the males is the epididymis. We currently are pursuing this hypothesis to help explain the mechanism responsible for the altered reproductive function.

Immunotoxicity Tests. Studies are underway by scientists in our Experimental Toxicology Division to evaluate the transgenerational effects of endocrine disruptors (specifically, dioxin) on immune function. After exposing pregnant rodents to dioxin, we are assessing immunotoxic responses in the pups, or fetuses, by

measuring the functional integrity of cells involved in immunity (e.g., natural killer cells and B and T lymphocytes). In studies conducted in FY96, we found that exposed pups display alterations in the proportion of T-cells in the thymus. We also found a persistent suppression of T-cell-mediated response. We are now trying to establish a linkage between these two immunotoxic events.

Ecological Tests. To better understand the ecological impact of endocrine disruptors on wildlife species, ORD has established a new program that addresses the ability of chemicals to disrupt hormonal control of reproduction and development in fish, amphibians, and aquatic invertebrates. One reproductive outcome of endocrine disruption in wildlife is the induction of vitellogenin in oviparous males (those belonging to species that hatch their eggs outside the body, such as fish, reptiles, and birds). Vitellogenin is an estrogen-inducible protein normally found only in females; its presence in males is taken as an indication of exposure to environmental estrogens. Conventional methods of detecting vitellogenin are performed *in vivo*, making them relatively expensive and labor-intensive, especially for use as screening tests. Consequently, NHEERL is attempting to develop *in vitro* assays that could serve as rapid, simple, and inexpensive substitutes for the *in vivo* tests.

For example, our Atlantic Ecology Division is developing a novel *in vitro* screening assay that uses laser cytometer technology to measure a chemical's affinity for the estrogen receptor in oviparous vertebrates. Initially, we applied the laser cytometer technique to a mammalian (rat) cell line in which the number of receptors is known; during FY96, we successfully detected receptor binding. In FY97, the method will be adapted to a fish cell line of cultured hepatocytes. Receptor binding affinity will be compared to vitellogenin production, with the goal of using the method to detect environmental contaminants that may interfere with egg production.

In FY97, investigators in our Gulf Ecology Division will begin studies to determine the suitability of an *in vitro* yeast estrogen system as a screening tool for estrogenic activity. Estrogenic chemicals will be tested in yeast cells engineered to contain genes that code for the human estrogen receptor and for a "reporter" protein that indicates receptor binding. Results will be compared to those obtained using the *in vivo* fish vitellogenin assay. Our analysis will determine the feasibility of using this *in vitro* assay in the pre-registration process for new chemicals.

Scientists in our Mid-Continent Ecology Division are developing *in vivo* toxicity tests that examine endpoints other than vitellogenin induction. Endpoints include those at the subcellular (hormone), tissue (histopathology), and whole organism (sexual differentiation, fecundity) levels. Test organisms include small fish (fathead minnow, medaka, and zebrafish) and representative freshwater invertebrates (e.g., molluscs, midges). A variety of estrogenic chemicals with known or suspected mechanisms of action are being tested to determine exposure windows and endpoints most susceptible to adverse effects. This research is in its initial stages of development, and results are not expected until FY97 or thereafter. An important emphasis of this project is the linkage of different endpoints to one another as well as the correlation of results from the organism to population-level effects.

Reported declines in regional and global populations of amphibians have raised concerns regarding the possible role of environmental contaminants on reproductive

and developmental outcome. Scientists in our Mid-Continent Ecology Division are conducting studies to characterize "normal" endocrine processes in amphibians and to identify species that could serve as models for assessing disruption of developmental/ reproductive processes. Currently, we are defining the "base-line" endocrine status for two amphibian species, *Rana pipiens* and *Xenopus laevis*, emphasizing endocrine systems modulated by thyroid hormones and retinoic acids. Chemicals with known or suspected modes of action are being administered to perturb the endocrine system. This is presently a laboratory-oriented project, but in the future it could involve field studies of indigenous species.

Transgenerational effects of endocrine disruptors in wildlife also are being investigated. These studies will provide valuable information on the potential of endocrine disruptors to produce latent reproductive or developmental effects. In a project initiated in FY94, we fed marine fish (mummichogs) a diet spiked with various concentrations of dioxin. Eggs from exposed females were hatched in clean sea water. Over the next two years, offspring were raised to adulthood and assessed for reproductive capacity (egg production, percent fertilization, larval survival, etc.). Data are in the process of being analyzed and will be used in a multigenerational model of dioxin effects on population dynamics.

In another transgenerational study, investigators in our Atlantic Ecology Division are evaluating the effect of EDCs on early development in estuarine fish by studying retinoid homeostatic regulation. Retinoic acid is a powerful teratogen and regulator of early development. Its balance in the body can be affected by changes in the activity levels of the cytochrome P450 enzymes, which metabolize chemical contaminants such as EDCs. During FY95-96, we developed a novel, non-destructive assay that permits measurements of P450 enzyme activity over time in embryos of fish exposed to EDCs. The embryos are allowed to mature to larval stage, and observations of effects (including hatch rate and success, growth and survival, and lesion characterization) are then made. This approach is unique in that it permits us to evaluate the relationship between EDC exposure, P450 enzyme induction, and transgenerational health effects. In FY96, this technique was applied to a field study in which fish collected from a Superfund site highly contaminated with PCBs were evaluated. Results are presently being analyzed. In FY97, we will begin to characterize retinoid status in fish embryos in an effort to correlate this endpoint with changes in enzyme activity levels and developmental abnormalities.

During FY94, a transgenerational study using freshwater fish (trout and other species) was initiated to investigate the effects of dioxin and related chemicals on early life stage development and survival. Different exposure scenarios, including translocation of dioxin from the adult female to oocytes, exposure of fertilized eggs to waterborne dioxin, and injection of dioxin into fertilized eggs, were studied by researchers in our Mid-Continent Ecology Division. In FY95, we reported that early life stages are the most susceptible to dioxin and that toxic potency is not influenced by exposure route. We also demonstrated that maternal transfer of dioxin was sufficient to cause dose-related effects in offspsring. Although we found trout to be the most sensitive species tested, a phylogenetic basis to species sensitivity was not apparent from our studies.

BIOMARKERS

In FY96, scientists in our Reproductive

Toxicology Division reported the discovery of a sperm membrane protein in the male rat that appears to be a biomarker for fertility. This protein, also common to humans, is compromised upon exposure to chemicals that disrupt endocrine status and is highly correlated with reduced fertility. Our findings have received widespread commercial interest and are the subject of a recent EPA patent application. Currently, we are attempting to develop an antibody to this sperm biomarker that will enable us to screen for endocrine disruptive activity and reduced fertility.

During FY95-96, scientists in our Gulf Ecology Division conducted *in vivo* studies in fish using vitellogenin induction as a biomarker for endocrine disruption. Two highly sophisticated analytical techniques–the Western blot assay, which screens serum for vitellogenin, and the ELISA assay, which quantitates the amount of vitellogenin present–were used to determine whether a fish was estrogenized. In FY96, we reported that male carp captured in an area known to be contaminated by estrogenic chemicals exhibit vitellogenin induction and reduced serum testosterone concentration.

The above-mentioned vitellogenin-induction assay utilizes blood samples. In many studies, however, the fish are too small to provide sufficient blood for testing. For this reason, we initiated research in FY96 to determine whether the liver, which is readily obtained even from small fish, can be used in the development of biomarkers. We are studying two endpoints: the liver estrogen receptor and vitellogenin gene mRNA. If we can determine that the estrogen receptor and/or the vitellogenin gene are activated prior to our ability to measure serum indicators of estrogenic activity, it may be possible to use these endpoints as early indicators of response to estrogenic chemicals.

Additional biomarkers of reproductive dysfunction in wildlife have been identified for future study, including plasma steroid hormone levels, liver estrogen receptor levels, and retinal necrosis in oviparous animals. In FY97, researchers in our Atlantic Ecology Division will initiate studies to determine which of these endpoints successfully reflects exposure to estrogenic chemicals. Initial studies will involve juvenile fish, which produce low levels of endogenous hormones, making results easier to analyze. This research will then be expanded to include adult fish, and we will determine whether any of the indicators presage an adverse effect (such as reproductive dysfunction). In future years, the diagnostic indicators will be applied in field situations to obtain a snapshot of the reproductive status of indigenous populations.

In a field study conducted by Oak Ridge National Laboratory with funding from EPA, fish from a river receiving pulp and paper mill effluent, which contains dioxin, were investigated over a seven-year period. The studies preceded and accompanied extensive modernization of mill facilities to reduce contaminant discharge. The goal of the project was to determine which biological indicators were predictive of population-level effects. The condition of fish prior to mill modernization was consistent with effects attributed to EDC exposure, including a sex ratio skewed towards male fish, alterations in reproductive hormone levels, and an absence of young fish suggesting near total reproductive failure. Following modernization of the mill, we observed that these endpoints trended towards normality, and fish communities have become more diverse coincident with decreases in body burdens of dioxin.

ENDOCRINE DISRUPTORS
PREDICTIVE MODELS RESEARCH PROGRAM

NHEERL defines ***predictive models research*** as research that produces data on mechanisms of action, pharmacokinetics, and dose-response for use in developing models that can estimate target dose and effect.

ISSUE

How can we better estimate the human health and environmental risks associated with endocrine disruptors?

Risk assessments often rely on predictive models that estimate dose and effect from experimental toxicity information. The reliability of the derived risk estimates depends on the soundness and accuracy of the models as well as the strength and scope of the toxicity data. To enhance the precision of risk estimates for endocrine-mediated effects, advances in modeling are required. These advances, in turn, rely on improved understanding of the basic processes involved in endocrine disruption. Another issue relevant to risk assessment involves exposure to mixtures of EDCs. One approach for assessing the risk of mixtures is the Toxic Equivalency Factor (TEF) method, which features an assumption of additivity for toxic effects. However, it is uncertain whether the TEF method, which is applied to mixtures of chemicals possessing a common mode of action (e.g., dioxin congeners), is valid for all EDC mixtures.

PROGRAM DESCRIPTION

The primary objective of this research program is to produce data that will facilitate the development of biologically plausible risk models. To accomplish this objective, our Laboratory has initiated research in three areas. Research is being conducted to understand the key events, or ***MECHANISMS OF ACTION,*** involved in endocrine-mediated toxicity. Both receptor- and non-receptor-based mechanisms of endocrine disruption are under study. ***PHARMACOKINETIC STUDIES*** are being conducted to describe the behavior of an EDC as it is metabolized, distributed to target tissues, and eliminated from the body. These data are vital to securing more accurate predictions of tissue and cellular dose, especially at critical and sensitive early life stages. Finally, we are producing better ways to assess the risks associated with exposure to ***MIXTURES*** of endocrine disruptors. We are developing TEFs for single chemicals, estimating the toxicity of EDC mixtures based on these TEFs, and comparing our predictions to observed effects.

PROGRAM PROGRESS

MECHANISMS OF ACTION

Reproductive and Developmental Toxicity. Significant new mechanistic data were reported during FY95 by scientists in our Reproductive Toxicology Division. They found that *p,p'*-DDE binds to the androgen receptor, thereby blocking the action of endogenous androgens in the body and resulting in abnormal reproductive development in male offspring. This important discovery represents a new mode of action for endocrine disruptors and will greatly facilitate the risk assessment process for this class of chemical.

We also are characterizing the mechanisms involved in responses mediated by the Ah receptor, its binding partner (Ah receptor

nuclear translocator, or ARNT), and the glucocorticoid receptors. Palate cells from embryonic tissues are being cultured *in vitro* and treated with developmental toxicants, such as dioxin. Gene expression patterns in the developing tissues are then analyzed across time, and dose-response profiles of gene expression are produced. Both mouse and human cells are included in our studies, permitting interspecies comparisons of response. During FY95, we characterized gene expression patterns in the human cell culture. Initially, we used immunohistochemical staining techniques and *in situ* hybridization to identify the proteins expressed in embryonic tissues. Currently, however, we are attempting to quantify gene expression through a more sophisticated technique called PCR (polymerase chain reaction). PCR analysis of the samples was completed during FY96, and statistical analysis of results is in progress. Our plans are to expand this work in FY97 to examine the mechanisms involved in the synergy between glucocorticoid hormones and dioxin and their effects on developing palates. This new avenue of research will help elucidate the interplay between glucocorticoids and EDCs, which is important because of the role hormones play as a trophic factor in normal development.

To complement this *in vitro* study, scientists in our Experimental Toxicology Division plan to assess the role of the Ah receptor and its binding partner *in vivo*. Studies will be initiated in FY97 in which several EDCs will be tested in a transgenic mouse model lacking the Ah receptor and in mice in which the Ah receptor is present. We will examine a variety of endocrine-related endpoints, such as developmental toxicity (expression of ARNT in palate shelves, hydronephrosis), reproductive dysfunction (sex differentiation) and immune response (functional integrity of immune cells). Ultimately, we plan to use this transgenic model to study the pharmacokinetics of EDCs, thereby building the basis for extrapolation of animal and *in vitro* test data to humans.

We also are studying Ah receptor-mediated mechanisms involved in the promotion of endometriosis, a painful reproductive disorder in women and a major cause of infertility. Several years ago, investigators in our Reproductive Toxicology Division developed a surgically-induced model of endometriosis in the mouse. We are now using this model to investigate how EDCs promote endometriosis and impair early pregnancy. Chemicals that mimic estrogen, including dioxin and dioxin-like compounds, are being studied. During FY96, we found that chemicals promote the growth of endometriotic lesions in a manner consistent with their relative affinity for the Ah receptor. Conversely, chemicals that do not bind to the Ah receptor have no effect on endometriotic lesions. Plans are now underway to develop an *in vitro* human endometrial cell line capable of detecting chemicals that disrupt uterine function.

In addition to receptor-based mechanisms of toxicity, we also are studying non-receptor-mediated mechanisms of endocrine disruption. Using female rodents, we are investigating the effects of EDCs on ovulation. Estrogenic chemicals that affect brain neurotransmitter events are being evaluated for their impact on the hormonal control of ovulation. Special attention is being paid to the importance of noradrenergic transmitter activity in the hypothalamus and its involvement in luteinizing hormone secretion from the pituitary, which represents a critical endocrine event required for oocyte release. During FY95-96, we found that when ovulation is disrupted by an EDC and the egg is overripe when fertilized, there are reductions in litter size and developmental effects, such as neural tube defects, in offspring.

Neurotoxicity. Mechanisms involved in EDC-induced developmental neurotoxicity are being studied by scientists in our Neurotoxicology Division. The objective is to examine the relationship between thyroid hormone disruption and developmental abnormalities of the central nervous system (CNS). Chemicals selected for study include dioxin, PCBs, and PCB mixtures. Experiments conducted during FY94-95 showed that developmental exposure to PCBs caused persistent hearing loss (ototoxicity) in adult rats; this effect was associated with reduced levels of circulating thyroid hormones. During FY96, we found that the hearing deficits could be partly ameliorated by replacing T4, a thyroid hormone, during exposure. This suggests that PCB-induced auditory deficits involve thyrotoxic actions. We plan to conduct additional tests to confirm whether decreases in T4 levels are accompanied by ototoxicity. If so, this will imply that there are important non-Ah receptor mechanisms underlying the neurotoxicity of some endocrine disruptors.

The effects of endocrine disruptors on regulation of body temperature and metabolism, both of which are controlled by the hypothalamus, also are being studied. During FY95, we found that exposing rats to dioxin during early development reduced their core temperature by decreasing the homeostatic set-point. This finding is important because it suggests that fundamental metabolic processes may be altered by developmental exposure to endocrine disruptors. It also suggests that the hypothalamus, and possibly the thyroid, are potential target sites for endocrine-mediated developmental neurotoxicity. To further explore this possibility, we have designed a series of tests to provide basic information regarding the role of endocrine disruptors, including thyroid inhibitors, in thyroid hormone production and brain development. Effects on the hypothalamic-pituitary-adrenal axis of the brain, on brain development, and on learning and memory are being evaluated in an effort to elucidate the relationship between neuroendocrine mechanisms and CNS function. Preliminary results from this research indicate that interfering with the actions of thyroid hormone can severely compromise brain development, leading to smaller brain size, cognitive deficits, and improper development of the auditory system.

Ecology. To help predict risks to wildlife from exposures to EDCs, NHEERL scientists will initiate two large projects in FY97. Investigators in our Gulf Ecology Division will be conducting an integrated assessment of endocrine disruptor effects on the life cycles of marine fish. The goal of this research is to develop well-characterized model test systems capable of analyzing a variety of endocrine-mediated effects. Our research will focus upon tests that identify specific reproductive, developmental, and physiological responses in the context of complete life histories. In this way, the gap between molecular definition, biochemical action, and ecological consequence can be bridged. A suite of small-sized fishes will be observed following exposure to selected EDCs, and effects on fertilization, growth, spawning, embryonic development, and reproductive success will be recorded. Behavioral as well as physiological functions will be studied.

Also in FY97, scientists in our Mid-Continent Ecology Division will begin developing *in vitro* test systems using cells and tissues from aquatic organisms to assess critical steroid receptor systems. Chemicals shown to activate receptor-mediated transcriptional processes in mammalian tests will be evaluated for their ability to activate receptors in aquatic organisms. The data will then be used to determine how closely mammalian tests predict perturbations of steroid receptor-based processes in aquatic species. These

tests will additionally be used to characterize various aspects of chemical metablic activation and bioavailability related to reproductive and developmental endpoints.

PHARMACOKINETIC STUDIES

Scientists in our Experimental Toxicology Division are in the initial stages of developing a physiologically based pharmacokinetic (PBPK) model in rodents that focuses on critical periods of development in the reproductive, immune, and central nervous systems. During FY95, we investigated the disposition and pharmacokinetics of dioxins in the adult female rat, and in FY96 we published our PBPK model. We are now broadening our investigation to study pharmacokinetics in pregnant and fetal rats. In FY97, we plan to characterize the tissue distribution of dioxin from the maternal to the fetal compartment. When developed, the PBPK model will be extended to humans in an effort to predict whether human fetal or neonatal target organs are exposed to potentially toxic levels of dioxins.

