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Accomplishments for Fiscal Year 2001
Research and Development
National Health and
Environmental Effects
Research Laboratory

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National Health and Environmental Effects Research Laboratory

An Annual Report of Accomplishments for Fiscal Year 2001

U.S. Environmental Protection Agency
Office of Research and Development
National Health and Environmental Effects Research Laboratory
Research Triangle Park, NC 27711

notice

The U. S. Environmental Protection Agency through its Office of Research and Development conducted and managed the research described in this report. It has been subjected to the Agency's peer and administrative review processes and has been approved for publication as an EPA document.

abstract

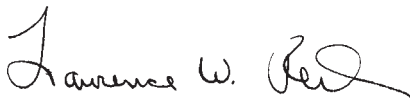
This Annual Report showcases some of the scientific activities of the National Health and Environmental Effects Research Laboratory (NHEERL) in various health and environmental effects research areas. Where appropriate, the contributions of other collaborating research organizations inside and outside EPA are acknowledged. The report is an indicator of progress and accomplishments that NHEERL has made in Fiscal Year 2001 in achieving the Agency's and ORD's strategic goals. NHEERL's highlighted research is organized under these goals. Specific research areas included for this year are: (1) Particulate Matter, (2) Air Toxics, (3) Drinking Water, (4) Aquatic Stressors, (5) Pesticides, (6) Global Change, (7) Ecosystems Protection, (8) Human Health Protection, and (9) Endocrine Disruptors.

letter from the director

EPA's mission is to protect human health and to safeguard the environment. As EPA's scientific arm, the Office of Research and Development (ORD) provides research, leadership, and advice on scientific issues to EPA. As one of five laboratories and centers in ORD, the National Health and Environmental Effects Research Laboratory (NHEERL) is charged with investigating the impacts of environmental stressors on both human and ecosystem health, the degree of harm caused by the stressors, and the factors that affect the degree of harm. The range of achievements highlighted in this report reflects NHEERL's support to ORD and EPA.

EPA's research efforts are organized according to strategic goals outlined in its Strategic Plan: *Clean Air (Particulate Matter and Air Toxics); Clean Water (Drinking Water and Aquatic Stressors); Safe Communities (Pesticides); Climate Change (Global Change); and Sound Science (Ecosystems Protection, Human Health Protection, and Endocrine Disruptors)*. ORD's research is planned and pursued to support EPA's strategic goals in an integrated fashion. NHEERL supports that plan with multidisciplinary teams of scientists dedicated to unraveling the many complex factors relating to a specific problem. For example, this report showcases the work of epidemiologists, toxicologists, analytical chemists, and others from across several divisions within NHEERL to determine the effects of arsenic in drinking water.

Using a risk-based investigative approach, NHEERL provides scientific data from its human health and environmental effects research to support ORD's mission to inform regulatory programs and make sound policies to fulfill EPA's efforts to safeguard human and ecosystem health. We are pleased to share some of our most important findings with you.



Lawrence W. Reiter, Ph.D.
Director, National Health and Environmental Effects Research Laboratory
Office of Research and Development
Research Triangle Park, North Carolina

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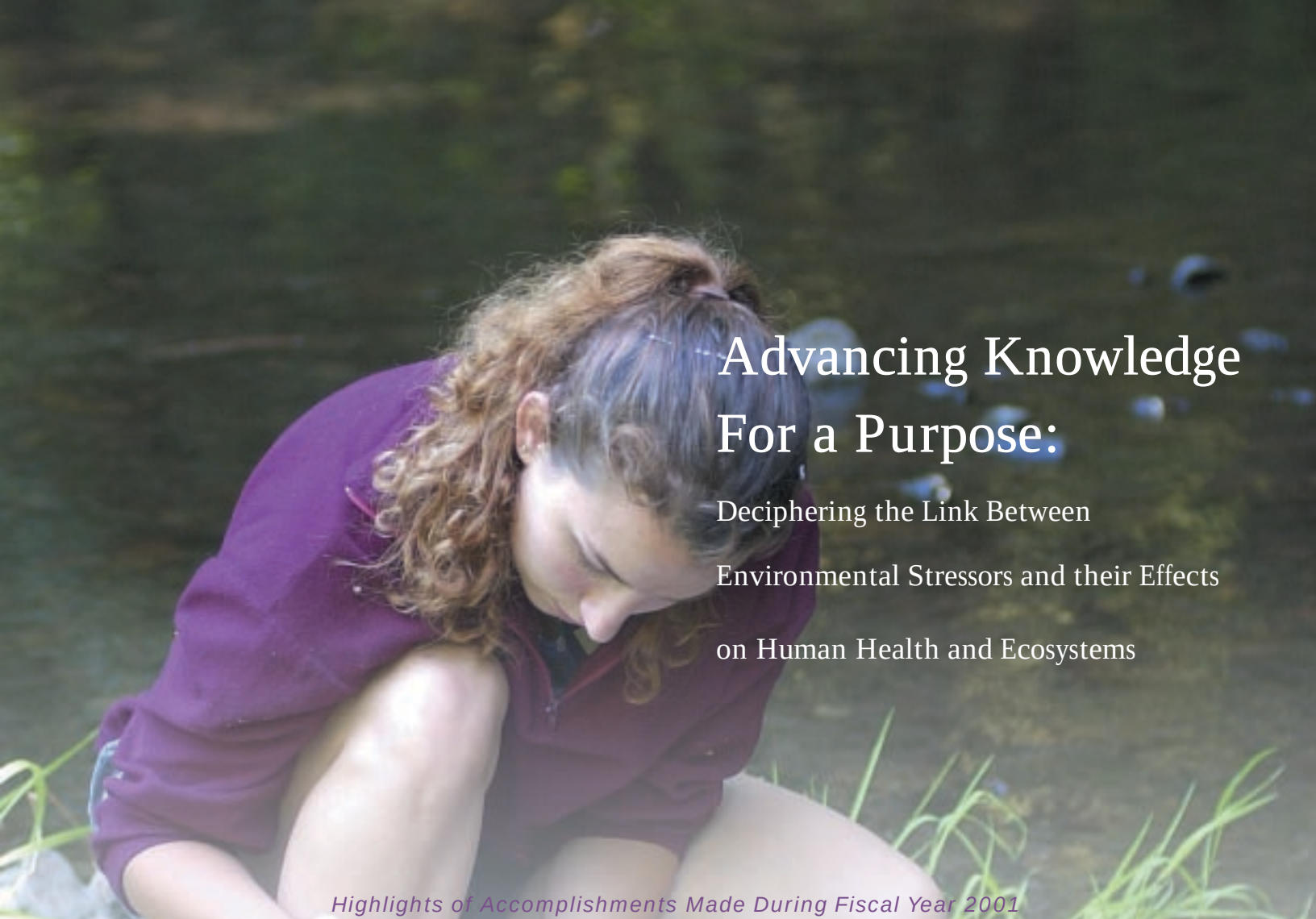


Advancing Knowledge For a Purpose:

Deciphering the Link Between Environmental Stressors and their Effects on Human Health and Ecosystems

Highlights of Accomplishments Made During Fiscal Year 2001

The National Health and Environmental Effects Research Laboratory (NHEERL) is an important arm of the U.S. Environmental Protection Agency's (EPA) Office of Research and Development (ORD). NHEERL is EPA's focal point for scientific research on the adverse effects of pollution and other stressors on human health and ecosystem vitality. Our scientists provide information essential to effective risk assessment, which is the scientific basis for regulatory and policy decisions.

NHEERL provides vital leadership in national and international research communities. Based in Research Triangle Park, NC, NHEERL has nine divisions in six states and a work force of over 700 federal employees. Five health divisions are centrally located in Research Triangle Park and Chapel Hill, NC, and four ecology divisions are based in ecologically significant regions (Atlantic seaboard, Pacific coast, Great Lakes, and the Gulf of Mexico) to address national and regional ecological risk assessment issues.

Our scientists conduct in-house research as well as participate in collaborative studies with academia, state governments, other federal agencies, and research organizations around the world. NHEERL research undergoes the highest levels of independent scientific review and scrutiny, and

NHEERL research centers are located in several geographically important areas around the nation.

our results are published in peer-reviewed journals, reports, and other media as a means of communicating our scientific progress and accomplishments to the public and scientific community. NHEERL scientists also regularly present research findings at symposia, hold membership and leadership positions on scientific committees and workgroups, and participate in various nationally and internationally recognized scientific organizations.

In researching health and ecological risk, NHEERL's organizational structure enables scientists to develop innovative methods and solutions to complex problems in an integrated manner. Data extrapolated from both animal and human studies are incorporated into computer models that are used in real-world applications. The combination of a scientifically diverse work force and highly specialized facilities enables NHEERL to stay on the cutting edge of health and environmental effects research. Currently, major research activities are focused on the harmful effects of particulate matter and endocrine-disrupting chemicals, and some of our most important projects are described and showcased in this report. It is important to note that this report is not a comprehensive summary of all research completed at NHEERL during this year, but rather, it highlights some of our recent accomplishments in the following areas:

- health effects of airborne particulate matter
- mechanisms of toxicity of air pollutants
- advances in drinking water safety research
- susceptibility of children to certain pesticides
- influence of pesticides on the developing immune system
- effects of global climate change on ecosystems and human populations
- assessment of the condition of aquatic and terrestrial ecosystems

As the largest research center within EPA's Office of Research and Development, NHEERL has nine divisions. NHEERL headquarters and five health research divisions are located in Research Triangle Park and Chapel Hill, North Carolina. Four ecology research divisions are located in ecologically significant areas around the country.

NHEERL Health Research Divisions

- Environmental Carcinogenesis (RTP, NC). Studies the associations among environmental contaminants and cancer.
- Experimental Toxicology (RTP, NC). Examines the toxicity of environmental contaminants to specific organ systems and bodily functions.
- Human Studies (Chapel Hill, NC). Conducts epidemiologic and clinical research on the human response to environmental contaminants.
- Neurotoxicology (RTP, NC). Studies the effects of chemical and physical agents on the nervous system.
- Reproductive Toxicology (RTP, NC). Develops methods used to study the reproductive and developmental effects of environmental contaminants.