To enhance our pharmacokinetic analyses, we are conducting studies using two transgenic mouse models, each of which lacks a different receptor system for EDCs. One model, discussed in the preceding section, lacks the Ah receptor. The other lacks the CYP1A2 receptor, which is the binding protein for dioxin in the liver. Without this protein, dioxin fails to bind to liver tissue, which affects both pharmacokinetics and resulting toxicity. Development of the CYP1A2 "knockout" model was initiated during FY95-96, and in FY96 we began our pharmacokinetic studies. Comparisons between transgenic and normal mouse models will enable us to better understand the pharmacokinetics of endocrine disruptors, thereby facilitating the development of more reliable risk models.

Because aquatic organisms ingest contaminants via food, their water milieu, and sediments, it is important to know the relative amount of chemical accumulation from each exposure route in order to estimate total dose and effect. During FY94-95, we began a series of studies evaluating the uptake and elimination kinetics of dioxin in medaka, a small fish species. Thusfar, our studies have predicted a steady-state bioconcentration factor from water to fish tissue of over 500,000. This value is much higher than previously reported in the literature, suggesting a greater potential for dioxin accumulation than previously estimated. We are now examining sediment contaminant bioavailability and dietary contributions in an effort to more accurately predict bioaccumulation and toxicological effect.

MIXTURES

To improve our ability to assess the risk of exposure to mixtures of EDCs, scientists in our Experimental Toxicology Division have conducted a study in which human body burdens of dioxins and dioxin-like congeners (dibenzofurans and PCBs) were estimated. During FY95, we determined the relative potencies of the congeners using subchronic studies in experimental animals and estimated the total toxic equivalency. We then compared the body burdens of dioxins that produce effects in experimental animals to body burdens associated with effects in humans. The TEF method was used to calculate body budens of dioxins in humans. We found that for effects that have been clearly associated with dioxin exposures, such as chloracne and the induction of CYP1A1, humans and animals respond at similar body burdens. These and additional estimations were central to the Agency's Dioxin Reassessment, enabling predictions of risk posed by the levels of exposure experienced by the general population.

Investigators in our Mid-Continent Ecology Division also are evaluating the feasibility of using TEFs for assessing the risk of chemical mixtures. Chemicals that share a common mode of action (congeners of polychlorinated dibenzodioxins, furans, and PCBs, all of which are thought to act through the Ah receptor) were selected for study. We are testing these chemicals for toxicity using an ecologically relevant endpoint: early lifestage mortality in rainbow trout. From the results, we have established tentative TEFs and have compared these values to TEFs for mammalian species. During FY95, we found that the TEFs for dioxins and furans in fish are similar to those in mammalian species. However, the potency of some PCBs is lower in fish relative to mammals. This suggests that inter-species differences should be taken into account when assessing the risk of exposure to mixtures of endocrine disruptors.

National Health and Environmental Effects Research Laboratory

DRINKING WATER

HEALTH EFFECTS RESEARCH

ANNUAL REPORT

OCTOBER, 1996

CONTENTS

INTRODUCTION

PURPOSE

The purpose of this report is to communicate results from the Drinking Water Health Effects Research Program of EPA's National Health and Environmental Effects Research Laboratory (NHEERL).

CONTENT

The report contains

- a summary of the NHEERL Drinking Water Research Program, including an explanation of its regulatory and programmatic context, its overall goal, the rationale for the program, and the research strategy

- a section that highlights recent accomplishments (FY95-96 Program Highlights)

- a description of the NHEERL Drinking Water Research Program, by program area, including a summary of research findings and anticipated progress for the near future.

COMMENTS WELCOME

The format of this report is still evolving, and we welcome feedback. Readers with comments or requests for further information are encouraged to contact

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DRINKING WATER RESEARCH PROGRAM SUMMARY

REGULATORY AND PROGRAMMATIC CONTEXT

The Safe Drinking Water Act (SDWA) requires EPA to identify and regulate drinking water contaminants that may pose a risk to human health. EPA's rule-making activities are supported by its Office of Research and Development (ORD), which maintains a multidisciplinary research program in drinking water. Research on health effects is conducted by NHEERL, a research arm of ORD. NHEERL provides critical scientific data, methods, and models that address major uncertainties in the Agency's risk assessment process for drinking water, leading to more scientifically sound, cost-effective regulations. Health research on drinking water contaminants at NHEERL is part of a multidisciplinary, multi-Laboratory/Center research program in ORD that includes the National Exposure Research Laboratory, the National Risk Management Research Laboratory, the National Center for Environmental Assessment, and the National Center for Environmental Research and Quality Assurance.

PROGRAM GOAL

To ensure that sound scientific information is available to characterize the nature and magnitude of the health risks posed by microbial pathogens, disinfection by-products (DBPs), and other priority drinking water contaminants.

RATIONALE

For almost 100 years, public water supplies have been treated with disinfectants, such as chlorine, to reduce the risk of infectious disease from waterborne pathogens. Water disinfection has been highly effective in reducing the incidence of certain diseases, such as cholera and typhoid. However, the continued occurrence of waterborne disease outbreaks demonstrates that contamination of drinking water with pathogenic bacteria, viruses, and parasites still poses a health risk when treatment is inadequate. The use of disinfectants, while reducing microbial risks, creates new potential risks as chemical by-products are formed during the treatment process. Some of these disinfection by-products (DBPs) have been shown to cause cancer and other toxic effects in experimental animals. In humans, however, the scientific evidence of adverse effects is inconclusive. Research is required to obtain sufficient understanding of the health risks posed by these and other drinking water contaminants.

RESEARCH STRATEGY

EPA has conducted health research on drinking water since the 1970s. Research has addressed an array of contaminants, including inorganics such as lead, industrial solvents such as trichloroethylene, microbial pathogens, and DBPs. The risk assessment paradigm of the National Academy of Sciences (NAS), which consists of four fundamental steps (hazard identification, dose-response assessment, exposure assessment, and risk characterization), provides the research context for NHEERL's drinking water research program. Emphasis is placed on health research in two areas of the risk assessment paradigm:

- Research in the area of ***hazard identification*** focuses on the development of methods and data that can demonstrate an association between exposure and

effects.

- Research supporting ***dose-response assessment*** seeks to explain the events that link exposure and effects. These events form the basis for the predictive models used to quantify risk.

NHEERL's research program in drinking water includes toxicological studies in experimental animals, clinical studies in human volunteers, and epidemiology studies in selected communities to resolve important drinking water issues. The toxicological studies are designed to characterize the various endpoints of potential concern (e.g., cancer, reproductive toxicity, neurotoxicity), and to evalute the biological processes by which key drinking water contaminants cause their effects. This information is then used to support the development of biologically based dose-response (BBDR) and physiologically based pharmacokinetic (PBPK) models of human response for the highest priority contaminants. The human studies, on the other hand, are designed to improve the tools we use in epidemiology studies and to improve our understanding of waterborne disease outbreaks and potential chemical-associated health risks. Drinking water contaminants under study include: **WATERBORNE PATHOGENS**, such as Norwalk virus and *Cryptosporidium*; priority **DISINFECTION BY-PRODUCTS**, such as haloacids and trihalomethanes; and **OTHER PRIORITY CONTAMINANTS**, such as arsenic, which occurs naturally in some source waters, and aluminum, which is widely used as a coagulant in water treatment. During FY95-96, a research plan for Microbial Pathogens and Disinfection By-Products in Drinking Water was developed by ORD. This plan is being used by NHEERL as a blueprint for its microbial and DBP health effects research program. An ORD research plan for arsenic is presently under development.

NHEERL DRINKING WATER RESEARCH FY95-96 PROGRAM HIGHLIGHTS

WATERBORNE PATHOGENS RESEARCH (pg 7)

The goal of this research is to characterize the human health risks associated with exposure to waterborne pathogens found in drinking water.

- *We made important advances in the development of serological tests that detect infection by* Cryptosporidium. *These tests will be used in drinking water epidemiology studies to help characterize population exposures to this important pathogen.*

DISINFECTION BY-PRODUCTS RESEARCH (pg 9)

The goal of this research is to develop data, methods and models to support assessments of the health effects caused by individual DBPs and mixtures of DBPs.

- *Our studies demonstrated for the first time that dichloroacetic acid is a hepatocarcinogen in the rat.*

- *We showed that dichloroacetic acid induces a unique* ras *oncogene mutation in hepatocellular carcinomas in rodents. This information may help elucidate the mechanism involved in the activation of the cancer process for this chemical.*

- *We discovered a novel, glutathione-mediated metabolic pathway for bromodichloromethane that leads to the generation of genetically active metabolites. This important finding may help characterize the genotoxic mechanism involved in the carcinogenicity of this DBP.*

- *We implemented a number of the research recommendations of a 1993 expert panel workshop and a 1995 work-in-progress workshop on potential reproductive and developmental effects of DBPs.*

OTHER PRIORITY CONTAMINANTS RESEARCH (pg 14)

The goal of this research is to assess the toxicity of arsenic, a contaminant found in some source waters, and aluminum, a widely used coagulant for removing solids during water treatment.

- *Our studies showed that glutathione is critical to a metabolic pathway leading to the detoxification of arsenic. This finding is an important step in understanding the factors that affect the variable sensitivity of humans to arsenic.*

WATERBORNE PATHOGENS RESEARCH PROGRAM

ISSUE

What are the human health risks associated with exposure to microbial pathogens found in drinking water?

Although drinking water disinfection has been highly effective in reducing the risk of certain waterborne diseases, microbial pathogens continue to cause occasional disease outbreaks when treatment is inadequate. While the disease symptoms caused by pathogens are generally known, limited information is available on the doses and conditions that produce disease.

PROGRAM DESCRIPTION

NHEERL's research program on drinking water pathogens is designed to improve our understanding of waterborne disease outbreaks in the U.S. population. Accordingly, we are conducting ***EPIDEMIOLOGY STUDIES*** to evaluate the causes of waterborne illnesses, the magnitude of risk, and the impact of water treatment alternatives and source water quality on disease rates. We also are performing ***CLINICAL STUDIES*** to determine the virulence (or infectious dose) of key pathogens and the impact of host factors, such as immune status, on infection.

PROGRAM PROGRESS

EPIDEMIOLOGY STUDIES

Health effects associated with differences in source water quality and treatment process. In FY93, NHEERL embarked on a multi-phased study to examine the impact of source water characteristics and water filtration on the incidence of waterborne disease. The basic study design involves a comparison of the health status of individuals before and after the addition of filtration units in the home or at the treatment plant. In the first phase of this research effort (FY93-94), NHEERL conducted a survey to identify communities planning to upgrade their treatment plants via filtration. Based on the survey, we selected eight study sites for possible inclusion in the project; an additional 10 were targeted for study in later years. In the second phase of the research effort, completed during FY95, data were collected on source water quality parameters and filter installation schedules. In FY96, we gathered information on community demographics, completed the site selection process, and initiated a pilot test of an epidemiological study design.

NHEERL and several outside organizations are assisting EPA's National Exposure Research Laboratory (NERL) in an analysis of the impact of water quality, treatment process, and distribution system contamination on endemic waterborne disease rates in a community in Quebec, Canada. A 35% excess risk of gastrointestinal illness was reported in this community in a previous investigation. The objectives of the study are to confirm or refute the original reports of illness, to determine the source of the illness, and to find suitable indicators of the health effects. Participating households have been grouped according to different water treatment practices, and in FY95, disease rates for these household groups were examined. A final report on the project is expected in FY97.

Immunological assays for use in epidemiology studies. NHEERL is exploring the possible use of serological tests in field studies of waterborne infectious disease. At issue is whether immunological tests, which can detect antibodies to pathogens in the serum of exposed individuals, can be used as a tool to characterize the prevalence of waterborne disease. In FY93, a collaborative effort involving NHEERL, NERL, the Centers for Disease Control and Prevention (CDC), and the Lovelace Medical Foundation was initiated. We compared the ability of two analytical tests (ELISA and Western Blot) to characterize the immune response to infection by *Cryptosporidium*, a protozoan parasite responsible for several serious outbreaks in the U.S. in recent years. Sera collected during a 1992 disease outbreak in Oregon were tested, and results showed that the Western Blot was the preferred method for identifying cases of cryptosporidiosis. Full-scale serosurveys are now underway at both the community and national levels to examine the risk of infection by *Cryptosporidium* as a function of water treatment method, source water quality and demographics.

Waterborne disease surveillance and reporting. Since 1971, EPA and the CDC have compiled information on waterborne disease outbreaks in the U.S. This surveillance program provides important information on deficiencies in water systems and on etiologic agents associated with outbreaks. During FY94-95, we developed and presented a training course on surveillance and investigation of waterborne disease outbreaks. In FY96, a report characterizing the status of reported waterborne disease in the U.S. for the years 1993-1994 was published, and a summary of waterborne disease surveillance activities in the U.S. was published.

CLINICAL STUDIES

Infectious dose of Norwalk virus. The Norwalk virus, which produces gastro-enteritis, is believed to be responsible for numerous waterborne disease outbreaks in the U.S. In Phase I of this project, begun in FY94, 45 adult volunteers were exposed to various doses of Norwalk virus in an attempt to characterize the virulence of the pathogen and evaluate the impact of immune status on infection and disease. After dosing, each individual was evaluated for clinical symptoms of gastroenteritis, shedding of virus in the stool, and immune response (sero-conversion). Results from Phase I are being used to characterize the dose-response of the virus, which will enable us to expand our research efforts to a larger study population and examine in greater detail the range of outcomes at low doses of the virus (Phase II).

Infectious dose of *Cryptosporidium*. EPA's National Exposure Research Laboratory, with assistance from NHEERL, is conducting a clinical study of *Cryptosporidium* to determine the infectious dose of this important waterborne pathogen. In FY94, scientists at the University of Texas exposed volunteers to *Cryptosporidium*, and infectivity and immune response were evaluated. Doses as low as 30 parasite oocysts were shown to cause infection in humans, and symptom occurrence and disease severity were found to be unrelated to dose. In FY95--one year after the initial exposure--individuals were re-challenged with the parasite to examine the ways in which additional exposure modulates response. Results currently are being analyzed, and in FY97 an assessment will be made of the possible protection from reinfection offered by an initial parasite challenge.

DISINFECTION BY-PRODUCTS RESEARCH PROGRAM

ISSUE

What are the toxic effects associated with disinfection by-products, and what are the toxicologic bases for these adverse effects? Significant gaps exist in our knowledge of the adverse health effects caused by DBPs in drinking water. Several epidemiology studies have suggested possible associations between exposure to disinfected water and cancer or adverse reproductive outcomes, but the findings have been inconsistent and causality has not been established. Toxicological studies using experimental animals have shown that a number of DBPs cause cancer, reproductive toxicity, and other effects, but the effects occur at concentrations higher than those typically found in drinking water. Moreover, the toxicity of many DBPs remains unknown or poorly characterized. Accurate assessments of the risks posed by DBPs and an understanding of the biological basis for observed effects are needed.

PROGRAM DESCRIPTION

NHEERL is conducting health effects research on DBPs in three distinct areas. ***HUMAN STUDIES*** are providing new data to characterize community risks, improve tools for epidemiology studies, and advance methods for managing health and exposure data. ***TOXICOLOGY STUDIES*** in laboratory animals are providing information on the toxicity (carcinogenic, neurotoxic, and reproductive/ developmental effects) of individual DBPs and on the biological and physiological processes involved in the toxic response. These data are then used to support the development and evaluation of predictive models of effect. Finally, we are evaluating ways in which ***MIXTURES*** of DBPs can affect toxicity. This area of research addresses the adequacy of the additivity assumption used in risk assessments of drinking water contaminants.

PROGRAM PROGRESS

HUMAN STUDIES

Development of improved tools for field research. NHEERL is supporting work to develop and validate models to improve estimates of exposure in epidemiology studies, thereby enhancing our ability to relate exposure to effects. A mathematical model for predicting individual household exposure to trihalomethanes (THMs) based on water treatment process, distribution system characteristics, and water transit time was evaluated in FY95. The model was used to estimate exposure at different points along the distribution system. A report is currently being drafted.

Improving methods for managing health and exposure data. The utility of a data management system (called Geographic Information Systems, or GIS) for studying the potential impact of DBPs on reproductive health is currently being analyzed. A pilot study using GIS has been conducted in two Colorado communities that use either chlorination or chlor-amination for water disinfection. The relationship between adverse reproductive outcomes (specifically, low birth weight) and the concentration of residual chlorine or other parameters at various points along the distribution system is being analyzed. In FY95, statistics on health outcomes were

transformed into GIS format, and preliminary epidemiologic analyses were conducted in the chlorinated community. During FY96, distribution system modeling data and health information were integrated into the analysis. A final report on this project is expected in FY97.

NHEERL also is addressing methodologic issues involving the merger of databases. National databases on health (National Maternal and Child Health Survey, 1988) and exposure (Federal Database Reporting System) are being used in our study. In FY95, in collaboration with EPA's National Center for Environmental Assessment, scientists began to merge information on adverse reproductive outcomes (e.g., fetal death and low birth weight) with exposure data (MCL violations for 1987-1989) to isolate possible associations. A final report on this analysis is anticipated in FY97.

Support for Ongoing Studies. The State of California, with partial EPA support, is conducting a prospective study of drinking water and spontaneous abortions. Three types of information will be used to assess potential risks: 1) quarterly reports of drinking water utilities; 2) self reports of subjects; and 3) analysis of DBPs based on drinking water samples from the tap. A final report is expected early in FY97.

The State of New Jersey also is conducting a study with partial EPA support to re-examine the findings of earlier investigations that suggested an association between neural tube defects and elevated levels of trihalomethanes, nitrates, and certain volatile solvents. This study is using refined methods, such as biomarkers, to relate exposure and effects. A final report will be submitted in FY97.

Workshops. In FY93, NHEERL and the International Life Sciences Institute (ILSI) convened an expert panel to review published epidemiologic and experimental data on the reproductive/developmental effects of DBPs and to help guide the development of a research strategy. The expert panel concluded that currently available data provide an inadequate basis for identifying DBPs as a reproductive or developmental hazard, but that specific types of research could be conducted to strengthen the scientific basis for such an assessment. Since this workshop, NHEERL has implemented a number of the research recommendations of the panel in the areas of epidemiology methods development and toxicology studies on individual DBPs. In FY95, a drinking water reproductive effects work-in-progress workshop was held to review ongoing laboratory and field studies being conducted by NHEERL and outside groups. It was noted that considerable progress had been made in improving the state-of-the-science since the FY93 workshop.