NHEERL Ecology Research Divisions

The ecology research divisions assess the condition of regional ecosystems—including terrestrial and aquatic environments—and study the effects of pollution and other stressors on these ecosystems.

- Atlantic Ecology (Narragansett, RI). Atlantic seaboard ecosystems.
- Gulf Ecology (Gulf Breeze, FL). Gulf of Mexico ecosystems.
- Mid-Continent Ecology (Duluth, MN and Grosse Ile, MI). Inland and freshwater ecosystems.
- Western Ecology (Corvallis and Newport, OR). Pacific coast ecosystems.


cleanair
particulate matter

Particulate matter (PM) is one of the six criteria air pollutants for which EPA has established National Ambient Air Quality Standards (NAAQS). The term particulate matter refers to airborne solid particles and liquid droplets. Based on particle size, particulate matter is categorized as ultrafine (0 - 0.1 micron in diameter), fine (0.1 - 2.5 micron), and coarse (2.5 - 10.0 micron). PM_{10} is an older term that refers to particles less than 10 microns in diameter and includes coarse, fine, and ultrafine particles. Generally, particles larger than 10 microns in diameter are trapped in the nasal passages and do not make their way into the lungs. EPA has established NAAQS for both fine and coarse particles (in the form of PM_{10}). Coarse particles can aggravate respiratory conditions such as asthma. Generally, coarse particles enter the air from dust generated by vehicles traveling on unpaved roads, materials handling, and crushing and grinding operations. The major source of fine particles is fuel combustion from vehicles, power plants, and industries.

Elevated levels of particulate matter have been associated with increased mortality and

hospitalizations for heart and lung conditions; these associations are stronger for fine particles than coarse particles. Because of its widespread distribution and potential impact on many people, particulate matter continues to be one of the highest research priorities at EPA.

Scientists at NHEERL are world leaders in particulate matter research.

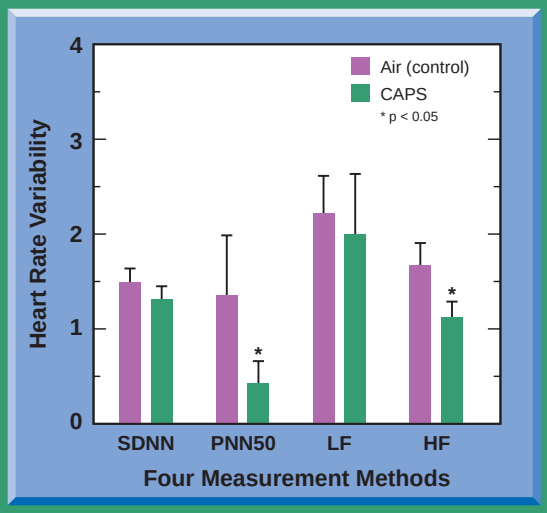
The NHEERL particulate matter research program is multidisciplinary and includes three major categories of studies: epidemiologic, human clinical, and laboratory studies using animals and tissue culture cells. The program is currently investigating the

- health effects associated with particulate matter,
- groups of people most likely to be affected,
- physiological mechanisms underlying these effects, and
- toxic components of particulate matter.

NHEERL researchers conduct epidemiologic studies in groups of people, toxicologic studies in the laboratory, and clinical studies in human volunteers, all of which contribute to the scientific understanding of how particulate matter causes health problems. Epidemiologic studies are often the first to point toward an association between an environmental exposure and a health problem. When this occurs, scientists conduct laboratory and clinical studies designed to clarify and characterize the apparent association. The results from these studies can then be used to identify additional important endpoints to measure when conducting future epidemiologic studies.

HEALTH EFFECTS IN VOLUNTEERS

A previous NHEERL epidemiologic study in a Baltimore, Maryland, retirement community found that elderly people experienced reduced heart rate variability when ambient particulate matter levels were elevated. To confirm this finding through clinical studies, NHEERL scientists exposed healthy young and elderly participants to concentrated ambient particulates (CAPS) in a highly sophisticated exposure chamber. Participants were under continuous medical supervision. Immediately after exposure, heart rate variability decreased while blood factors that promote clot formation increased (Figure 1.) Both reduced heart rate variability and blood clot formation are associated with increased risk of heart attacks. Therefore, these findings point toward potential mechanisms by which elevated particulate matter levels increase the risk of acute cardiovascular disease. They are also consistent with a recent published report that people can suffer heart attacks as soon as two hours after exposure to elevated particulate matter levels. Future epidemiology studies will look for associations between particulate matter and blood components involved in blood clot formation.



Heart rate variability is the ability of the heart rate to speed up or slow down in response to the body's needs or external stimuli. This variability reflects nervous system control over the heart. When heart rate variability is reduced, the nervous system has less control over the heart, therefore, the heart is less able to respond appropriately to stressful conditions.