TOXICOLOGY STUDIES

Reproductive and Developmental Toxicity. Reproductive screening studies of DBPs are being conducted both intramurally and in collaboration with the National Toxicology Program (NTP) at the National Institute of Environmental Health Sciences (NIEHS). A wide range of chlorinated, brominated, and chlorobrominated by-products are being evaluated in male and female rodents. One DBP of particular interest is dibromoacetic acid, which has been shown to produce effects on both the male reproductive system and the developing fetus at experimental exposure levels. We are performing a thorough evaluation of the reproductive and developmental toxicity of this chemical in the male rodent. Studies utilize a broad range of exposures and multiple reproductive assessments. Observations have included alterations in

sperm morphology and motility, as well as in mating and spermatogenesis, at experimental doses. Research is now underway to determine whether these effects are the result of compromised endocrine balance. Findings were published in FY95 on reproductive competence and sperm quality. We are presently investigating developmental mechanisms that may be involved in DBP-induced neural tube and heart defects. Specifically, we are attempting to determine whether these effects may be a consequence of changes in protein kinase activity during critical stages of embryonic development. Also during FY95, reproductive and developmental toxicity studies showed that experimental doses of bromate and dichlorobromomethane reduce sperm counts in rodents. These chemicals are now scheduled for more rigorous testing for effects on fertility. Finally, we evaluated reproductive function in rodents exposed to bromodichloromethane and found a significant impairment in sperm motility.

To prioritize chemicals for more comprehensive testing *in vivo* (and to hypothesize a mechanistic basis for developmental toxicity), we conducted a Quantitative Structure-Activity Relationship (QSAR) study during FY95 on a series of mono-, di-, and tri-haloacetic acids. QSAR analyzes the relationship between key structural properties of a chemical and its outcome in toxicity tests. We are focusing specifically on dichloroacetic acid (DCA) and its chloro/bromo analogues, which we have shown to be embryotoxic in the *in vitro* whole mouse embryo culture system. QSAR modeling is being carried out in tandem with ongoing experimental studies. Our results in FY95 showed that lipophilicity and electronic properties are important determinants in the induction of neural tube defects. Based on these findings, we are conducting *in vivo* developmental screening tests in which comprehensive fetal evaluations (assessments of soft tissue and skeletal abnormalities) are being performed.

Carcinogenicity. Tests conducted by NHEERL during FY94-95 showed that bromodichloromethane induced hepatocellular (liver) cancer, but only at the lowest dose tested. Studies are now underway to try to explain this unexpected finding. A chronic bioassay using potassium bromate was completed in FY95, and our pathological analyses confirmed reports by others that bromate induces cancer of the kidney, thyroid, mesentery, and large intestine. Mechanistic studies are planned, with an emphasis on the ability of bromate to induce oxidative damage in DNA, alter thyroid function, and affect cell proliferation and death. Finally, one of our most important findings in this research area involves dichloroacetic acid. During FY95, we demonstrated for the first time ever that DCA is a hepatocarcinogen in rats.

Several priority DBPs will be evaluated by the NTP in their two year (chronic) cancer bioassay program in rodents. This research will be conducted in collaboration with scientists from NHEERL. In FY95, we initiated a subchronic carcinogenicity study of sodium chlorate in rodents to assist in the design and implementation of a chronic bioassay, which is scheduled to be performed by NTP in FY97.

Neurotoxicity. During FY95, we completed a series of experiments in rodents on the neurotoxic effects of dichloroacetic acid (DCA), an important haloacid commonly found in disinfected drinking water. These studies involved acute, subchronic, and chronic exposure of male rats to DCA. Following acute exposure, effects were negligible; however, following subchronic and chronic exposures, there was

preliminary evidence of neurotoxicity (limb weakness and deficits in gait). High concentrations of DCA produced hindlimb paralysis, and although neuropathological assessments indicated localized damage to the spinal cord, no damage to the nerve that innervates the hindlimbs was observed. A more extensive assessment of the neuropathological effects is in progress to correlate nervous system damage with behavioral effects. This analysis will be completed in FY97.

Biologically based dose-reponse (BBDR) models. NHEERL is actively engaged in the development of BBDR models to help explain the biological/physiological events involved in toxic responses to drinking water contaminants. In one project, we are developing a BBDR model for carcinogenesis by conducting tumor studies in rodents while simultaneously measuring biochemical, immunohistological, pathological, genetic, and mechanistic endpoints. Special attention has been focused on dichloroacetic acid (DCA) in an effort to explain the progression of DCA-induced liver lesions and cancer. During FY95, we helped define several critical parameters required for a BBDR model for DCA. In studies of *ras* oncogene activation (which can initiate the cancer process) in the liver cells of mice, we showed that a unique mutation occurs in response to DCA. This finding is significant because it may be possible to use this "fingerprint" of genetic damage to demonstrate exposure to DCA and to explain a possible mechanism(s) for carcinogenesis. Our research on apoptosis (programmed cell death) showed that DCA suppresses cell death in liver cells, thus increasing the number of cells at risk of transformation and possibly enhancing the chances for tumorigenesis. And finally, research in FY95 examining the role of endocrine disruption in DCA induction of liver cancer in rodents has indicated alteration of steroid hormone levels and changes in steroid metabolism and receptor activity. Our model development is an ongoing effort, and as additional mechanistic results become available, we will integrate these data with the tumor data to develop a mathematical construct that represents a BBDR model for humans.

Physiologically based pharmacokinetic (PBPK) models. PBPK models, which describe the behavior of a chemical as it is metabolized, distributed to target organs and tissues, and eliminated from the body, are being developed by NHEERL to facilitate the extrapolation of toxicity data from animals to humans. In FY95, considerable progress was made to support the development of a PBPK model for bromodichloromethane (BDCM). Parameters being studied include tissue partition coefficients, primary pathways of metabolism, metabolic rate constants, and macromolecular binding in target organs. During FY95, we found that glutathione plays an important protective role in acute BDCM toxicity in rodents, presumably due to macromolecular binding. In addition, one of our most important findings was the discovery of a novel, glutathione-mediated metabolic pathway for BDCM that leads to the generation of genetically active metabolites. Though believed to be a minor pathway, this finding is significant because it suggests there may be a genotoxic mechanism of action for BDCM carcinogenicity that is different from that of chloroform, another important trihalomethane. Ongoing studies are also examining the involvement of cytochrome P450 isozymes on BDCM metabolism and toxicity. An initial PBPK model is expected in FY97, and in subsequent years, we hope to characterize human brominated trihalomethane metabolism to refine our model.

DBP MIXTURES

Drinking water invariably contains mixtures of DBPs. In an effort to improve our understanding of the interactions that may occur in these mixtures, NHEERL is investigating binary and quaternary mixtures of DBPs. The first class of drinking water contaminants selected for testing is the trihalomethanes (THMs). In a collaborative effort with the EPA's National Center for Environmental Assessment, we are examining hepatotoxicity using various combinations of THMs. A matrix of dose levels has been constructed so that effects can be observed at different combinations of dose. Experiments on binary mixtures (BDCM and chloroform) were initiated in FY95 in rodents, and results are in the process of being analyzed. In subsequent years, other binary combinations of THMs will be assessed. For those mixtures that exhibit synergistic effects in experimental animals, pharmacokinetic and pharmacodynamic studies will be conducted to elucidate underlying mechanism(s). We also have begun to study additivity for mixtures of four THMs (BDCM, chlorodibromomethane, bromoform, and chloroform). We are predicting the response of the mixture *a priori* based on additivity of the dose-response curves for each individual chemical; the response of the mixture is then determined experimentally and compared to the expected response. We initiated the single-chemical experiments in FY95, and, based on the dose-response information obtained from these studies, we began our mixtures experiments in FY96. The purpose is to evaluate the adequacy of the additivity assumption for dose and effect.

Additional mixture studies have been conducted by NHEERL in collaboration with EPA's National Risk Management Research Laboratory (NRMRL) using a simple assay for genetic toxicity. The use of genotoxicity testing in evaluations of alternative disinfectants received increased attention recently when Finnish studies demonstrated a positive correlation between the mutagenicity of chlorinated drinking water and certain human cancers. In FY94, drinking water samples were collected by NRMRL from treatment plants utilizing a variety of water disinfection practices (ozonation, chloramination, chlorination, and treatment with chlorine dioxide). In FY95, scientists from NRMRL and NHEERL analyzed the samples for mutagenicity. Ozonation did not enhance the mutagenic potency of raw water, but treatment with either chlorine or chloramine greatly increased mutagenic potency. Using molecular techniques, we then identified for the first time the spectrum of mutations produced by these samples. We found that the mutation spectra of the drinking water samples resembled the spectrum produced by MX, a compound present at low concentrations in drinking water but extremely important to its mutagenicity. This indicates that the mutation spectrum produced by a complex mixture reflects the dominance of one (or a few) class(es) of chemicals within the mixture.

OTHER PRIORITY CONTAMINANTS RESEARCH PROGRAM

ISSUE

What health effects result from exposure to arsenic and aluminum in drinking water?
Arsenic, a naturally occurring source water contaminant, is important from both a regulatory and public health perspective. Several studies have linked arsenic-tainted drinking water to cancers of the skin and internal organs, such as the bladder. Aluminum compounds, which are used as coagulants for removing solids during water treatment, are generally considered safe at levels found in drinking water. However, concerns have been raised regarding the possible involvement of aluminum in neurodegenerative diseases, such as Alzheimer's or Parkinson's disease.

PROGRAM DESCRIPTION

The objective of this research program is to provide quantitative toxicity data on the adverse health effects caused by ***ARSENIC*** and ***ALUMINUM***. We are studying the metabolism of arsenic and the biochemical mechanisms involved in its toxicity to help explain how it induces cancer and other effects in humans. For aluminum, our research is aimed at understanding the modes of action involved in neurotoxicity.

PROGRAM PROGRESS

ARSENIC

NHEERL is addressing several key scientific issues that impact the risk assessment for arsenic in drinking water. Studies are being conducted to provide a better understanding of the dose-response relationship for arsenic, the relationship between arsenic metabolism and toxicity, and the factors that may affect the variable sensitivity of humans to arsenic. Recent accomplishments in this area include the development of improved analytical methods for the study of arsenic disposition and the development of an *in vitro* system to characterize its methylation. During FY94, we studied the enzymatic basis of methylation to evaluate factors that may influence inter-individual variation in arsenic metabolism. In FY95, we published important information on the role of glutathione in the methylation and detoxification of arsenic. We currently are drafting major portions of a research plan for ORD to guide future work in this area.

ALUMINUM

A review of the literature conducted in FY95 by our laboratory concluded that the association between aluminum and Alzheimer's disease is weak. Although aluminum is neurotoxic, no mechanism has been offered to explain the wide variety of effects observed in experimental animals. We are therefore conducting research to identify biochemical mechanisms of action and to develop data for use in BBDR models to explain aluminum-induced neurotoxicity in humans. In FY95, we showed that aluminum alters signal transduction mechanisms in brain tissue *in vitro*. These results may help us understand how aluminum causes deficits in learning and memory. We also showed during FY95 that aluminum *per se* does not alter long-term potentiation, indicating that aluminum may have more general, non-specific effects on brain function. Future research will examine behavioral and neurochemical changes *in vivo* following developmental exposure to aluminum in drinking water.

CONCLUSIONS

The accomplishments of NHEERL scientists on the health effects of drinking water contaminants discussed in this report reflect the considerable progress that has been made in improving the scientific basis for decision-making. These advances also lay the foundation for future progress in resolving key uncertainties in health risk assessment. It is through the implementation of a multidisciplinary, mission-oriented drinking water research program, which emphasizes the study of high risk, high uncertainty public health problems of relevance to EPA's Office of Water, that our goals are being achieved.

National Health and Environmental Effects Research Laboratory

AIR TOXICS

HEALTH EFFECTS RESEARCH

ANNUAL REPORT

SEPTEMBER, 1996

CONTENTS

INTRODUCTION

PURPOSE

The purpose of this report is to communicate results from the Air Toxics Research Program of EPA's National Health and Environmental Effects Research Laboratory (NHEERL).

CONTENT

The report contains

- a summary of the NHEERL Air Toxics Research Program, including an explanation of its regulatory and programmatic context, its overall goal, the rationale for the program, and the research strategy
- a section which highlights recent key findings (FY95 Program Highlights)
- a description of the NHEERL Air Toxics Research Program, by program area, including a summary of research accomplishments and anticipated progress for the near future.

COMMENTS WELCOME

The format of this report is still evolving, and we welcome feedback. Readers with comments or requests for further information are encouraged to contact:

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AIR TOXICS RESEARCH PROGRAM SUMMARY

REGULATORY AND PROGRAMMATIC CONTEXT

The Clean Air Act (CAA) authorizes EPA to regulate a wide variety of toxic air pollutants, including motor vehicle emissions and hazardous air pollutants (HAPs) emitted from stationary sources. It requires EPA to set technology-based standards for pollutant sources and, if warranted, health-based standards for residual risk following installation of Maximum Achievable Control Technology. To help the Program Office meet these requirements, EPA's Office of Research and Development (ORD) maintains an Air Toxics Research Program that develops methods and models to improve air toxics risk assessment and provides chemical-specific data on priority contaminants.

PROGRAM GOAL

To develop improved methodologies for assessment of health risks from exposure to air toxics.

RATIONALE

Millions of Americans, particularly those living in urban environments, are exposed to hazardous air pollutants. The health risks posed by these exposures are potentially high, according to the National Academy of Sciences (NAS), but substantial scientific uncertainty remains regarding these risks. For example, although some air contaminants are known to be toxic in laboratory studies, little is known about the effects that may be caused in humans at the relatively low concentrations normally present in ambient air. Research is needed to address the poorly understood issues surrounding the health risks of air toxics.

RESEARCH STRATEGY

To ensure that the Agency is equipped with scientific and technical data relevant to the formulation of sound environmental policy, ORD operates a research program founded on the principles of risk assessment. In the area of health effects, the program is patterned after the risk assessment paradigm of the National Academy of Sciences (NAS). This paradigm consists of 4 steps--hazard identification, dose-response assessment, exposure assessment, and risk characterization--that provide information for risk management decisions. NHEERL's research programs in Air Toxics adhere to this risk-based strategy, emphasizing three types of research activities:

- Research in the area of ***hazard identification*** focuses on the development and utilization of methods to identify health hazards.
- Research supporting ***dose-response assessment*** seeks to explain the events linking exposure to effects. These events form the basis for the predictive models used to quantify risk.
- There also may be instances when scientific data on a particular contaminant is required. In such cases, short-term ***problem-specific*** research is conducted to systematically collect and analyze information regarding specific gaps in knowledge.

The objective of NHEERL's research program in Air Toxics is to provide toxicity data and new methods for evaluating the cancer and non-cancer risks for HAPs. Our efforts are focused on a set of chemicals and source categories believed to present the greatest potential threat to public health and/or which serve as the best models for

methods development. In the area of **HAZARD IDENTIFICATION,** we are exploring new areas in air toxics epidemiology. We are developing molecular/biologic methods, such as biomarkers, to strengthen conventional epidemiology approaches, and we are utilizing existing health databases to help shape epidemiology research questions. Research in **DOSE-RESPONSE ASSESSMENT** is designed to anticipate and address the most problematic air toxics risk assessment issues faced by EPA, such as estimating exposure-response relationships over a range of exposure concentrations and durations, predicting human response to these exposures, and estimating risk from common air pollutant mixtures. Finally, we are conducting **PROBLEM-SPECIFIC STUDIES** to provide human health data for a fuel additive (MTBE) recently associated with health complaints in some parts of the country.

NHEERL AIR TOXICS RESEARCH FY95 PROGRAM HIGHLIGHTS

HAZARD IDENTIFICATION RESEARCH (pg 7)

The goal of this research is to develop methods that will improve our ability to characterize the health hazards associated with exposures to air toxics.

- *We demonstrated that urine metabolite analysis is a highly sensitive measure of recent exposure to polycyclic aromatic hydrocarbons (PAHs) and may serve as an effective biomarker for PAH exposures in epidemiology studies.*

DOSE-RESPONSE ASSESSMENT RESEARCH (pg 9)

The goal of this research is to better understand the factors that influence exposure-response relationships for a set of representative air toxics so that improved empirical methods and quantitative, biologically based risk models can be developed.

- *In studies of respiratory toxicity, we demonstrated that phosgene impairs pulmonary host defenses and enhances sensitivity to bacterial infection in the lungs of mice.*

- *We found that inhalation of trichloroethylene (TCE), a volatile organic solvent, causes a unique mid-frequency hearing loss in test animals.*

- *We showed that the peak concentration of TCE in blood, as estimated by our PBPK model, is a good indicator of acute neurotoxic effects.*

- *Using rodents, we found a direct link between DNA adducts formed in lungs and induced mutations in lung tumor oncogenes for certain PAHs. These findings were substantiated when we linked airborne combustion products with human carcinogenesis via DNA adducts in target tissues.*

PROBLEM-SPECIFIC STUDIES (pg 13)

The goal of this research is to assess the human health effects of a fuel additive, methyl tertiary butyl ether (MTBE).

- *In a study investigating complaints of illness associated with MTBE, our scientists demonstrated that ambient levels of MTBE have no significant effect on normal, healthy individuals. This study was awarded the EPA bronze medal.*

- *We sponsored a workshop on MTBE in which a panel of scientific experts from industry, academia, and government provided guidance to EPA on the feasibility and design of epidemiology studies of MTBE-exposed populations.*

AIR TOXICS
HAZARD IDENTIFICATION RESEARCH PROGRAM

NHEERL defines ***hazard identification research*** as research to characterize the association between environmental exposure and adverse effect.

ISSUE

Does exposure to ambient concentrations of HAPs result in demonstrable health effects? At present, there is virtually no direct observational evidence (i.e., epidemiologic data) to link ambient HAP exposures with health effects. This is due, in part, to the relatively small population sizes exposed to HAPs (compared to criteria pollutants, for instance), to limited exposure information, and to the expense of adequate epidemiology studies. Better tools are needed to identify and characterize these risks.

PROGRAM DESCRIPTION

NHEERL is engaged in two research projects to identify the health hazards associated with exposure to HAPs. In the first project, we are developing **MOLECULAR/BIOLOGIC METHODS** to identify potentially hazardous air pollutants and to facilitate field studies of urban air pollution. Computational chemistry and molecular modeling techniques are being used to explore correlations between the structural properties of a chemical and its toxicity. Biomarkers are being developed to bridge the association between exposure and effects. Biomarkers under study include DNA and protein adduct formation in target tissues and metabolite analysis in human body fluids. In the second research project, **HEALTH DATABASES** on disease incidence in urban areas are being evaluated to relate exposure and effects. We are using a data management tool, Geographic Information Systems (GIS), to interface existing health effects databases (e.g., State Birth Defect Registries) with emissions monitoring information to identify geographic locations that qualify for more detailed field studies.

PROGRAM PROGRESS

MOLECULAR/BIOLOGIC METHODS. NHEERL is using quantum mechanical studies of the structure and reactivity of PAHs (and their metabolites) to identify biologically active chemicals and to describe the interaction that occurs between the reactive chemical and its biological target. In FY95, we found that the electrostatic properties of diol epoxides (metabolites) of a PAH called benzo(c)phenanathrene influence its ability to interact with DNA and may explain observed differences in carcinogenic potency. These studies are giving us insight into the chemical features associated with enhanced toxicity. We will continue to apply this method to other air toxics and their metabolites in an effort to identify classes of chemicals that pose a potential health hazard and to better understand the mode of action at the target site.

We also are developing tools (biomarkers) for use in environmental epidemiology studies to improve estimates of human exposure and biologically effective dose. In FY95, we made several advances in biomarker development. We improved methods for separating and identifying DNA adducts using highly sophisticated analytical techniques (^{32}P-postlabeling and HPLC analysis). These techniques permitted us to

identify DNA adducts formed in mouse skin and lung tissue following topical treatment with diesel exhaust extracts. These adducts were formed by nitroaromatic compounds present in the exhaust.

Another focus for biomarker research by NHEERL is analysis of body fluids for indicators of exposure to air toxics. In FY95, we published results from a study in which we analyzed urine collected from individuals belonging to a population associated with high lung cancer mortality. We found a correlation between exposure to unvented smoke and PAH metabolites in urine. Further evaluation of the data showed that urine metabolite analysis is a highly sensitive measure of recent exposure to PAHs.

HEALTH DATABASES. An emerging area of research for NHEERL is the use of health databases to help shape epidemiology research questions and hypotheses. Reproductive/developmental and pulmonary health effects will be considered first because existing databases appear to be more suitable for evaluating these effects. Our overall objective over the next couple of years is to determine whether a geographic area exhibiting a high incidence of health effects is also likely to be characterized by high emissions/ concentrations of specified HAPs.

AIR TOXICS
DOSE-RESPONSE ASSESSMENT RESEARCH PROGRAM

NHEERL defines ***dose-response assessment research*** as research to identify and describe the basis for the effects caused by exposure to environmental stressors or chemical contaminants.

ISSUE

How can risk assessments of toxic air pollutants be strengthened?

EPA is mandated by the Clean Air Act to assess the risks posed by toxic air pollutants. Risk assessments frequently rely on predictive models that estimate human risk using experimental toxicity information. The reliability of the derived risk estimates depends on the soundness and accuracy of the models as well as the strength and scope of the toxicity data. Improved methods and models are needed to enhance the precision of risk estimates, and new toxicity data are needed to facilitate model development.

PROGRAM DESCRIPTION

The primary objective of this research program is to develop new approaches to risk assessment for air toxics that anticipate and address the problematic issues faced by EPA's Office of Air and Radiation (OAR). Specifically, we are examining various combinations of exposure concentration and duration to explain **EXPOSURE-RESPONSE RELATIONSHIPS.** A range of health effects and chemical class combinations have been selected for initial analysis: for respiratory toxicity, we are using a representative irritant gas (phosgene); for neurotoxicity, a solvent (TCE); for developmental toxicity, a non-chlorinated hydrocarbon (methanol); and for cancer, polycyclic organic matter (POM). Using these data, we are developing empirical and biologically based **RISK MODELS** to provide quantitative estimates of response as a function of dose. We also are conducting research to determine how interactions among chemicals in air **MIXTURES** affect risk.

PROGRAM PROGRESS

EXPOSURE-RESPONSE RELATIONSHIPS. Short-term, high-concentration exposures to toxic air pollutants may induce toxicity disproportionate to that caused by long-term, low-level exposures. Recognizing this distinction, ORD has anticipated difficulties in assessing risks from short-term exposures. To address this problem, NHEERL is conducting research to improve the scientific basis for risk assessments from acute exposures and is working with the National Center for Environmental Assessment (NCEA) to apply results in development of new acute risk assessment methodologies. One approach to improved assessment is to analyze toxicity at various combinations of chemical concentration (C) and time (t). According to Haber's "Law," the product of this relationship (Cxt) should yield a constant effect on health; this assumption underlies current risk assessment efforts. Our research is designed to determine whether this assumption is valid across short-term and intermittent exposures to air toxics.

Respiratory Toxicity: Irritant Gases (Phosgene). Phosgene is a respiratory irritant and a highly reactive air toxic. Using various chemical concentrations and exposure times, NHEERL is examining the effects of phosgene on pulmonary host defenses and disease susceptibility, with an emphasis on animal-to-human extrapolation. In FY95, scientists in our Experimental

Toxicology Division used an established rodent model for allergic sensitization to show that phosgene impairs the immune system. Acute exposure was shown to decrease resistance to bacterial infection and to enhance susceptibility to tumor cell challenge; these effects appear to be due to impaired alveolar macrophage activity and natural killer cell activity, respectively. This valuable mechanistic information will be used to improve extrapolations of human response. Subchronic exposure studies showed that phosgene impairs clearance of bacteria from the lungs and increases inflammatory response. We also found during FY95 that the concentration of phosgene is more important than time of exposure in determining response.

Neurotoxicity: Organic Solvents (Trichloroethylene, or TCE). Exposures to volatile organic compounds (VOCs), such as solvents, can cause neurotoxic and behavioral effects in humans. In FY94, we initiated a project to better define the exposure-response relationship for solvents. TCE was selected as the model compound. Our objective is to describe the relationship between C and t for a variety of neurotoxic endpoints--including indices of complex behavior, visual function, and ototoxicity (hearing loss)--as a function of acute inhalation exposures in rodents.

Behavior was measured using a vigilance test (response time to a signal light for food reward) developed by our Neurotoxicology Division in FY94. In FY95, we applied this test to airborne TCE and evaluated Cxt relationships. Preliminary findings suggest that TCE toxicity results from a combination of concentration and time, but that concentration is more important than time in determining the strength of response.

The effect of TCE on visual function was analyzed by measuring visual evoked potentials (VEPs). Using an inhalation chamber, we exposed rats in FY95 to TCE vapors while simultaneously recording VEPs. Collected data are now being analyzed.

Ototoxicity tests of inhaled TCE were conducted during FY94-95 to characterize hearing loss across a range of hearing frequencies. While most previous studies of ototoxicity to organic solvents have demonstrated hearing deficits at high frequencies, we found during FY95 that TCE causes a unique hearing loss in the mid-frequency range, sparing function at lower and higher frequencies. Research in this area continues as we determine the Cxt relationship for a variety of exposure durations (acute to subchronic).

Developmental Toxicity: Non-chlorinated Hydrocarbons (Methanol). Methanol represents an important chemical under the Clean Air Act because it has been proposed as an alternative fuel for motor vehicles and is a likely fuel for fuel cell technology cars of the future. Moreover, it is listed among the 189 toxic air pollutants requiring regulation by EPA. In the early 1990s, scientists in our Reproductive Toxicology Division showed that methanol adversely affects fetal development: inhalation of methanol by pregnant mice--at concentrations relevant to humans--resulted in multiple birth defects. Research on this phenomenon continues, and in FY95 we completed work on the toxicity of methanol's principal metabolite, formic acid. Using an *in vitro* embryo culture system, we showed that methanol, not formic acid, is the proximate developmental toxicant. We also completed a blood profile of Cxt effects for a variety of methanol exposure scenarios, and we defined the critical period for induction of skeletal and visceral malformations. In the upcoming year, we will be narrowing the search for the early events in the pathogenesis of the birth defects caused by methanol, with specific emphasis placed on Cxt studies and gene expression in exposed embryos.

Cancer: Polycyclic Organic Matter/Polycyclic Aromatic Hydrocarbons

(POM/PAHs): PAHs are toxic components of many environmental contaminants, including combustion emissions. In an effort to uncover the mode of action for PAH toxicity, we are studying a set of PAHs and their metabolites for their ability to form DNA adducts and to morphologically transform cells. Using mouse embryo fibroblasts, we studied an extremely potent PAH, dibenzo(a,l)pyrene (DBA) in FY94 and compared its toxicity to that of benzo(a)pyrene (BAP). We concluded that DBP is significantly more active as a morphological transforming agent than BAP. In addition, its adducts are different from those of BAP, which may provide a clue to its higher carcinogenic potency. In FY95, we expanded our study to include dibenzo(a,h)anthracene (DBA). Collectively, the results from these analyses will help explain the mechanisms underlying PAH carcinogenesis.

In an important project in humans conducted by scientists in our Human Studies Division using human autopsy samples, we reported in FY95 that POM (combustion products) can be linked to human carcinogenesis via the formation of DNA adducts.

RISK MODELS. Using exposure-response data obtained from the research projects described above, NHEERL is developing non-cancer and cancer risk models to improve the scientific basis for health risk assessment.

Scientists in our Experimental Toxicology Division are in the initial stages of developing a biologically based dose-response (BBDR) model for ***pulmonary toxicity*** using phosgene as the prototype for irritant gases. We are collecting exposure-response data for various Cxt combinations in order to understand why concentration, rather than time, drives the toxic response. NHEERL scientists are working with NCEA to reflect these results through development of an acute reference exposure (ARE) methodology. The use of ARE methodologies will assist in assessing the health risks of acute exposures for phosgene as well as for other compounds.

To improve predictions of the ***neurotoxic effects*** of volatile organics, NHEERL is developing a physiologically based pharmacokinetic (PBPK) model in rodents to estimate human risk. During FY95, we used our model to estimate blood and brain concentrations of TCE and its metabolites in order to predict neurotoxicity. The accuracy of these predictions is being determined based on various measures of neurotoxic outcome, including complex behavior, visual function, and hearing loss. During FY95, we showed that model estimates of the peak concentration of TCE in blood were a good indicator of neurotoxic risk across exposure scenarios and neurotoxic outcome measures. In the future, parameters of the PBPK model will be scaled up to produce rational predictions of human risk based on laboratory findings.

As a result of our discovery in the early 1990s that inhalation of methanol caused multiple birth defects in mice, scientists in our Reproductive Toxicology Division proposed a 5-year plan to develop the Agency's first BBDR model for ***developmental toxicity*** to be used in methanol risk assessment. During FY95, NHEERL moved closer to achieving its scheduled development of this model by confirming the heightened sensitivity of the mouse embryo culture system relative to the rat in terms of developmental toxicity. This evidence supports the relevancy of the mouse model for predicting effects in humans exposed to methanol. When completed, this model will represent a significant step forward in efforts to evaluate environmentally induced birth defects.

One of the uncertainties in ***cancer*** risk assessment of PAHs is the validity of

the assumption that all environmental PAHs are equal in potency to benzo[a]pyrene (BaP). To help reduce this uncertainty, scientists in our Environmental Carcinogenesis Division have studied the lung tumorigenic potency of five environmental PAHs in mice. The relationship between lung DNA adducts, specific mutations in lung tumor oncogenes, and lung tumorigenic potency induced by the PAHs was evaluated. We found that for certain PAHs, there is a direct link between their ability to form DNA adducts, mutate oncogenes, and induce tumors. A set of DNA adduct(dose)-tumor(response) relationships has been developed for these 5 PAHs for use in risk assessment; this information will be used to develop cancer potency estimates.

MIXTURES. Urban air is a mixture of gases and pollutants; rarely, if ever, does exposure involve a solitary contaminant. For this reason, NHEERL is studying the effects of chemical interactions in air using mixtures of common urban air contaminants. Scientists in our Neurotoxicology Division are examining the interactive effects of mixtures of VOCs on hearing. Several volatile solvents--including TCE, xylene, and toluene--were selected for testing. A multifactorial study in rodents was designed, and a matrix of concentration combinations was tested. During FY95, we found that inhalation exposure to mixed xylene, toluene and TCE caused hearing deficits in the mid-frequency range, sparing function at lower and higher frequencies. This information is useful because it suggests a common mechanism of toxicity for environmentally related solvents.

AIR TOXICS
PROBLEM-SPECIFIC STUDIES

NHEERL defines ***problem-specific studies*** as research to characterize the effects induced by a specific environmental perturbation or chemical contaminant.

ISSUE

What are the adverse effects associated with exposures to the fuel additive, MTBE? Areas of the country unable to meet the National Ambient Air Quality Standard (NAAQS) for carbon monoxide must, according to the Clean Air Act of 1990, consider addition of oxygenates to auto fuels to reduce carbon monoxide emissions. In 1992, the addition of an oxygenate called methyl-tertiary-butyl ether (MTBE) coincided with complaints of illness, including headache, nausea, sore throat, and eye irritation. Concerns about the potential health risks of MTBE have prompted research in this area.

PROGRAM DESCRIPTION

In response to emerging concerns regarding the health risks posed by MTBE and other fuel additives, ORD organized a joint government-industry research program for health risk assessment. To address the uncertainties identified by the assessment, NHEERL has initiated a series of clinical research studies to evaluate the health effects of MTBE in normal, healthy individuals and has designed a study of sensitive subgroups of individuals (those who complain of MTBE symptoms). Study participants are exposed for short duration to environmentally relevant concentrations of MTBE, and various measures of sensory and physiological response are recorded and analyzed.

PROGRAM PROGRESS

During FY94, a group of healthy volunteers was recruited by our Human Studies Division to participate in a clinical study of acute health effects induced by exposure to MTBE. Individuals were exposed in specially designed air chambers to levels of pure MTBE approximating a typical exposure during refueling. Cognitive testing was conducted during exposure, and objective measures of eye and nose irritation were obtained pre- and post-exposure. Pharmacokinetic studies of blood levels of MTBE and its principal metabolite, tertiary butyl alcohol (TBA), also were performed. Symptom questionnaires were administered. In FY95, we published the findings of this important study, which earned the prestigious Bronze Medal from EPA. Our results showed that MTBE at ambient levels has no significant effect on normal, healthy subjects. These findings have allayed some concerns about the potential risks posed by MTBE and have facilitated its continued use in efforts to control carbon monoxide and its attendant health effects. In FY96, we plan to follow up our pharmacokinetic studies to determine whether TBA persists in the blood following repeated exposure to MTBE.

We also conducted a workshop on MTBE in FY95. Panel members included experts from industry, academia, and government. Results from studies of MTBE exposure and related health effects were presented, and a report was prepared by the panel providing guidance to EPA on the feasibility and design of epidemiology studies on populations exposed to MTBE.

National Health and Environmental Effects Research Laboratory

INDOOR AIR RESEARCH

ANNUAL REPORT

JUNE, 1996

CONTENTS

INTRODUCTION

PURPOSE

The purpose of this report is to communicate results from the Indoor Air Research Program of EPA's National Health and Environmental Effects Research Laboratory (NHEERL).

CONTENT

The report contains

- a summary of the Health and Environmental Effects Research Program for Indoor Air, which describes the regulatory and programmatic context of the research program, the overall program goal, the rationale for the program, and the research approach
- a section which highlights recent key findings (FY95 Program Highlights)
- a more detailed description of the NHEERL Indoor Air Research Program, by program area, including a summary of research accomplishments and anticipated progress for the near future.

COMMENTS WELCOME

The format of this report is still evolving, and we welcome feedback. Readers with comments or requests for further information are encouraged to contact

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INDOOR AIR RESEARCH PROGRAM SUMMARY

REGULATORY AND PROGRAMMATIC CONTEXT

EPA's Indoor Air Research Program is authorized by the Radon Gas and Indoor Air Quality Research Act of 1986 (Title IV of the Superfund Amendments and Reauthorization Act, or SARA). The Agency has no regulatory jurisdiction over indoor air; rather, its goal is to inform the public debate. EPA's Office of Air and Radiation (OAR), which disseminates advisory information on indoor air quality to consumers, building owners, and public health professionals, relies on the Office of Research and Development (ORD) to provide scientific data for this process. The bulk of ORD's resources for indoor air research are appropriated to areas of greatest uncertainty: health effects, exposure assessment, and risk management. Health effects research, which is designed to improve understanding of the noncancer health risks associated with indoor air pollutants, is conducted by NHEERL.

PROGRAM GOAL

To determine the health risks posed by low-level mixtures of organic vapors and indoor allergens.

RATIONALE

Individuals in the United States spend 90% of their time indoors; however, surprisingly little is known about the health risks of indoor air, particularly for noncancer endpoints. Diverse symptoms are associated with contaminants from indoor air sources, but neither the determinants of these symptoms nor the long-term consequences of exposure are known. These factors have led EPA's Science Advisory Board (SAB) to rank indoor air as one of the top 5 health risks in comparative risk studies.

RESEARCH STRATEGY

To ensure that the Agency is equipped with scientific and technical data relevant to the formulation of sound environmental policy, ORD operates a research program founded on risk-based principles. In the area of health effects, the program explicitly conforms to the risk assessment paradigm described by the National Academy of Sciences (NAS) in 1983. This paradigm consists of 4 steps (hazard identification, dose-response assessment, exposure assessment, and risk assessment) that drive risk management decisions. NHEERL's research programs adhere to this risk-based strategy. In our Indoor Air Research Program, we are studying the predominant classes of biological and chemical indoor air contaminants suspected of causing adverse health effects. Our approach is (1) to characterize the determinants of allergic response to **BIOCONTAMINANTS**, and (2) to understand the relationship between exposure and effect for indoor **ORGANIC VAPORS**. The guiding philosophy of the program is to progress from identifying the health effects of concern to developing methods that measure effects to producing dose-response models that relate exposure and response.