The graph shows reduced heart rate variability following controlled exposure of elderly volunteers to concentrated ambient particulates (CAPS). Each pair of bars represents a different way of measuring heart rate variability. Combining the results of a series of related studies, NHEERL researchers showed that heart rate variability immediately following CAPS exposure was reduced significantly according to two measures of heart rate variability (PNN50 and HF). The HF measurement of heart rate variability was also significantly reduced in studies of elderly volunteers in retirement communities. This agreement between community exposure studies and controlled laboratory exposure studies builds evidence for one mechanism by which particulate matter may cause adverse cardiovascular effects.

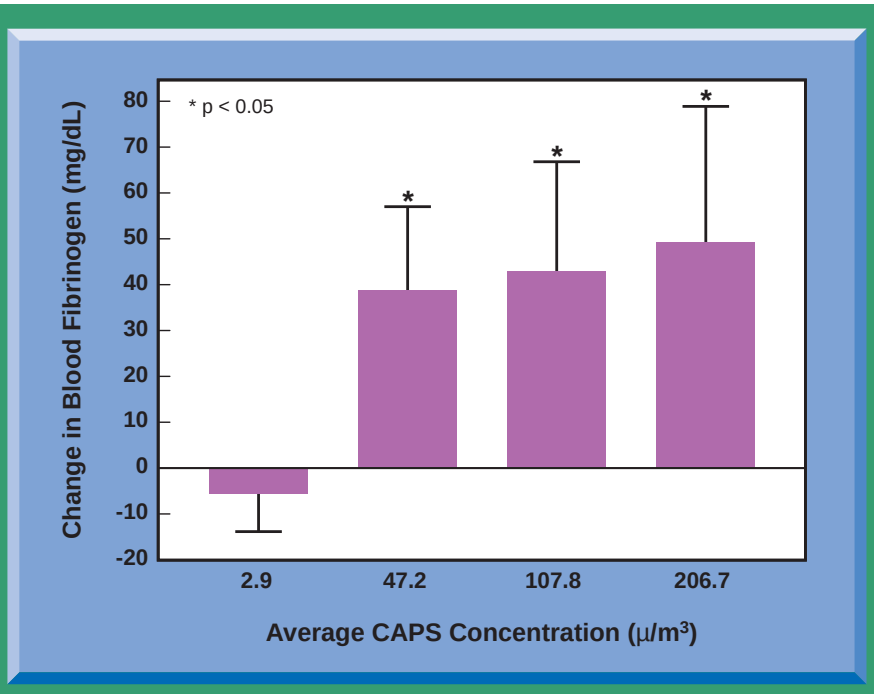


FIGURE 1. Fibrinogen is a protein important in blood clotting. Note that blood fibrinogen level increased as particulate matter concentration increased. * indicates statistical significance.

TOXIC COMPONENTS OF PARTICULATE MATTER

A series of epidemiologic studies conducted by university scientists in Utah Valley, Utah, found an association between health problems and high PM_{10} levels, which were associated with operation of a steel mill in the valley. The health problems included increased hospital admissions for respiratory conditions and increased deaths due to respiratory and cardiovascular diseases.

Subsequently, to pinpoint the factors responsible for this observed association, NHEERL scientists obtained air quality monitoring filters from the Utah Valley for the time period overlapping that of the epidemiologic study. This included one year during which the steel mill was closed due to a labor dispute (year 2), the year preceding the closure (year 1), and the year following mill reopening (year 3). The researchers used water to extract particulate matter components from the filters. These extracts were used in a variety of studies. The NHEERL Annual Report of Accomplishments for Fiscal Year 2000 described the results of several studies, the findings of which were consistent with the epidemiologic studies. Both clinical and laboratory studies found that extracts of particulate matter collected when the steel mill was operating caused more lung inflammation than the particulate matter collected when the steel mill was closed. (Lung inflammation reversed in all study participants.) Since the same amount of material from each of the three years was used for the experiments, these results demonstrate for the first time that the chemical composition of particulate matter plays an important role in its toxicity.

Smoke and haze can be seen covering the region from the Los Angeles Basin and San Diego, to north of the Salton Sea and across the Mojave Desert.

Image STS006-114-063 courtesy of Earth Sciences & Image Analysis Laboratory, NASA Johnson Space Center (http://eol.jsc.nasa.gov)

Research published in 2001 revealed a mechanism whereby particulate matter causes lung inflammation. A study conducted by scientists from NHEERL and the University of North Carolina at Chapel Hill using epithelial cells lining the human airway identified a specific cellular signaling pathway that increased the production of inflammatory proteins when it was activated by exposure to Utah Valley particulate matter extracts.

Also in 2001, NHEERL scientists and collaborators at CIIT Centers for Health Research reported that rats developed significant acute lung injury and inflammation after extracts of years 1 and 3 were put into the lungs. (The effects were largely resolved within 96 hours.) Year 2 extract did not cause appreciable lung damage. Because the degree of lung damage was correlated with metal content of the extracts, researchers hypothesized that soluble metals may be responsible for some of the adverse health effects observed.

This research approach links associations first identified in epidemiologic studies to animal toxicologic studies and human clinical studies, which–taken together–provide information on the mechanisms by which particulate matter causes health problems.

Other projects conducted by researchers at NHEERL, the University of Rochester School of Medicine and Dentistry, and the University of North Carolina at Chapel Hill investigated the extracts' metal content, the specific effects of the extracts on respiratory cells, and the mechanisms of cellular injury. The researchers found that, compared to year 2, years 1 and 3 extracts contained more soluble iron, copper, and zinc. A study examining the response of human respiratory tract cells in the extracts found that those cells exposed to extracts from years 1 and 3 generated the highest levels of oxidants (Figure 2) and inflammatory proteins (Figure 3). These findings add to the mounting evidence that the type and amount of water-soluble metals present in particulate matter may be an important factor determining the toxic effects of particulate matter in humans.

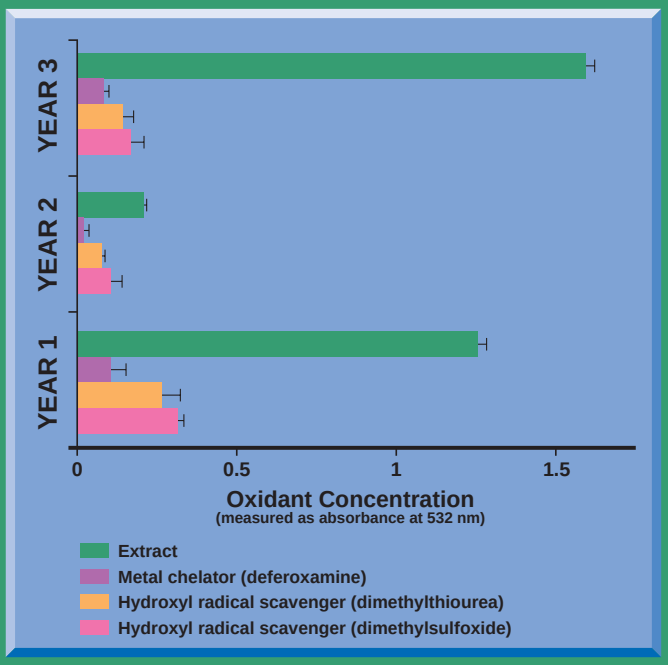
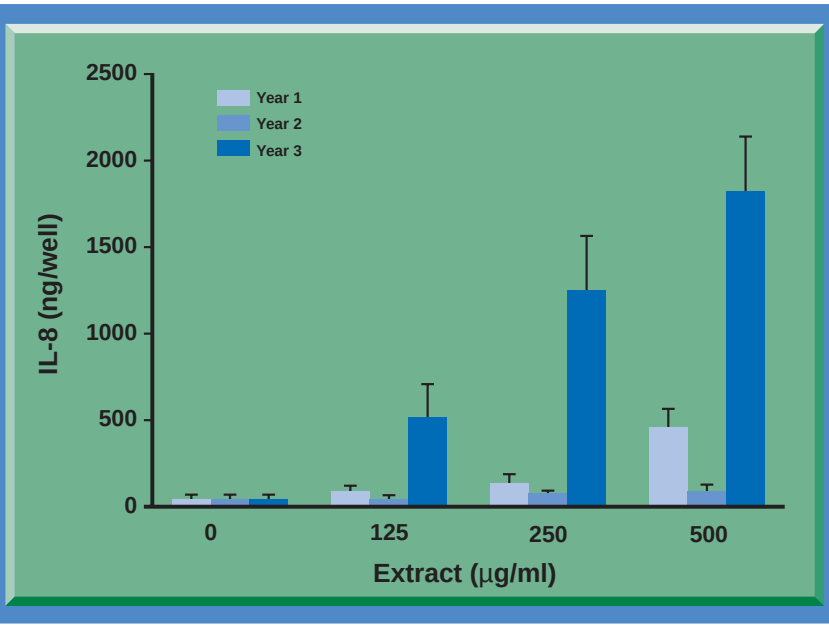


FIGURE 2. Oxidants, also known as free radicals, are highly reactive molecules that can damage cells and DNA. (Household bleach and hydrogen peroxide are familiar oxidants.) In the body, antioxidants neutralize or "scavenge" oxidants, rendering them harmless. In this cell culture test, adding a metal chelator or a hydroxyl radical scavenger to the extract before exposing cells to particulate matter extracts dramatically reduced oxidant production by cells exposed to years 1 and 3 extracts. (The metal chelator removed the metals from the extract, whereas the hydroxyl radical scavengers neutralized hydroxyl radicals, one type of oxidant, as they were produced by the cells.)