NHEERL INDOOR AIR RESEARCH FY95 PROGRAM HIGHLIGHTS

BIOCONTAMINANTS RESEARCH (pg 6)

The goal of this research is to characterize the health risks posed by indoor allergens, with special emphasis on the house dust mite.

- *Scientists in NHEERL provided the first direct evidence of a link between NO_2 and asthma. Using a rodent model of dust mite-induced asthma, NO_2 was found to exacerbate various biochemical events associated with immune response and to produce clinical measures of asthmatic symptoms (decreased lung function).*

ORGANIC VAPORS RESEARCH (pg 9)

The goal of this research is to develop methods that measure the health effects of indoor organic vapors and to apply these methods to the study of potentially susceptible subpopulations.

- *Our published findings on the health effects of carpet emissions contradicted reports by a commercial testing firm showing severe signs of toxicity in mice. We replicated the tests conducted by the commercial firm and demonstrated that the carpet emissions caused no significant adverse effects in test animals.*

- *A NHEERL-sponsored workshop identified promising methods for assessing the neurotoxic health complaints associated with indoor air exposures.*

- *We identified a subpopulation of humans with alleged increased susceptibility to the effects of indoor air pollution (individuals reporting Multiple Chemical Sensitivity, or MCS). A detailed evaluation of the health of these individuals in the unexposed state was completed. This cohort will be compared to other groups in upcoming indoor air studies.*

INDOOR AIR BIOCONTAMINANTS RESEARCH PROGRAM

NHEERL defines ***biocontaminants research*** as research that leads to a better understanding of the relationship between biocontaminants and associated health effects, including the extent to which biocontaminants and other pollutants may interact to alter the health risk posed by either individually.

ISSUE

What are the health risks posed by indoor biological contaminants, or allergens? What is the involvement of cofactors in allergic response?

Asthma morbidity and mortality are on the rise in the United States and other industrialized countries. Mounting evidence, led by epidemiology studies, suggests that indoor allergens are involved in causing or exacerbating asthma. Of particular interest is the house dust mite, which is one of the most prevalent indoor allergens. These microscopic organisms are known to trigger allergic reactions; they also have been associated with asthma. Other pollutants, such as NO_2 or ozone, may exacerbate allergic response. For example, it has been shown that children in homes with gas stoves or kerosene heaters, which emit NO_2, have more respiratory illnesses than children in homes without these features.

PROGRAM DESCRIPTION

The purpose of this research program is to provide a clearer understanding of the health effects associated with indoor exposure to biocontaminants, including allergens (such as the dust mite), molds, and other microbials. The role of biocontaminants in causing and exacerbating asthma is a primary research focus. The research combines field, clinical, and laboratory studies to 1) develop quantitative tools to measure ***BIOLOGICAL RESPONSE*** to indoor allergens, and 2) determine the importance of ***COFACTORS*** in the exposure-dose-response relationship. The near-term goal is to resolve these issues for one of the most prevalent indoor allergens, the house dust mite. Dust mites were selected because significant indoor human exposures occur, the exposures have been associated with asthma, and the antigen can be measured quantitatively.

PROGRAM PROGRESS

BIOLOGICAL RESPONSES. To improve understanding of the biological effects produced by allergens, NHEERL is engaged in research that will lead to better measures of effect and more accurate models for predicting dose-response relationships. Our approach includes the development of animal models of allergic lung disease and clinical and field studies to evaluate human response to allergen challenge.

Animal models -- Because many important research questions are difficult to answer in humans, animal models for allergy to indoor biocontaminants are essential. Scientists in our Experimental Toxicology Division are developing two rodent models of allergen-induced asthma: the rat model, which has been under development for several years and is near completion, and the mouse model, which began its development in FY95.

Our rat model successfully mimics the pattern of allergy-induced asthma in

humans, exhibiting features such as eosinophilic inflammatory response, mucous secretion, and hyperreactivity to antigens leading to constriction of the airways (similar to the wheezing response in humans). The production of immunologic disease in our model was confirmed in FY94 when we demonstrated that the allergic state could be transferred to naive (nonallergic) rats. During FY95 we began to learn more about allergic response at the cellular level by using our rat model to characterize the events correlated with development of allergic disease. Specifically, we began to study cytokines. Cytokines are soluble factors produced by cells that modulate immune and inflammatory response. We are using PCR (polymerase chain reaction) techniques to detect the induction of a variety of cytokines, which helps explain the mechanism(s) involved in immune response and facilitates extrapolation of biochemical response to the disease state. It is anticipated that the rat model will be completed in FY96, and it will be used in subsequent years to characterize the effects and dose-responses for a variety of other biocontaminants.

We initiated a similar effort in FY95 to develop a model of allergen-induced asthma in the mouse, which offers the advantage of having a more clearly defined set of immunological parameters to measure. The development of this model will continue in FY96.

Human studies -- With humans, as with our animal models, the objective of our research is to better understand the allergic/inflammatory response to an indoor allergen challenge. In FY93, scientists in our Human Studies Division launched a clinical study of allergic asthmatics to characterize their response to the house dust mite antigen. The antigen is administered via nasal challenge (i.e., applied directly into the nose rather than breathed into the lungs). One of the responses, reported in FY94, is an increase in eosinophils, a blood cell that signals allergic response. We continued our nasal challenge studies in FY95 by evaluating whether the lower airways respond similarly to the upper airways. If a similar response to dust mite allergen is demonstrated in the lower airways, it will imply that the dust mite can increase allergen-induced morbidity in asthmatics.

This clinical study was coupled with a field study (also initiated in FY93) in which we collaborated with investigators at the University of North Carolina's Center for Environmental Medicine and Lung Biology to measure house dust mite antigen in the homes of allergic asthmatic children. We found that the degree of mite contamination in the homes did not account for differences in wheezing, bronchial hyperrreactivity, or lung function. During FY94, we shifted our focus from children to adults, where controlled inhalation exposures to allergens are feasible. Initial studies, using grass allergy as a prototype, verified that exposure to grass allergens can produce a substantial decline in pulmonary function in asthmatics. Future plans call for continued clinical research on allergic, asthmatic, and normal children to better define the biological response to allergen challenge.

COFACTORS. Although cofactors such as gender, age, and pollution are known or presumed to affect dose-response relationships, little is known of the involvement of these cofactors in indoor allergen-induced disease. We are testing, in laboratory animals and in humans, the hypothesis that exposure to ubiquitous pollutants, such as NO_2, O_3, and volatile organics, predisposes an individual to the development of indoor biocontaminant allergies and/or exacerbation of symptoms.

Laboratory animals -- In FY95,

NHEERL, in collaboration with the University of North Carolina, provided the first direct link beween NO_2 and asthma. Although exposure to NO_2 alone does not trigger an asthmatic attack, we demonstrated that it exacerbates asthmatic responses when administered to rodents sensitized with dust mite antigen. Various measures of immune response, including antibodies, allergen-activated lymphocytes, and inflammatory cells in the mucous, indicated enhanced sensitivity. Another critical observation was decreased lung function, which is a clinical measure of asthmatic symptoms. We are currently exploring the mechanism(s) by which NO_2 exacerbates allergic disease. Building upon what we have learned about cytokines in our rodent model, we have begun exploring cytokine responses in co-exposure studies. In this way, we hope to better correlate cellular response with the disease state.

Similar co-exposure studies are being conducted with ozone to examine its interaction with dust mite antigen. This research was initiated in FY94, and results thusfar indicate that ozone is not as potent as NO_2 in modulating immune response.

Human studies -- In FY93, we began an important study in humans to evaluate the interactions between indoor allergens and chemical air pollutants. Using nasal challenge, we exposed dust mite-sensitive asthmatics to ozone, a lung irritant. In FY95, we published several key findings. A comparison of the pulmonary responses of asthmatics and non-asthmatics showed that asthmatics appear to be more susceptible to ozone than normal, healthy individuals. Ozone seems to sensitize asthmatics to the effects of biological contaminants, predisposing them to a more severe allergic reaction. Moreoever, we found that asthmatics experience a different kind of inflammation (eosinophil-driven) than normal individuals (neutrophil-driven).

While the issue of asthma morbidity and mortality is far from resolved, our studies in both animals and humans indicate that interactions between common indoor pollutants may produce unexpectedly severe adverse effects.

INDOOR AIR
ORGANIC VAPORS RESEARCH PROGRAM

NHEERL defines ***organic vapors research*** as research that leads to a better understanding of the relationship between organic vapors and associated health effects, including the extent to which organic vapors and other pollutants may interact to alter the health risk posed by either.

ISSUE

Do indoor organic vapors pose a human health risk? If so, what is the magnitude of this risk and what factors influence risk? Exposures to organic vapors indoors are common, and many signs/symptoms have been attributed to such exposures. Predominant among these symptoms is sensory irritation. However, despite many apparent associations, the relationship between sensory irritation and exposure to organic vapors is unclear. Part of the difficulty in establishing relationships between exposure and effect results from the lack of adequate means to quantitatively measure effects. An additional difficulty is that not all individuals exposed to indoor pollutants are equally susceptible; allergics, asthmatics, and individuals who report multiple chemical sensitivity (MCS) may be at greater risk of adverse effects than the general public. To protect the health of individuals who may be most vulnerable to the effects of indoor pollutants, it is necessary to identify and study susceptible subpopulations and to understand the determinants of susceptibility.

PROGRAM DESCRIPTION

The primary emphasis of this research is to develop objective, ***QUANTITATIVE METHODS*** with which to measure the symptom-based effects of organic vapors. Our approach is to use laboratory and clinical studies to assess sensory irritation, neurotoxicity, immunotoxicity, and respiratory health effects associated with indoor exposures to organic vapors. As adequate assessment methods become available, they are enlisted to address questions regarding the toxicity of ***VAPOR MIXTURES*** and to evaluate responses in ***SUSCEPTIBLE SUBPOPULATIONS*** who may be at greater risk of adverse response than the general population.

PROGRAM PROGRESS

QUANTITATIVE METHODS. Using experimental animals and human volunteers, we are developing quantitative tools to measure the effects of organic vapors. Our laboratory is interested in sensory irritation--which manifests itself in the eyes, nose, and throat--as well as pulmonary, or lung, irritation. A collateral goal of our research is to understand and describe the neurological and immune components of these responses, which often defy detection by conventional assessment means.

Experimental animals -- During FY94-FY95, NHEERL successfully tackled a politically charged indoor air problem. A commercial animal-testing firm claimed that it had scientific evidence to validate anecdotal accounts of adverse health effects resulting from exposure to carpet emissions. The testing facility stated that it had found an association between carpet emissions and severe signs of toxicity (heart and nervous system conditions) in

mice. In FY94, NHEERL initiated an effort to replicate the findings described in the report. In a study marked by extensive scrutiny from both the scientific community and the public, NHEERL and other ORD scientists characterized the chemistry and microbial emissions from three "problem" carpets. Following a carefully controlled experimental paradigm designed to replicate the procedures used by the private firm, we were able to demonstrate that exposure of mice to these emissions did not result in any significant adverse effects. In FY95, we published our findings, and the controversy abated.

NHEERL is also interested in identifying promising experimental approaches to assess the complaints of neurotoxicity associated with indoor exposure to organic vapors. To this end, we sponsored a workshop in FY95 to provide scientific input to EPA's research planning process with regard to the development of animal models of nervous system vulnerability to indoor air pollutants. Workshop participants discussed experimental design schemes and susceptibility and offered recommendations for future research efforts. Their recommendations included the development and use of animal models of susceptibility to indoor air, the identification of objective outcome measures for human health consequences, and evaluation of mechanisms involved in susceptibility.

Human volunteers -- Through clinical studies, our laboratory is attempting to derive quantitative methods for assessing sensory irritation and to understand the relationships among the assessment measures. In FY94, we invented a device (the video corneascope) to assess eye irritation that measures, noninvasively, tear film break-up time. We also developed a quantitative, computer-based method for estimating eye redness. These objective measures of eye irritation are now being applied to other research studies in which irritancy is an outcome.

In addition to being uncomfortable, irritation may interfere with performance and productivity. To address this issue, we initiated studies in FY94 to evaluate the impact of sensory irritation on task performance in humans. Two types of studies are being performed. In one, we are producing sensory irritation with electrical stimulation, and in the other we are using noise as a surrogate for irritation. The effect of both stressors on performance is being assessed. Preliminary experiments were completed in FY95, and data are undergoing analysis.

VAPOR MIXTURES. The goal of this research is to develop methods that predict the critical features of mixtures that contribute to the adverse health effects from indoor air. Our intent is to better understand the mechanisms of mixtures toxicity. Previous work by our Laboratory (prior to 1993) showed that a mixture of organic vapors could produce subjective responses of eye, nose and throat irritation in normal healthy males; we also showed, for the first time, that exposure to a vapor mixture could produce objective signs of inflammation in the nose. In FY93, our data showed that perception of odor and sensory irritation were independent of one another, and that eye, nose, and throat irritation were independent from one another. These differences suggested that dissimilar mechanisms were at work, and that the onset of one type of sensory irritation did not presuppose another. In FY94, we collaborated with investigators at Yale University to study the factors involved in these responses by examining whether mixture components behave additively or synergistically with regard to sensory irritation. The results, published in FY95,

suggest that the degree of additivity among components of a vapor mixture varies from sub- to supra-additivity, and the factors affecting response may include carbon-chain length, lipid solubility, and the number of compounds comprising the mixture.

SUSCEPTIBLE SUBPOPULATIONS. Among those who may be especially sensitive to the effects of indoor air pollutants are individuals who report multiple chemical sensitivity (MCS). MCS is a condition described by individuals who allege that their health has been impaired by chemical exposure and that low-level exposures can trigger the adverse reactions. The goal of this research project is to identify a subset of individuals who claim to have MCS, to conduct careful clinical characterizations of the condition, and to utilize this experimental population in studies of response to indoor pollutants. During FY94, we collected data on persons reporting MCS symptomatology based on exposure history and symptoms, medical history and examination, psychiatric evaluation and a profile of medication, and psychological and physiological tests. During FY95, individuals who met the criteria for MCS were identified, and we completed a detailed evaluation of the health of these individuals in the unexposed state. In FY96 we plan to collect additional information that will help characterize the differences between this group and healthy individuals. It is anticipated that the MCS cohort will be used as a subgroup in a number of upcoming indoor air studies in which researchers will investigate responses, such as nasal irritation, of MCS and control populations to organic vapors and other indoor pollutants. These studies will help further define the factors contributing to MCS symptomatology.

National Health and Environmental Effects Research Laboratory

GLOBAL CLIMATE CHANGE

HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH

ANNUAL REPORT

MAY, 1996

CONTENTS

INTRODUCTION

PURPOSE

The purpose of this report is to communicate results from the Global Climate Change Research Program of the EPA's National Health and Environmental Effects Research Laboratory (NHEERL).

CONTENT

The report contains

- a summary of the Health and Environmental Effects Research Program for Global Climate Change, which describes the regulatory and programmatic context of NHEERL's research program, the overall program goal, the rationale for the program, and the research approach

- a section which highlights recent key findings (FY95 Program Highlights)

- a more detailed description of the NHEERL Global Climate Change Research Program, by program area, including a summary of research accomplishments and anticipated progress for the near future

COMMENTS WELCOME

The format of this report is still evolving, and we welcome feedback. Readers with comments, suggestions, questions, or requests for further information are encouraged to contact

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GLOBAL CLIMATE CHANGE RESEARCH PROGRAM SUMMARY

REGULATORY AND PROGRAMMATIC CONTEXT

As a result of the Global Change Research Act of 1990, a comprehensive government-wide program (the U.S. Global Change Research Program, or USGCRP) was instituted to improve our ability to understand and respond to global change. EPA supports this effort through its Global Change Research Program. Several EPA offices, including the Office of Research and Development (ORD) in cooperation with the Office of Policy, Planning and Evaluation (OPPE) and the Office of Air and Radiation (OAR), are assessing the ecological impacts of climate change to inform the policy-making process.* NHEERL is contributing to this effort by conducting research to characterize the effects of global climate change on ecological resources.

**EPA is also a member of the Intergovernmental Panel on Climate Change (IPCC). In October of 1995, this United Nations-sponsored group released a report acknowledging for the first time a link between human activities and climate change and noting the vulnerability of ecosystems and human health to such changes.*

PROGRAM GOAL

To provide information for policy makers that will reduce the scientific uncertainties surrounding the nature, rate, and magnitude of ecological response to global climate change.

RATIONALE

Levels of greenhouse gases in the atmosphere are irrefutably on the rise. Atmospheric CO_2, for example, has increased 13% in less than 40 years. There is prevailing scientific agreement that the continued accretion of greenhouse gases will lead to significant changes in climate-driven conditions, such as rises in temperature and sea level, shifts in the composition and geographic distribution of ecosystems, and adverse human health effects. However, uncertainty remains regarding the rate and magnitude of the projected changes, as well as the processes that regulate or modulate change.

RESEARCH STRATEGY

To ensure that the Agency is equipped with scientific and technical data relevant to the formulation of sound environmental policy, ORD operates a research program founded on risk-based principles. In the area of health effects, the program explicitly conforms to the risk assessment paradigm described by the National Academy of Sciences (NAS) in 1983. This paradigm consists of 4 steps (hazard identification, dose-response assessment, exposure assessment, and risk characterization) that drive risk management decisions. For ecological effects, the research program follows the framework for ecological risk assessment developed by EPA in 1992, consisting of problem formulation, analysis (characterization of exposure and effects), and risk characterization. NHEERL's research programs adhere to these risk-based strategies. Three types of research activities are emphasized:

- Research in the area of hazard identification, or ***problem formulation***, focuses on the development of methods that can provide evidence of an association between exposure and effects.
- Research characterizing dose-response seeks to identify and describe the events linking exposure to effects. These events, referred to here as ***determinants of effect***, form the basis for the predictive models used to quantify risk.
- There also may be instances when scientific data on a particular contaminant or

stressor are required. In such cases, short-term ***problem-specific*** research is conducted to systematically collect and analyze information that will fill specific gaps in knowledge.