FIGURE 3. In cell cultures, one measure of the inflammatory response is the amount of inflammatory protein released following cell injury. This graph shows production of the inflammatory protein interleukin-8 (IL-8) following exposure to control (0 dose) and three doses of Utah Valley dust extracts. Note that IL-8 production increased as dose increased. Production of interleukin-6, another inflammatory protein, followed a similar pattern.




clean air
air toxics

The Clean Air Act and Amendments identified 188 hazardous air pollutants (HAPs, also known as air toxics) that are emitted from a variety of stationary, mobile, and indoor sources. Significant doubts remain regarding the health effects of hazardous air pollutants. Of the 188 HAPs, EPA has not yet developed cancer risk information for 143, reference doses (RfD, for ingestion exposure) for 134, or reference concentrations (RfC, for inhalation exposure) for 167. Appreciable uncertainty accompanies the risk estimates for many HAPs for which EPA has some health risk information. Over the past decade, EPA's air toxics regulatory program established technology-based standards for specific industry categories. Future regulatory actions and nonregulatory guidelines will require an improved understanding and quantification of the health risks that air toxics from single and multiple sources pose at multiple geographic scales (e.g., local, regional).

To reduce uncertainties in future risk assessments, EPA needs more research on adverse health effects of air toxics. To this end, EPA has an Air Toxics Research Strategy designed to meet four long-term goals of EPA's Air Program providing:

1. methods and information to support assessment of the health effects and risks from exposure to air toxics at national, regional, and local scales;
2. measurements and models that will reduce uncertainty of estimated mobile source emissions and estimates of human exposure and health effects associated with mobile sources of air toxics;
3. methods to support residual risk assessments and risk management strategies of stationary sources, including major and area source categories; and
4. health effects information and validated emissions characterization and transport models that estimate and provide guidance on management of risks from indoor air toxics based on building type and indoor activities.

AIR TOXICS IMPLEMENTATION PLAN

In support of EPA's Air Toxics Research Strategy, NHEERL is developing an Air Toxics Implementation Plan to guide its research in this area. Most of NHEERL's air toxics research efforts address the first goal mentioned above. NHEERL

scientists conduct research to determine the health and ecological risks and dose-response relationships associated with exposure to air toxics. Researchers at NHEERL are working to fill current knowledge gaps regarding mode of action, dose-response relationships for acute and chronic exposures, susceptibility of sensitive subpopulations, and mixture interactions. Historically, approaches to filling these gaps have relied upon toxicity data obtained from laboratory animals or human studies. NHEERL is also taking a newer approach through development of physiologically based pharmacokinetic (PBPK) models that facilitate dose-response assessments.

Current areas of air toxics research activity at NHEERL include

- irritant and pulmonary effects of aldehydes and halides,
- neurotoxic effects of volatile organic compounds, and
- carcinogenic effects of polycyclic organic matter and hydrocarbons at low doses.

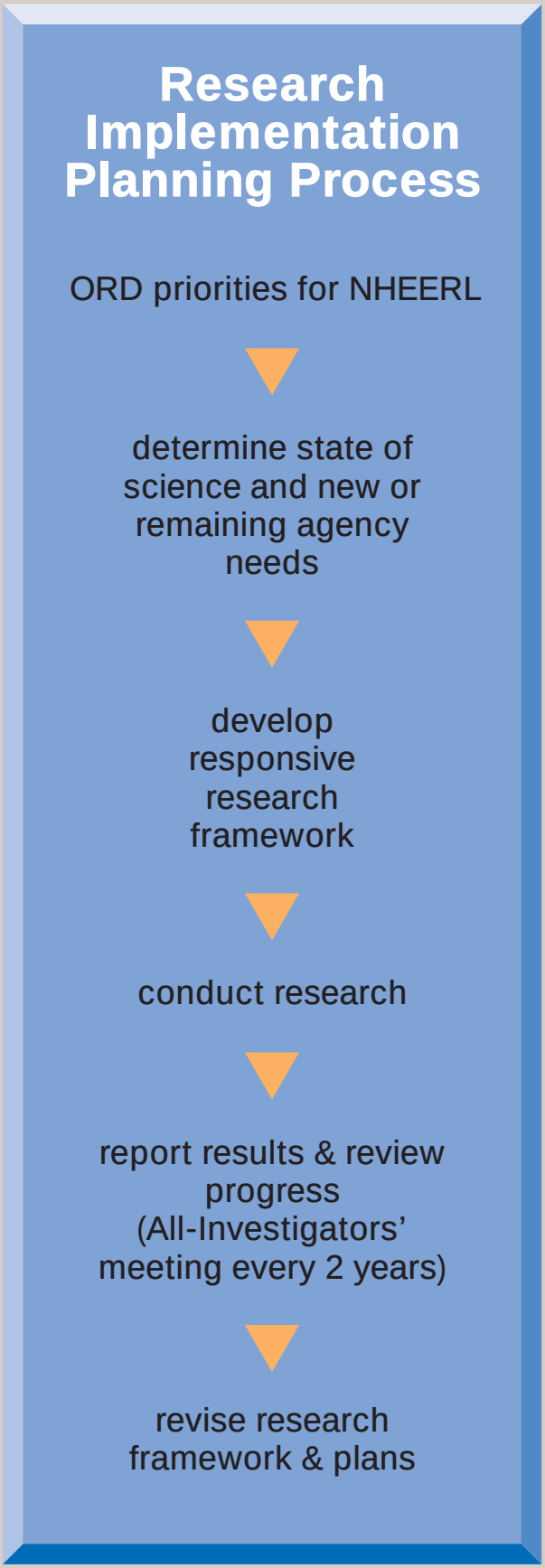
NHEERL's Research Planning Process

For each high-priority research area, NHEERL develops an implementation plan. Once ORD has identified the priority topics, these multi-year plans provide a mechanism for prioritizing research projects and link directly to EPA goals and ORD strategic plans. To enhance research integration with EPA programs and goals, representatives from all ORD Laboratories and Centers participate in developing implementation plans. Also, representatives from EPA Program Offices and Regions participate to ensure that NHEERL is conducting research that supports EPA's mission. At two-year intervals, each implementation plan is reviewed and revised based on new scientific findings and changing research needs.