To characterize response to global climate change, NHEERL has established three research areas that follow the precepts discussed above. In the area of **PROBLEM FORMULATION**, we are assessing the ecological effects of climate change and are identifying early warning signs to alert us to ecological decline. Our **DETERMINANTS OF EFFECT** research is designed to evaluate the events responsible for the interactions that occur between ecosystems, atmospheric CO_2, and climate change, and we are developing and utilizing predictive models to describe the dynamics of global change. Finally, **PROBLEM-SPECIFIC STUDIES** have been conducted to provide qualitative and quantitative data on the adverse health and environmental effects caused by the depletion of stratospheric ozone.

GLOBAL CLIMATE CHANGE RESEARCH FY95 PROGRAM HIGHLIGHTS

PROBLEM FORMULATION (pg 8)

The goal of this research is to improve our ability to identify and foresee the ecological consequences of global climate change.

- *Our studies of a fish species (*Rivulus marmoratus*) common to mangrove ecosystems indicated that its distribution may serve as a biological indicator of global climate change.*

- *A technique for determining thermal requirements for freshwater fishes was developed and utilized to demonstrate that redistribution of fish species may represent a major impact of climate change.*

- *Scientists from our Western Ecology Division were invited to lead two international scientific teams responsible for preparing key chapters in the report released this year by the IPCC.*

DETERMINANTS OF EFFECT RESEARCH (pg 11)

The goal of this research is to better understand the factors that influence climate change, atmospheric CO_2 concentrations, and ecosystem vitality and productivity.

- *As one of the major accomplishments of the entire U.S. Global Change Research Program, our Atlantic Ecology Division, in collaboration with extramural scientists, showed that the net loss of carbon from the terrestrial biosphere during the late 1980s may be close to zero, indicating that carbon losses due to deforestation must be offset by carbon accretion elsewhere in the terrestrial biosphere. Following the Mt. Pinatubo eruption in 1991 and a transient period of global cooling, the biosphere became a net sink for CO_2.*

- *Along with extramural collaborators, we demonstrated that atmospheric concentrations of CO_2 are modulated by changes in the atmospheric supply of iron to remote ocean areas; limitations in aeolian flux of iron constrain the growth of phytoplankton, thereby reducing marine CO_2 uptake.*

- *We used our predictive models to show that over the next 100 years, increasing amounts of CO_2 could be added to the atmosphere due to accelerated changes in the distribution of global vegetation.*

PROBLEM-SPECIFIC STUDIES (pg 14)

The goal of this research is to investigate the effects of stratospheric ozone depletion.

- *We showed that pigmentation in humans, which safeguards against sunburn and skin cancer caused by UV exposure, does not protect against immune suppression; however, some types of sunscreen are effective in mitigating suppression of the immune system.*

- *Whereas studies using greenhouses and growth chambers had shown that UV-B had significant effects on rice production, our studies of rice grown under realistic field conditions (with a higher UV-A to UV-B ratio believed to stimulate cell repair) demonstrated that UV-B does not pose a major risk to crop yield.*

GLOBAL CLIMATE CHANGE PROBLEM FORMULATION RESEARCH PROGRAM

NHEERL defines ***problem formulation research*** as research to demonstrate a causal connection between exposure and effect.

ISSUE

What are the enduring ecological consequences of global climate change? Are there early warning signs in the ecosystem that presage change?

Changes in the global climate have begun to have a significant impact on ecosystems. For example, rising water temperatures resulting from global warming are responsible for coral dieback and bleaching in the tropics. Although our knowledge regarding the impact of climate change is improving, our understanding of effects is limited. Changes that naturally or seasonally occur within an ecosystem can confound our ability to discriminate between effects that are short-term and those that are more enduring, and research is needed to clarify these differences. In addition, indicators are needed to alert us to impending ecological decline. Bioindicators quantify the health of a population or ecosystem and can help diagnose the most probable cause(s) of the observed effects.

PROGRAM DESCRIPTION

To characterize ecosystem response to climate change, NHEERL has established two complementary areas of research. We are conducting ***ECOLOGICAL EFFECTS*** research to evaluate the protracted response of terrestrial and aquatic ecosystems to climate variability and change, and we are developing methods that hasten detection of the effects of global climate change, known as ***INDICATORS*** research. Indicators research is an emerging field of interest and one that represents an area of growth for NHEERL. At present, we are evaluating biological indicators that possess a high degree of sensitivity and specificity for ecosystem damage. The goal is to identify and validate relevant measurements that can serve as prognosticators of change.

PROGRAM PROGRESS

ECOLOGICAL EFFECTS. One of the ways in which we are characterizing the abiding effects of climate change on ecosystems is through experimental assessments of forest productivity. Forest productivity is a gauge of *terrestrial ecosystem* health and is measured in terms of ecophysiological responses, such as the rate of tree growth, death, and replacement. The life stage most vulnerable to climate change is seedling establishment; therefore, we have selected Douglas fir tree seedlings for our studies of productivity. We are exposing the seedlings to elevated levels of CO_2 (at varying temperatures) in controlled environmental chambers maintained by our Western Ecology Division for studying the effects of environmental stresses on plant and soil ecological processes. These chambers, collectively referred to as the Terrestrial Ecophysiological Research Area (TERA), measure plant shoot, plant root, and litter/soil processes in real time. Our research is unique in that the effects of changes in both CO_2 and temperature (as well as other variables, such as moisture) can be studied simultaneously. Studies were initiated in 1993, at which time the seedlings were planted and measurements of survival, growth, and CO_2 uptake were begun. In FY95, we reported that as temperatures rise (along with enhanced CO_2), there is an increase in soil respiration with an accompanying release of CO_2 into the atmosphere. Because this release is greater

than the uptake of CO_2 by the plant during growth, there is a net increase in atmospheric CO_2. This result suggests that increases in temperatures will exacerbate climate change by increasing atmospheric CO_2. Increases in temperature also were shown to cause asynchronous tree growth; trees "break bud" at an irregular rate when temperatures increase, and the result is a distorted, deformed tree. These chamber studies are due to be completed in FY97, at which time the trees will be harvested.

In a related vegetation study, NHEERL scientists, working in cooperation with scientists from the Desert Research Institute in Reno, NV, conducted a year-long investigation beginning in FY93 on the effects of elevated CO_2 (in combination with nitrogen fertilization) on the growth of the ponderosa pine. Scientists used a miniaturized camera system to study root proliferation and asssociated fungal structures. In FY95 we published our findings. We reported that, contrary to some assumptions, higher levels of atmospheric CO_2 enhanced seedling growth, rooting depth, intensity of rooting and fungal colonization. Apparently, CO_2 stimulates processes in the rhizosphere (the root/soil complex) that enable the plant to gain access to additional nutrients. Our data support accumulating evidence suggesting that processes in the rhizosphere are key to the response of vegetation to stress (see "Indicators of Climate Change" below).

We are characterizing the effects of climate change on *freshwater ecosystems* by studying shifts in the composition and/or geographic distribution of aquatic species. We are attempting to predict such shifts using estimates of the tolerance of aquatic organisms--specifically, freshwater fishes--to changing habitat conditions, such as rising water temperatures. Researchers in our Mid-Continent Ecology Division developed a technique for determining the thermal requirements for freshwater fishes based on available field measurements, and then utilized this technique to evaluate the potential effects of rising water temperatures on the distribution of 57 species of freshwater fish. Our results, published this year, suggest that redistribution of fish species may emerge as a major impact of climate change. We found that temperature shifts predicted by a doubling of atmospheric CO_2 would result in a 50% reduction in the existing habitat for cold- and cool-water fishes. EPA's Office for Policy, Planning and Evaluation used these findings to estimate losses in revenue spent on sport fisheries in U.S. streams of up to $320 million annually.

Also, we are examining *coastal ecosystem effects* of climate change. Scientists in our Atlantic Ecology Division are addressing future ecological vulnerabilities along the U.S. Atlantic coast in a project called "coastal futures." The goals of our coastal futures research are to anticipate the environmental implications of human demographic and climate-related changes along the Atlantic coast and to reduce key uncertainties regarding the ecological risks associated with these changes. Currently, research is being conducted to address the combined effects to coastal ecosystems of projected increases in nutrient loading resulting from coastal zone development and increases in temperature resulting from global warming. This research will contribute to advances in regional and state level vulnerability assessments.

INDICATORS OF CLIMATE CHANGE. NHEERL is conducting research to identify biological indicators that may herald ecosystem vulnerability to climate change. In cooperation with researchers at the University of Miami, scientists in our Gulf Ecology Division are studying a coastal zone habitat--the mangrove ecosystem--to identify aquatic indicators of climate change. Mangrove habitats buffer insular and continental coastlines, and their response to climate-induced changes could influence future coastal zone environments. The mangrove ecosystem, which is endemic to the central

Florida coast, was identified in the IPCC report as an ecosystem particularly at risk. We are studying this model coastal wetland at different levels of scale (species, habitat, and landscape) to enhance our ability to identify ecosystem processes that may presage climate change. We have begun by studying a fish species (*Rivulus marmoratus*) common to mangrove ecosystems to determine whether it can be used as a surrogate for mangrove ecosystem sensitivity. During FY95, we reported that the distribution of this fish may serve as a biological indicator of global climate change. These findings, along with those generated at the habitat and landscape levels, will be used to develop models that address extrapolations of scale, i.e., how effects compare as one moves from species to habitats to landscapes.

GLOBAL CLIMATE CHANGE DETERMINANTS OF EFFECT RESEARCH PROGRAM

NHEERL defines ***determinants of effect research*** as research to identify and describe the bases for the health and/or environmental effects caused by exposure to environmental stressors or chemical contaminants.

ISSUE

What factors control, temper, or regulate the ecological effects caused by climate change? Levels of atmospheric CO_2 and other greenhouse gases are on the increase, driving a global-scale climate change that is stressing ecosystems. Ecosystems respond to climate-induced stress in a variety of ways, some of which further impact CO_2 concentrations. For example, increases in CO_2 are known to stimulate plant growth, increasing the sequestration of carbon by the biosphere. However, other factors, such as tree mortality, offset these processes and drive CO_2 back into the atmosphere. Until the fundamental bases for the ecological effects of climate change are understood and the processes affecting the flux of CO_2 between the biosphere and the atmosphere are explained, substantial uncertainties will remain with regards to climate change projections.

PROGRAM DESCRIPTION

The objective of this research program is to achieve a better understanding of the interactive processes linking climate change, atmospheric CO_2 (and other climate-induced stressors), and ecosystem response. We are especially interested in ascertaining the ways in which ecosystems modulate atmospheric CO_2 and thereby accelerate climate change. To achieve our objective, we are conducting ***MECHANISTIC RESEARCH***, in which we are analyzing the events that regulate these interactive processes, and we are developing ***PREDICTIVE MODELS*** that integrate information on the changing features of ecosystems, atmosphere, and climate to explain the dynamics of change. Much of our research focuses on the factors that control the flux of carbon, a surrogate for tracking greenhouse gases, through the global system. Our research is helping to identify major sources and sinks for greenhouse gases and is closing critical scientific gaps in our understanding of the global carbon budget, which will increase scientific confidence in climate change predictions.

PROGRAM PROGRESS

MECHANISTIC RESEARCH. *Marine ecosystems* represent an important component of the global carbon cycle. To explain their role in modulating atmospheric CO_2, we are studying factors (and associated mechanisms) that influence the oceanic uptake of CO_2. One factor is phytoplankton, which utilize CO_2 for growth and thereby remove it from the atmosphere. It is believed that growth of phytoplankton is constrained in several open ocean areas by low levels of iron flux from the atmosphere. Our Atlantic Ecology Division along with extramural collaborators have tested this hypothesis to determine whether changes in iron flux in the ocean in these remote areas modulate marine production and, consequently, atmospheric CO_2 concentrations. This year, we reported that carbon fixation in major regions of the open sea is indeed limited by low aeolian flux of iron; therefore, past and future changes in iron flux to the ocean surface may affect atmospheric concentrations of CO_2.

NHEERL also is conducting mechanistic research on *terrestrial ecosystems* by studying the movement and sequestration of

carbon in soils and in the rhizosphere of plants (the root/soil complex). Specifically, scientists in our Western Ecology Division are examining processes that occur within the rhizosphere to affect plant growth, metabolism, and carbon utilization. During FY93 and FY94, we used a unique culturing system to quantify the uptake and allocation of carbon by conifer (ponderosa pine) seedlings. We were among the first to directly measure carbon flux through all major pools of the rhizosphere. In FY94, we reported that the symbiotic associations that exist between the root system and its associated fungi have a profound effect on the cycling rate and size of forest carbon pools. This infers that the role of forests in the global carbon cycle would be altered world-wide if these rhizospheric associations were affected by global climate change. At the regional scale, these associations could be affected by other anthropogenic stressors, such as tropospheric ozone. We therefore initiated a project in FY94 to obtain a mechanistic understanding of rhizosphere response to stressors using tropospheric ozone as the test agent. In FY95 we demonstrated that ozone alters the movement of carbohydrates in ponderosa pine seedlings, often reducing the amount of carbohydrate allocated to roots and their associated fungi. As a result, the seedlings are more susceptible to nutrient and moisture stress. These findings collectively suggest that perhaps the most significant mechanism for carbon release in plants exists within the rhizosphere. Moreover, anthropogenic stressors such as ozone may magnify the effects of increases in temperatures and regional drought predicted to result from climate change.

Chemistry methods developed by extramural collaborators of our Atlantic Ecology Division are improving our understanding of the processes involved in CO_2 cycling and are enabling us to more precisely estimate trends in biologically mediated carbon fluxes. In the past eight years, carbon measurements have been made at a growing network of monitoring stations, with joint support from EPA and the National Science Foundation. The results suggest that in the late 1980s, the net loss of carbon from the terrestrial biosphere may have been close to zero, and that following the eruption of Mt. Pinatubo in 1991, the biosphere became a net sink for CO_2. It has been postulated that following the Mt. Pinatubo eruption, increased iron fluxes to the Southern Ocean stimulated marine production, and the subsequent transient global cooling during the next few years reduced respiratory CO_2 losses from the terrestrial biosphere. Post eruption, the rate of atmospheric CO_2 increase slowed dramatically as a result of these biospheric responses. This finding was recognized as one of the major accomplishments for the entire U.S. Global Change Research Program in FY95.

PREDICTIVE MODELS. NHEERL is actively engaged in the development of global climate models that improve our ability to extrapolate experimental findings to real life situations. Whereas current climate models treat the biosphere as a static system, we are pioneering the development of more comprehensive and dynamic models that integrate information on changing features of the biosphere, the atmosphere, and climate to more accurately describe the associations between ecosystem structure, carbon content, vegetation, hydrology, and atmospheric composition.

Since 1992, scientists in our Western Ecology Division have been involved in the development of models that simulate the *transient response* of terrestrial ecosystems to climate change. A key feature of these models is their ability to integrate the time lag between climate change and response. We simulated the lags associated with forest dieback, migration, and regrowth to predict the timing and nature of forest response to climate change. In FY95 we published our findings, which revealed that over the next 100 years, increasing amounts of CO_2 could

be added to the atmosphere due to accelerated changes in the distribution of global vegetation. Redistribution of vegetation will likely be driven by forest dieback and wildfires. We also developed and applied another model that simulates the transient effects of delayed migration of species and ecosystems under rapid climate change. During FY95, results from our model projected decreases in the diversity of tree species as changes in climate and land use accelerate. This suggests that most of the species that dominate (and hence control) ecosystem integrity are vulnerable to dramatic reductions in number over the next 50 to 100 years.

We also are developing models that simulate *spatial responses* of terrestrial ecosystems to climate change, e.g., geographical shifts in vegetation. From these models we are able to predict changes in terrestrial carbon storage and displacement. This year, we developed spatial data on the distribution of young to late secondary forests in tropical forested regions; these data will be used to describe how carbon requirements change as vegetation is redistributed. We also applied spatial analysis techniques to describe factors influencing ecosystem vitality and productivity. We combined regional vegetation models with estimates of predicted human use of forest resources and identified regions and timber products vulnerable to climate change. Much of this research was conducted in support of the IPCC. Finally, we developed and published in FY95 a method for measuring and mapping uncertainty in spatial simulation model results; this method improves our ability to extrapolate experimental findings to ecological assessments.

An important issue largely ignored by current climate models is the *linkage* that exists between terrestrial and aquatic ecosystems. We are conducting hydrological modeling to relate terrestrial ecosystem structure and function with aquatic community characteristics. The goals of this research are to predict the response of the hydrological cycle to climate-induced changes in precipitation and to understand the interactions between hydrological systems and regional vulnerabilities. We are collecting and mapping information on soil erosion, sediment runoff, and nutrient transport to evaluate the impact of hydrological change on the terrestrial landscape. In FY95, these data were entered into models that simulate regional moisture flow through terrestrial ecosystems and into streams and rivers. Our results will be used to project habitat changes and to help explain the dynamics that exist at the interface between terrestrial and aquatic ecosystems.

Delineating geographic areas of ecological similarity based on multiple landscape characteristics has become a powerful tool for evaluating patterns of regional ecological response and for extrapolating observations from individual sites to regional scales. This approach was developed by a NHEERL scientist and is referred to as "Omernik ecoregions." In FY95, we continued to refine this approach and applied our techniques to develop a classification for the western corn belt. These ecoregion classifications advance the scientific basis for evaluations of response to global climate change.

GLOBAL CLIMATE CHANGE
PROBLEM-SPECIFIC STUDIES RESEARCH PROGRAM

NHEERL defines ***problem-specific studies research*** as research to describe the nature and magnitude of the effects induced by a specific environmental perturbation or chemical contaminant.

ISSUE

What are the adverse effects associated with the depletion of stratospheric ozone?
Stratospheric ozone shields the earth from ultraviolet (UV) radiation, thereby providing protection against possible health and ecological effects. As levels of stratospheric ozone decrease, the integrity of this protective atmospheric layer is threatened, increasing the potential for UV exposure. Some of the adverse effects resulting from UV-B exposures are well known, such as skin cancer. However, other effects, such as the suppression of immune function, are not clearly understood. Moreover, many uncertainties remain with regard to ecological responses to increased levels of UV.