An implementation plan consists of a research framework and research plans for specific projects. The research framework is usually established at workshops and meetings attended by scientists and program managers. The research framework

- describes the problem EPA faces,
- discusses how the NHEERL mission relates to the problem,
- identifies principal scientific uncertainties,
- lists the uncertainties that NHEERL can address or problems NHEERL can solve, and
- outlines major research approaches/steps needed.

Research plans for specific projects are developed by NHEERL divisions in response to the implementation plan's research framework. They are reviewed by a steering committee to ensure their relevancy and responsiveness to EPA needs.



PBPK MODEL FOR MTBE VALIDATED

NHEERL scientists recently validated a PBPK model for methyl tertiary butyl ether (MTBE), which is added to automotive fuel in various percentages for three purposes:

- at 15% to reduce carbon monoxide (CO) emissions from automobiles in areas where CO levels exceed CO standards;
- at 10% to reduce ozone precursors in areas where ozone levels exceed ozone standards; and
- at 2 to 6% to enhance octane in other localities.

By reducing ozone and carbon monoxide levels to nonhazardous concentrations in areas where these pollutants are a significant problem, the addition of MTBE to gasoline can reduce episodes of ozone- or carbon monoxide-induced health problems. In addition, MTBE use in gasoline reduces the concentration of benzene, a known human carcinogen.

In 2000, approximately three billion gallons of MTBE were added to gasoline in the United States. As with all supplies of gasoline, it is inevitable that spills, leaks, and evaporative losses occurred that contaminated drinking water with MTBE in some areas of the country. In addition to bathing in or ingesting MTBE-contaminated water, people may be exposed by inhalation while pumping gasoline or while using contaminated water. Because of MTBE's widespread use and Agency concern about the agent, EPA continues to explore the human health risks of exposure.

The pharmacokinetics of MTBE in the human body are important to the risk assessment process by which public health guidelines are established. Also, modeling of MTBE exposure by various routes will aid in estimating the relative

PBPK MODELS

Physiologically based pharmacokinetic (PBPK) modeling is a powerful tool used to estimate the concentration and associated toxicity of a toxic substance in target tissues such as brain, kidney, or liver, based on specified exposure conditions. PBPK models predict uptake, metabolism, tissue distribution, and elimination of a toxic substance following ingestion, inhalation, or dermal routes of exposure. Because PBPK models are founded on physiologic, chemical-specific, and species-specific processes, they provide a scientific basis for risk managers and other decision makers who set standards for safe exposure limits to toxic substances. PBPK models may also be helpful in predicting chemical interactions for exposures to toxic mixtures.

The PBPK model can be used to improve risk assessment for unintentional exposures to MTBE.

contribution of each route of exposure to observed MTBE body burdens.

Scientists at NHEERL, the National Exposure Research Laboratory (NERL), and the Centers for Disease Control (CDC) collaborated to study the pharmacokinetics of MTBE and its primary metabolite, tertiary butyl alcohol (TBA). Studies in rats formed the foundation for human PBPK modeling. Based on the measurements made in these rat studies, the researchers developed a rat-to-human extrapolation model and subsequently validated this PBPK model with human volunteers. MTBE was most rapidly absorbed following ingestion and inhalation exposure. Dermal uptake was slower and dependent on the MTBE concentration. Compared to inhalation or dermal exposure, a greater TBA:MTBE ratio was observed following oral ingestion, providing evidence of significant first-pass metabolism for this route of exposure. Regardless of the route of exposure, about half of the MTBE was exhaled, with the rest metabolized to TBA.

Twenty-four hours after exposure, blood MTBE concentration was very low, but blood TBA concentration was still about 27% of peak, indicating that TBA was metabolized and eliminated more slowly than MTBE. Figure 1 shows the correlation between observed blood MTBE concentrations and the levels predicted by the PBPK model. The model accurately simulated MTBE and TBA blood concentrations for all three routes of exposure. Therefore, the PBPK model can be used to improve risk assessment for unintentional exposures to MTBE via inhalation, dermal contact, and ingestion. With further refinement, the model is expected to play an important role in policy decisions that attempt to balance the health benefits of using MTBE to reduce ozone and carbon monoxide in the air with the potential increased risk of cancer from MTBE exposure.