PROGRAM DESCRIPTION

NHEERL's stratospheric ozone research program seeks to resolve key questions surrounding the ***HUMAN HEALTH*** and ***ENVIRONMENTAL EFFECTS*** from exposure to excess UV radiation. We have studied suppression of human immune function, ocular effects, effects on crop yields, the effect on the oceanic food chain, and possible synergistic effects between UV radiation and chemical toxicity. This research supports the periodic effects assessments required by the Montreal Protocol and provides data to the Program Office to explain the implications of ozone depletion.

PROGRAM PROGRESS

HUMAN HEALTH EFFECTS. The goals of these research efforts were to assess the impact of UV radiation on the human immune system and to characterize the health implications of immune suppression. Using a variety of experimental techniques, we evaluated whether the effects to the immune system from UV-B exposures are local or systemic, and we evaluated the capability of sunscreens and Vitamin A to protect against suppression of immune function. Our research to date has shown that UV-B does indeed suppress human immune function and that the suppression is systemic in nature. Additional studies in humans conducted this year demonstrated that pigmentation, which is a safeguard against sunburn and skin cancer, does not protect against immune suppression; however, some types of sunscreen are effective in mitigating suppression of the immune system.

To enhance our understanding of the immunological effects of UV radiation, we developed animal models to study immune function. We identified a strain of laboratory mice with mechanisms of immune supression similar to those found in humans and used this animal model to study the effect of immune suppression on disease susceptibility. In FY94 we found that several types of infection were altered by exposure to UV-B, and in FY95 we demonstrated that a laboratory diet supplemented with Vitamin A provided some protective effect against UV-induced immune suppression.

We also have conducted field studies in southern Chile, an area that offers a unique opportunity to study the health effects of increased UV exposure due to its location near the seasonal Antarctic stratospheric

ozone "hole". During FY95, we reported that it is possible to perform standardized ocular, dermatologic, and immunologic testing in the field. Despite anecdotal reports of blind sheep, our studies of ocular effects in animals found no short-term effects produced by UV exposure.

ENVIRONMENTAL EFFECTS. During FY95, NHEERL scientists resolved a critical question regarding the effects of enhanced UV radiation on the production of rice, the world's most important crop species. Previous studies by others using greenhouses and growth chambers had shown that UV-B had significant effects on rice production. However, research conducted this year by NHEERL found no such effects when rice was grown under realistic field conditions. This suggests that field-grown rice is less susceptible to the effects of UV-B (possibly due to higher UV-A to UV-B ratios, which stimulate cell repair). Consequently, increased UV-B does not appear to pose a major risk to rice yield.

Marine phytoplankton are at the base of the oceanic food chain. Reductions in their productivity could have major impacts on the structure and/or function of marine ecosystems. Phytoplankton are especially vulnerable to increases in UV-B because they inhabit the surface zones that are highly exposed to UV radiation. During FY95, NHEERL scientists found that ambient levels of UV-B may inhibit the light harvesting efficiency and production of phytoplankton in clear waters typical of the open sea. Shipboard experiments were conducted on Antarctic phytoplankton during the period of the ozone "hole" to assess maximum UV-B impacts under field conditions. Our results, which are currently in press, indicate that increased UV-B alters the pigment composition of phytoplankton. These findings support laboratory-based data. However, because the effect is mitigated by cloud cover and mixing of phytoplankton in the water column, direct application of laboratory-derived dose-responses to the Antarctic may overestimate effect. To assess the sensitivity of different phytoplankton physiological processes to UV-B, we tested the effects of UV-B on nitrogen uptake. We reported this year that ecological assessments based solely on reductions in carbon fixation without consideration of nitrogen may underestimate the extent of damage to phytoplankton.

Finally, in studies of the effect of sunlight on the toxicity of chemical compounds, we reported during FY95 that the toxicity of some aromatic hydrocarbons can be increased by several orders of magnitude in the presence of sunlight. UV light activates some of these compounds such that toxicity increases directly with increases in light intensity and energy. Quantitative Structure-Activity Relationship (QSAR) studies are permitting us to predict the relationship between the structure of a chemical and the extent to which sunlight increases its toxicity.

National Health and Environmental Effects Research Laboratory

CRITERIA AIR POLLUTANTS: PM_{10}

HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH

ANNUAL REPORT

NOVEMBER, 1995

CONTENTS

INTRODUCTION

PURPOSE: The purpose of this report is to communicate results within EPA from the Office of Research and Development (ORD) Particulate Matter (PM_{10}*) Health and Environmental Effects Research Program.

CONTENT: The report contains (1) a summary of the Health and Environmental Effects Research Program for PM_{10}, which describes the ORD context of NHEERL's research program, the overall program goal, the rationale for the program, and the research approach; (2) a section that highlights some recent key findings (FY95 Program Highlights); and (3) a more detailed description of the NHEERL PM_{10} Research Program, by program area, including a summary of research accomplishments and anticipated progress for the near future.

COMMENTS WELCOME: The format of this report is still evolving, and we welcome feedback. Readers with comments, suggestions, questions, or requests for further information are encouraged to communicate them to Ila Cote, Assistant Director, National Health and Environmental Effects Research Laboratory (MD-51A), U.S. EPA, Research Triangle Park, N.C. 27711. [Phone: (919) 541-3644 or FAX: (919) 541-0642]

* *PM_{10}* refers to the most respirable air particles--those 10 micrometers and smaller.

PM_{10} HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH PROGRAM SUMMARY

EPA/ORD CONTEXT OF NHEERL'S PM_{10} RESEARCH PROGRAM: Sections 108-109 of the Clean Air Act (CAA) require EPA to periodically review and revise National Ambient Air Quality Standards (NAAQS) for ambient air pollutants, including particulate matter (PM). EPA's Office of Research and Development (ORD) provides scientific support for this process. ORD's PM_{10} research program, which is based upon a peer-reviewed PM_{10} strategy, is designed to reduce uncertainties in risk assessment and to provide sound scientific data to guide decision-makers in possible revisions of the standard. Four areas where substantial uncertainties exist have been identified by ORD and are the focus of its research efforts: health effects research, exposure research, source characterization, and risk assessment. This document summarizes the health effects research.

PROGRAM GOAL: To provide credible PM health effects data that reduce the uncertainties in risk assessment and thereby guide revisions of the PM standard.

RATIONALE: Recent epidemiological studies of urban populations have suggested that exposures to particulate matter at levels below the current NAAQS may lead to increased morbidity from pulmonary disorders and increased mortality from cardiopulmonary diseases and cancer. Age and pre-existing cardiopulmonary disease appear to be important factors in PM susceptibility. PM research is needed to provide a more cogent explanation of the public health burden incurred by exposure to particulate matter.

APPROACH: Research is being conducted to (1) improve **PROBLEM CHARACTERIZATION** by providing more detailed analysis of existing epidemiologic data and by initiating new epidemiologic studies utilizing more thorough assessments of particle composition and exposures; (2) evaluate **DOSIMETRY** (exposure-dose relationships) by measuring and modeling particle deposition in the lungs; and (3) investigate **CAUSAL MECHANISMS** by determining the role of PM composition, size, and physical properties on health effects.

NHEERL PM_{10} RESEARCH FY95 PROGRAM HIGHLIGHTS

Problem Characterization Research (pg 6)

The goal of this research is to improve our understanding of the epidemiological observations suggesting a relationship between increased mortality/morbidity and PM exposure.

> *▸We assembled a peer-review advisory panel of widely respected extramural scientists to provide epidemiological guidance to EPA investigators.*

Dosimetry Research (pg 8)

The goal of this research is to develop animal and human dosimetric models to better understand the role of particle size and pre-existing conditions on the health effects of PM and to facilitate animal-to-human extrapolation.

> *▸ We demonstrated that breathing pattern and airway resistance, not age or gender, are the most significant factors affecting particle depostion in the lung.*

> *▸We formulated an artificial lung lining fluid that simulates human lung lining fluid; it is being used to improve dosimetry estimates and to elucidate the mechanisms of oxidative stress in lung disease.*

Causal Mechanisms Research (pg 10)

The goal of this research is to explain the biological mechanisms that evoke the health effects associated with exposures to PM and its components.

> *▸ We found that the "fingerprint" of mutations induced by PM in human lung tumors differs according to the source of PM exposure.*

> *▸ We developed two rodent models of enhanced susceptibility to permit the evaluation of pre-existing inflammation as a risk factor in PM responsiveness.*

> *▸ We found that the amount of transition metal present on air particles is directly related to the severity of oxidative lung damage.*

> *▸ We showed that urban air particles are much more cytotoxic and inflammatory than natural particles, such as asbestos or silica.*

PM_{10} PROBLEM CHARACTERIZATION RESEARCH PROGRAM

NHEERL defines ***problem characterization research*** as research to identify and describe the health and environmental risks posed by exposure to environmental contaminants.

Issue

Do exposures to PM at levels below the current national standard produce increased mortality and morbidity? If so, what health effects are caused by PM and its components?

Recent assessments of epidemiological data have shown significant associations between various measures of ambient particulate matter and excess mortality and morbidity, raising serious concerns that exposure to PM at levels below the currently accepted standard may impose a heavy burden on human health. However, uncertainties in the data, such as limited characterizations of exposure and possible artifacts in the statistical methodology, have clouded our understanding of these observations.

Program Description

This research is designed to help EPA understand the relationship between PM exposures and health effects (mortality and morbidity) using more advanced biostatistics, more coherent mortality and morbidity measures, and improved characterization of exposures. Epidemiologists in NHEERL are collaborating with investigators in EPA's National Exposures Research Laboratory (NERL), the National Center for Evaluation and Assessment (NCEA), and other public and private organizations to ***re-analyze existing epidemiological databases*** using better exposure data and additional statistical applications and to ***construct additional morbidity/mortality data sets*** using improved exposure monitoring and biomedical data.

Program Progress

Re-analyze existing epidemiological databases. We are re-evaluating particle data collected by EPA from 1991-1992 using improved characterizations of exposure, and we are integrating these data with mortality figures for the same period. This new analysis will significantly enhance evaluations of the relationship between mortality and PM exposures.

Construct additional data sets. The objective of this research is to collect additional data for use in epidemiology studies of the health effects of PM. To support this

effort, we undertook a 10-year survey of size-specific ambient PM levels in 36 U.S. locations; the survey was completed this year. The PM data collected in this survey will be coupled with data on mortality and morbidity (lung function endpoints) to evaluate health effects relative to PM size.

New epidemiology studies, coordinated by NHEERL and jointly funded by Federal, State, and academic contributions, are underway. We assembled an advisory panel of expert extramural scientists to provide peer review and technical guidance for these studies, and the panel is due to submit a final report on NHEERL's epidemiology research plan in FY96.

Our multi-year cooperative venture on health research in China continues and is near completion. We have collected data on lung cancer mortality in Xuan Wei, China (where a high incidence of lung cancer has been observed) and are relating these health statistics to various measures of PM exposure. This analysis will improve our understanding of the relationship between lung cancer and PM. In another study underway in China, the relationship between PM and morbidity is being assessed using health endpoints such as pulmonary function and respiratory illness. During 1995, we were engaged in the sampling of PM in 4 cities of China with disparate levels of ambient PM exposures. Biomedical-demographic-socioeconomic questionnaires were administered to households taking part in the study, and lung function data were collected by performing spirometry tests in the children of participating households. This project, scheduled for completion in 1999, is being performed in collaboration with NCEA, which will assume responsibility for the remainder of its execution.

In cooperation with researchers in the Czech Republic, we instituted a multidisciplinary health research program in 1992 to document the relationship between human health effects and PM exposures in a heavily industrialized region of Eastern Europe. We are studying respiratory, neurobehavioral, reproductive, and genetic effects in the Teplice District, an area of exceptionally high levels of PM. Results generated this past year indicate an increase in the prevalence of respiratory symptoms in school children (compared to children living in less polluted districts); neurobehavioral performance in this same population of children was not found to be related to air pollution exposure. Studies are underway to characterize the relationship between PM exposures, exposure to carcinogenic constituents in the air, and biomarkers of dose and genetic damage.

PM_{10} DOSIMETRY RESEARCH PROGRAM

NHEERL defines ***dosimetry research*** as research that elucidates the relationship between exposure and dose at the site of toxicity.

Issue

What is the relationship between PM exposure, dose, and the effects observed in sensitive subpopulations? How do the effects observed in laboratory animals relate to human response?

Substantial uncertainties exist regarding the relationship between PM exposure, dose, and observed effects. It is believed that air flow in the lungs of healthy and susceptible individuals differs, thereby influencing the pattern of particle deposition in the lung. Particle deposition, in turn, determines dose and contributes to the effects of PM. Understanding deposition patterns and the clearance of particles from the lungs of healthy and susceptible individuals is critical for assessing the potential risks of PM. Of additional importance is the attendant development of mathematical or computational models to explain dose distribution in the airways and to link data collected in animals to humans. Of particular interest are dosimetric models for susceptible subpopulations; such models do not presently exist, in part because toxicology data on PM has been generated only in healthy animals.

Program Description

To improve our understanding of PM exposure-dose relationships, we are examining ***particle deposition and clearance*** in the lungs of humans and laboratory animals exhibiting normal and abnormal lung function. Both monodispersed aerosols (aerosols composed of particles of uniform size) and polydispersed aerosols (particles of varying sizes and more representative of real-world exposures) are included in our studies. An important component of our research is the effect of factors such as airway obstruction, age, and pulmonary disease on particle retention and deposition. Our findings are then used to develop ***dosimetric models*** to estimate dose distribution in the human lung. These models are helping us predict exposure-dose relationships in individuals with abnormal lung function and are assisting our extrapolation of animal data to humans.

Program Progress

Particle deposition and clearance. The objective of this research is to provide human exposure-dose data for use in risk assessment and to help relate this data to the effects observed at the site of toxicity. We are using particles differing both in size (ultrafine, fine, and coarse) and in size distribution (mono- versus polydispersed aerosols) to measure deposition dose in models of the human airway, in experimental

animals, and in human volunteers. Collectively, these research efforts will help us understand the distribution and clearance kinetics of PM in the human respiratory tract.

The *airway models* allow us to perform mathematical analyses of dose distribution and to study how particle distribution is influenced by changing conditions, such as airway obstructions, air flow rates, branching patterns, etc. In addition, we have developed an *in vitro* model in which we formulated an artificial lung lining fluid to simulate conditions in the human lung; results from this study are improving our dosimetry estimates.

Our *animal experiments* are helping to explain particle clearance kinetics. This year, we completed a pilot study in dogs in which we evaluated the retention of particles in the lung following intrabronchial deposition. Our research demonstrated complete clearance of particles from large airways within 24 hours, which confirms the conventional understanding of particle clearance kinetics.

Our *human studies* are elucidating differences in particle deposition between normal and abnormal lungs. We have been able to refine our research by developing a technique that can measure minor airways obstructions, which are ordinarily difficult to detect using conventional lung function tests. This year, we showed that the most significant factors affecting fine-particle deposition in individualss with normal lung function are breathing pattern and airway resistance. We are also studying the role of alveolar macrophages and cellular response in particle clearance, and these data are being analyzed. In studies of particle clearance kinetics in humans, we demonstrated this year that "inert" particles cause a significant inflammatory reaction in the human lung, lending credence to the theory that urban air particles may be potent inducers of lung damage and inflammation. We also have examined variability in particle deposition with respect to age (children vs. young adult vs. elderly) and found that age appears to have only minor effects on deposition. Over the next two years, we plan to study particle deposition in three additional susceptible population groups: heavy smokers, asthmatics, and persons with chronic obstructive pulmonary disease.

Dosimetric models. The objective of this research is to develop more realistic estimates of dose distribution in the human lung. Using the Cray supercomputer, we are incorporating the data obtained from the studies described above to modify and expand existing dosimetry models. The improved models are assisting predictions of exposure-dose relationships under a variety of conditions, such as obstructed airways and uneven ventilation. Our findings during the past year indicate that the total lung deposition of particles increases with airways obstruction, suggesting an enhancement of particle deposition in individuals with abnormal lung function. These models also will aid in the extrapolation of animal data to better define exposures of concern. As human clinical and animal studies progress and more data become available, the theoretical models will be validated and improved.

PM_{10} CAUSAL MECHANISMS RESEARCH PROGRAM

NHEERL defines ***causal mechanisms research*** as research to identify and characterize the physical, chemical and/or biological mechanisms whereby an environmental agent may induce health or environmental effects.

Issue

What are the causal mechanisms that can provide a biologic explanation for the epidemiologic observations of excess mortality and morbidity?

Despite human evidence linking low-level PM exposures with adverse health effects, no plausible biological mechanisms for the observed toxicity have been offered. Understanding causal mechanisms would help explain the recent findings relating PM toxicity to particle size and composition and would help account for variability in susceptibility to PM. The issue of susceptibility is important because sensitive subpopulations, including individuals with physiological abnormalities, pre-existing disease, or weakened physical condition, may be predisposed to unusual response, injury, or death when exposed to particles.

Program Description

Our research on causal mechanisms is focused in two areas. First, we are assessing the effects of ***particle size and composition*** on PM toxicity. *In vitro* methodology is being used to test specific mechanistic hypotheses of injury and response at the cellular level; experimental animals are being used to investigate the role of particle characteristics in lung inflammation and cancer; and human volunteers are being used to evaluate specific biochemical and physiologic events resulting from PM exposures. Two particle-associated agents purported to play a role in toxicity -- metals and organic matter -- are receiving special attention. Secondly, we are evaluating the role of ***susceptibility factors***, such as pre-existing lung disease, on response to PM. We are developing animal models of respiratory disorder and disease to represent sensitive human subpopulations and are using these models to study enhanced susceptibility to PM. Data also are being collected in humans with respiratory disease (e.g., asthmatics) to explain predisposition to the effects of PM.

Program Progress

Particle size/composition. *In vitro techniques* are being used to describe injury at the cellular level and to devise mechanistic explanations of toxicity. We are exposing lung cells and tissues (both animal and human) to a wide range of PM sources and sizes to test specific hypotheses regarding the relationship between toxicity and particle size/composition. Toxicity endpoints include oxidant formation, inflammation,

and DNA damage (discussed below under human studies). Results this year demonstrated that urban air particles are much more cytotoxic and inflammatory than natural particles, such as asbestos or silica. In addition to providing a database on the relative toxicities of particles from many sources, results from the *in vitro* studies will strengthen animal-to-human extrapolations.

We are conducting *animal studies* to investigate the role of surface-related metals in oxidant lung injury and inflammation and to evaluate the effect of particle-associated organics on the induction of cancer. In our studies of surface-related metals, we are exposing animals to particulate matter high in metal content (residual oil fly ash) and are measuring various physiological and biochemical indicators of damage, such as oxidant formation. We also have developed a method to adhere metals to inert particles to mimic the fly ash and have used these metal-coated particles in our animal studies. Our findings indicate that the amount of metal present on air particles is directly related to the severity of injury and that lung damage appears to occur through an oxidant mechanism. With regard to cancer, our studies have shown that PM contains organic matter that is carcinogenic in animals. We also have produced evidence that particle size may play a role in cancer induction. These experimental studies will serve as the basis for subsequent human studies.

Results from our *human studies* suggest that exposure to increasing levels of PM results in elevated levels of genetic damage (DNA adduct formation). To better characterize this relationship, we are developing biomarkers of PM exposure (urine metabolites and DNA adducts) and biomarkers of molecular effect (DNA adducts and mutations at the *k-ras* gene in human lung tumor samples), with complementary studies conducted in animals. By relating biomarkers of exposure to biomarkers of effect and further linking these data to PM_{10} exposure profiles, a better understanding of the sequence of events leading to health effects is being attained. Results have been promising thusfar. Our group published findings in 1994 suggesting that small particles may induce cancer via cell proliferation or other pathways not involving the formation of DNA adducts. Within the past year, we have shown that the spectrum of mutations at the *k-ras* gene from lung tumors differs depending on the source of PM exposure, indicating the possible involvement of different mechanistic pathways.

<u>Susceptibility Factors</u>

Due to the apparent strong association between pre-existing cardiopulmonary disease and excess mortality, we are developing *animal models* of emphysema, pulmonary and systemic hypertension, asthma, and infectious disease to represent sensitive human subpopulations. This year, two rodent models of enhanced susceptibility (pulmonary hypertension and pre-existent inflammation) were developed to help explain the risk factors involved in PM responsiveness. Studies of *sensitive human subpopulations*, such as asthmatics and persons with chronic obstructive lung disease, are just getting underway. Individuals will be exposed to ambient particles, and we will measure indices of lung function and cardiovascular function.

Development of measurement techniques. The objective of this research is to develop a continuous monitoring method to measure deposition of polydispersed aerosols in the lung using laser optics adapted for inhalation studies. This technique will permit a more accurate assessment of particle inhalation, respiratory tract deposition, and dose distribution of ambient PM.

National Health and Environmental Effects Research Laboratory

PESTICIDES IN THE DIETS OF INFANTS AND CHILDREN

Peer-Reviewed Research on Priority Issues

September, 1995

CONTENTS

INTRODUCTION

EPA scientists have long been concerned that infants and children might be especially vulnerable to the toxic effects of pollutants. In 1993, these concerns were reinforced by the National Research Council (NRC) in its report entitled *Pesticides in the Diets of Infants and Children.* The NRC found that children may be at greater risk than adults to the toxic effects of pesticide residues on foods. It also acknowledged that before sound estimates of the scope and magnitude of this risk could be made, important gaps in the scientific database on pesticides had to be filled. The NRC identified areas where information was needed and proposed a broad-based research strategy to characterize the unique susceptibility of infants and children to the toxic effects of dietary pesticides.

Research on the differential risks associated with exposures to pesticides is an integral part of the intramural program at the National Health and Environmental Effects Research Laboratory (NHEERL). A multidisciplinary program addressing age-related differences in pesticide toxicity is fundamental to NHEERL's on-going research efforts. In addition, NHEERL recently initiated a targeted, peer-reviewed program to expand the scope of existing pesticide research to determine how young and mature animals differ in their responses to exposure to pesticides. Scientists throughout NHEERL submitted research proposals in a competition for funding under this program. The best research ideas are now being implemented. Findings will supplement the database on age-dependent toxicity to pesticides and explain some of the principles guiding developmental toxicity. Results also will be used to revise current testing guidelines and to reduce uncertainty in risk assessment.

This document summarizes

- the overall NHEERL research program in this area
- the project selection process implemented by NHEERL
- the NRC recommendations
- research progress made by NHEERL in addressing the NRC recommendations
- the individual projects contributing to the research progress reported

RESEARCH PROGRAM SUMMARY

An integrated and multidisciplinary research program addressing age-related quantitative and qualitative differences in pesticide toxicity is part of the on-going research program at NHEERL. This program consists of several research projects within the Neurotoxicology, Developmental Toxicology, and Environmental Toxicology Divisions which compare the toxicity of representative pesticides in adult and immature animals. Studies are currently underway to:

- provide more complete toxicity data in animals exposed to pesticides during development
- characterize qualitative and quantitative differences in response between young and adult animals
- provide data to improve current testing guidelines

NHEERL RESEARCH PROJECT SELECTION PROCESS

During FY95, NHEERL expanded the scope of its existing pesticide program by initiating several new projects that address the research needs outlined in the NRC report, *Pesticides in the Diets of Infants and Children.* These projects were selected based on the following process and will provide the foundation for NHEERL's future research in this area.

- A Steering Group was formed to develop a comprehensive research strategy responsive to the recommendations made by the NRC. The strategy pivots on 3 key issues identified by the NRC (described in greater detail on page 6):
  - age-related differences in sensitivity to pesticides
  - toxicity testing guidelines
  - reducing uncertainties

- A Request for Proposals (RFP) was developed and distributed internally to NHEERL scientists.

- An extramural panel was convened to review the proposals for scientific merit.

- A panel consisting of Assistant Laboratory Directors and Program Office Representatives reviewed the proposals for programmatic relevance.

- Proposals were prioritized according to scientific merit and programmatic relevancy and funded as a function of available funds.

- Five projects were selected and were initiated late in FY95.

A description of the proposals for which resources were allocated begins on page 10.

SUMMARY OF NRC RECOMMENDATIONS

Among the recommendations set forth by the NRC[1] to safeguard the health of infants and children were the following:

Assess Age-related Differences in Sensitivity to Pesticides (pp. 42-44; 105-110; 152-156)

According to the NRC report, toxicity data on mature animals may not sufficiently characterize the toxic effects of pesticides in young animals. **The report recommended that studies be conducted to resolve the issue of potential differences in susceptibility between young and adult animals, and that this research lead to the development of appropriate models for evaluating pesticide toxicity in infants and children.** The NRC also found a paucity of data related to pesticide toxicity in developing organisms, particularly with regard to neurotoxic, immunotoxic, and endocrine responses. **It was recommended that research be conducted to fill these data gaps and to explain the underlying principles guiding developmental toxicity (such as pharmacodynamics, metabolism, and mechanisms of action).**

Develop Toxicity Testing Procedures (pp. 105-110; 152-156)

The NRC observed that current testing guidelines do not include standard tests on immature animals as part of the basic evaluation of pesticides for toxicity. **It recommended that a standard developmental assessment protocol be established to facilitate the systematic interpretation of pesticide toxicity studies in immature animals. A redesign and expansion in scope of current test methods would be required, especially in the areas of neurodevelopmental effects, immunotoxicity, and reproductive/developmental toxicity. In cases where no such guidelines exist, as in the visual system, procedures would need to be developed and validated. It was recommended that several representative classes of pesticides be included in validation studies to compare responses in adult and immature animals.**

Reduce Uncertainty (pp. 359-363)

The evaluation of potential risks to infants and children due to dietary pesticide residues requires consideration of several factors that can impact the risk assessment process. To reduce the degree of uncertainty in estimates of risk, **the NRC recommended that physiological parameters and biochemical measurements in young and adult animals be compared to assess age-dependent differences, that evaluations of age-related differences in absorption, metabolism, detoxification, and excretion of pesticides (called PBPK modeling) be made, that the use of the benchmark dose for risk assessment applications be explored, and that toxicological data be generated to help evaluate the adequacy of the uncertainty factor used in calculations of risk assessment.**

[1] *Pesticides in the Diets of Infants and Children*, National Academy Press, Washington, DC, 1993.

AGE-RELATED DIFFERENCES IN SENSITIVITY TO PESTICIDES RESEARCH PROGRAM

Program Description

A multidisciplinary evaluation of quantitative and qualitative age-related differences in pesticide toxicity is an integral part of the existing research program within NHEERL. Organ systems, behavior, and biochemical responses are being examined to enhance our understanding of the principles of developmental toxicity. Special emphasis is being placed on neurotoxic, immunotoxic, and endocrine responses; at issue are reproductive competency and function, neurobehavioral changes, neurochemistry, neural growth and differentiation, allergic response, and immune function. New animal models are being developed that may more closely approximate responses in humans. Results from these studies also will address a number of issues of concern to risk assessors, including the extrapolation of animal data to human populations, the adequacy of the current uncertainty factor used in calculations of risk assessment, the identification of the most appropriate adverse effect for calculations of the reference or benchmark dose, and the determination of safe levels (tolerances) of pesticide residues. In addition, studies of mechanisms of action will enhance biologically-based risk assessment models.

RESEARCH PROGRESS

Recent NHEERL Findings

- Younger animals can be up to 21 times more sensitive than adults to the neurotoxic effects of a pyrethroid pesticide.

- Younger animals are more sensitive to the lethal effects of some cholinesterase-inhibiting pesticides; however, the difference is usually less than a factor of 10.

- The developing visual system is sensitive to the effects of cholinesterase-inhibiting pesticides.

- Some pesticides affect steroid hormones during development in rats, which can alter the onset of puberty.

Current Activity

- Studies are underway to examine toxicokinetic and toxicodynamic factors

that might explain age-related differences in sensitivity to the cholinesterase-inhibiting pesticides.

- On-going studies are testing the hypothesis that exposure to pesticides during development results in myopia and that the effect is age-dependent.

- Cholinesterase-inhibiting pesticides have been shown to affect learning and memory in adult rats; studies to examine these effects in young animals are underway.

Near Future

- Age-related effects will be evaluated using
  Physiological parameters (semen evaluations, reproductive organ changes, neural growth and differentiation, brain weight, and lung inflammation)
  Behavioral changes (effects on learning and memory)
  Biochemical measurements (endocrine markers, immune response endpoints, DNA and protein synthesis, enzyme activity, and hormonal measurements)

TOXICITY TESTING GUIDELINES RESEARCH PROGRAM

Program Description

Studies are underway that could lead to the revision of current testing guidelines for developmental toxicological endpoints, including teratogenic, reproductive, and neurotoxicological effects. Established rodent models are being used to study exposure strategies, biochemical changes, and behavioral endpoints in an effort to improve existing test procedures. In addition, new test methods are being developed and validated to augment current guidelines. Representative classes of pesticides are being used to facilitate our understanding of the differences in response between young and adult animals.

RESEARCH PROGRESS

Recent NHEERL Findings

- NHEERL research has helped OPPTS develop testing guidelines for assessing the effects of pre- and perinatal exposures to pesticides to predict outcome in infants and in children.

- NHEERL scientists helped draft the developmental neurotoxicity testing guidelines.

Current Activity

- Studies to further refine and validate the procedures described in the testing guidelines continue.

- Research on the visual toxicity of cholinesterase-inhibiting pesticides is underway.

Near Future

- New test procedures for assessing differential sensitivity to pesticides will be developed, including
  - a rabbit model for reproductive toxicity testing
  - new tests for learning and memory

NHEERL RESEARCH PROJECTS

The Effects of Pesticides on Reproductive Toxicity

General Approach

This research will use male rabbits as a model to determine how pesticides (o,p-DDT and p,p'-DDE) administered at a young age affect the development and function of the mature reproductive system. The rabbit, with its prolonged period of reproductive development, closely approximates the infancy-adolescence phase in humans. However, the rabbit model has not been used routinely in studies of this type; therefore, a subordinate objective of this research is the establishment of the rabbit as a relevant animal model for study of male reproductive competency. Analyses will include endocrine markers, semen evaluations (sperm number, motility, and morphology), fertility, and reproductive organ changes.

Research Questions

Is the reproductive system of the young more susceptible to pesticides than that of the mature animal?

What exposure levels pose a risk?

Is the rabbit a good model for reproductive toxicity?

What are the effects of pesticides on endocrine markers, on semen (sperm motility, morphology), on fertility, and on reproductive organ development?

NRC Recommendations Addressed

This study should provide qualitative information on the susceptibility of the developing reproductive system to pesticides, it should provide quantitative (dose-response) data that will enhance biologically-based risk assessment models, and it should provide mechanistic explanations useful for human risk assessment. Moreover, results from this study will be used to determine the appropriateness of the rabbit model for reproductive toxicity testing; these methods could then be applied to OPPTS testing guidelines.

Progress

Project will be initiated late in FY96.

For more information contact: Gary R. Klinefelter, Ph.D.

NHEERL RESEARCH PROJECTS

The Effects of Pesticides on Learning and Memory

General Approach

Research will be conducted to assess age-related differences in the neurobehavioral effects of pesticides. Cholinesterase-inhibiting pesticides (carbaryl and chlorpyrifos) shown previously to affect learning will be administered to male and female rodents perinatally. The animals will then be raised to adulthood and evaluated for learning deficits and memory impairments. Complementary evaluations will be carried out in rodents exposed to pesticides as adults. Studies will be conducted to determine whether changes in learning and memory can be correlated with changes in neurochemical responses.

Research Questions

Does early exposure to pesticides produce long-lasting effects on learning and memory?

Can better tests be developed to detect the effects of pesticides on learning and memory?

NRC Recommendations Addressed

By addressing age-related differences in sensitivity to the developing nervous system, this proposal responds to an area of concern specifically identified by the NRC report. Different heirarchical levels of learning and memory will be studied, the results of which may be used to document differences in risk based on age of exposure. Tests for cognitive function, if found useful for evaluating the neurotoxic potential of pesticides, may lead to a revision of testing guidelines for new and existing pesticides.

Progress

Project initiated late FY95.

For more information contact: Robert C. MacPhail, Ph.D.

NHEERL RESEARCH PROJECTS

The Biochemical Effects of Pesticides on the Central Nervous System

General Approach

Research will determine whether exposure to cholinesterase-inhibiting pesticides (carbaryl and chlorpyrifos) results in toxic effects to the central nervous system (CNS) that are expressed differently in developing and adult animals. The specific aims of the study are the development of an exposure strategy, the measurement in young and adult animals of diverse endpoints associated with growth and differentiation of the CNS, and the determination of mechanisms of action.

Research Questions

Are the biochemical effects of exposure to pesticides different in the young and adult CNS?

Can mechanisms of action be identified?

What is the effect of early exposure to pesticides on growth and differentiation of the CNS?

NRC Recommendations Addressed

Due to the broad range of biochemical assessments that will be made, this research will contribute to the database on pesticide toxicity, which addresses the NRC's concern regarding paucity of data. Qualitative (and possibly quantitative) differences in response between the mature and developing CNS will likely be observed. The in-depth assessment of biochemical markers of effect will be useful for risk assessment and will complement similar projects with predominantly behavioral components.

Progress

Project initiated late FY95.

For more information contact: Stanley Barone, Ph.D.

NHEERL RESEARCH PROJECTS

The Effects of Pesticides on the Immune System and Allergic Response

General Approach

The impact of pesticide exposure on the development of allergy to house dust mites will be studied in adult and young animals using the pesticides dieldren and carbaryl. An established rodent model for allergic sensitization will be used to test two hypotheses: 1) that exposure to pesticides promote the development of allergic sensitization to house dust mites, and 2) that this effect is greater in young animals than in mature animals. Investigators will focus on a tightly drawn and clinically relevant set of immunological parameters to evaluate immune function, pulmonary hyperactivity, and lung inflammation.

Research Questions

Does early exposure to pesticides impair the immune system?

Can pesticides help trigger asthma?

Are there biochemical changes (biomarkers) that represent an early indication of exposure to pesticides?

NRC Recommendations Addressed

Data from these studies should indicate whether the young represent a sensitive subpopulation for immune response and whether pesticide exposures play a role in the development of allergic lung disease. Dose-response relationships will be used to extrapolate information from animal toxicology studies to pesticide-exposed human populations. Finally, mechanistic information on immune responses will prove useful to risk assessment.

Progress

Project initiated late FY95.

For more information contact: MaryJane Selgrade, Ph.D.

NHEERL RESEARCH PROJECTS

Neurochemical Changes and Behavioral Effects Induced by Pesticides

General Approach

Research to test the behavioral and neurochemical effects of exposure to pesticides (chlorpyrifos and methoxychlor) will be performed in young and adult rats to explore age-related differences in neurotoxicity. The effects of both acute and chronic (repeated) exposure to pesticides will be studied, and long-term and immediate effects will be observed. Changes in behavioral endpoints (cognitive development and learning) will be related to neurochemistry in the brain and thyroid.

Research Questions

What are the long-term and immediate effects of exposures to pesticides and do they differ in mature and immature animals?

How do behavioral effects (changes in learning and memory) relate to effects on neurochemical processes?

What are the effects of early exposure to pesticides on the endocrine system?

NRC Recommendations Addressed

This project will evaluate pesticide effects on the neural and endocrine systems, both of which were identified as areas of concern by the NRC. Empirical evidence regarding qualitative and quantitative differences in the effects of pesticides across age will be generated, thereby helping to fill gaps in data. By linking behavioral effects with effects on underlying neurochemical processes, this research will contribute to the development of biologically-based approaches to risk assessment. This research will also help determine the adequacy of current animal testing guidelines by addressing the importance of age of exposure and by possibly developing more sensitive biological or behavioral methods for use in these evaluations.

Progress

Project initiated late FY95.

For more information contact: Mark E. Stanton, Ph.D.