MTBE Blood Concentrations

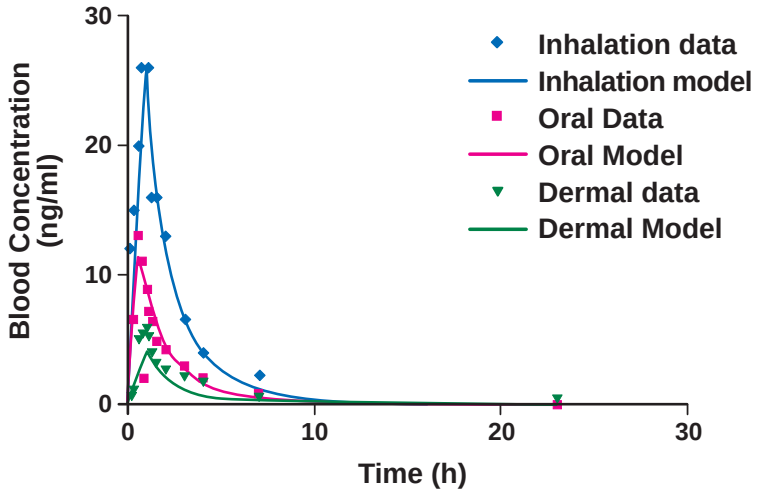


Figure 1. Correlation between MTBE blood concentrations and modeled MTBE blood levels for one participant.


cleanwater
drinking water

The 1996 Amendments to the Safe Drinking Water Act (SDWA) require EPA to conduct research to provide a scientific foundation for regulatory standards that limit contaminants in drinking water. Contaminants that EPA is studying include waterborne pathogens, man-made chemicals, naturally occurring elements, and disinfection by-products.

Arsenic is an element that occurs in several types of rock formations, particularly in areas of past or present volcanic or geothermal activity. In several regions of the United States, arsenic leaches from rock into groundwater that is used for drinking water. Health problems associated with arsenic ingestion include cardiovascular disease, strokes, peripheral neuropathy (a disorder of the nerves), diabetes, abnormal fetal development, and several types of cancer. While studies in foreign countries have demonstrated that very high levels of arsenic in drinking water can be harmful, the minimum dose and minimum duration of exposure required to cause health problems have not been established.

Studying the adverse health effects of exposure to arsenic in drinking water is a major research priority at EPA. At NHEERL, scientists are conducting cutting-edge research investigating the metabolic processes by which the body transforms arsenic and the molecular mechanisms by which arsenic causes cancer and other health problems. Other scientists are characterizing arsenic exposure in specific communities in the United States.

ARSENIC MODE OF ACTION AND METABOLIC FATE

A cooperative effort studying the mechanism of action of arsenic (As) compounds and the role of metabolism in the element's toxicity and carcinogenicity involves scientists from NHEERL, the University of North Carolina at Chapel Hill, the University of British Columbia in Vancouver, and the Polytechnical Institute of Mexico.

New analytic technique developed

This research team developed an analytic technique to distinguish between methylated arsenic compounds with arsenic in the +3

(trivalent, As^{III}) and +5 (pentavalent, As^{V}) oxidation states. This is important because the oxidation state of an element in a compound may dramatically affect the compound's reactivity and toxicity. In studies using this technology, the research team identified and quantified methyl As^{III} and dimethyl As^{III} in cultured cells, urine specimens from people exposed to inorganic arsenic, and other biological samples. The oxidation state (As^{III} *versus* As^{V}) of arsenic metabolites influences their toxicity. Therefore, the ability to distinguish between and measure As^{III} and As^{V} metabolites in tissue cultures, tissue samples, and urine is critically important to studies of arsenic metabolism and toxicity. This analytic ability is also important to epidemiologic studies of people exposed to naturally occurring arsenic in drinking water and/or food.

Methylation, the addition of a methyl group to a substance, is a common step in the body's metabolism of foreign or toxic substances. Methylated compounds are often less toxic to the body than the original chemical. Scientists have long thought that the methylation of arsenic is a detoxifying reaction that produces compounds less toxic than inorganic arsenic. New research conducted by NHEERL scientists and collaborators suggests that some methylated arsenic compounds may be more toxic than inorganic arsenic.

Toxic activity investigated

In 2001, the collaborating scientists reported that three trivalent arsenic compounds—inorganic As^{III}, methyl As^{III}, and dimethyl As^{III}—inhibited the enzyme thioredoxin reductase in cultured rat liver cells. As a key enzyme in the cellular response to oxidative stress, thioredoxin reductase plays a critical role in the response of cells to a wide range of toxic agents. Exposing cells to various doses of inorganic As^{III} and methyl As^{III} caused a concentration-dependent inhibition of thioredoxin reductase. As the concentration of inorganic As^{III} increased in the medium, the concentrations of methyl arsenic and dimethyl arsenic metabolites inside the cells also increased.

As the intracellular concentration of arsenic compounds increased, thioredoxin reductase inhibition increased. Methyl As^{III} was more potent than inorganic As^{III} and inhibited thioredoxin reductase activity at much lower concentrations (see Figure 1). The consequences of thioredoxin reductase inhibition are unknown. Thioredoxin reductase and thioredoxin (one of the molecules with which thioredoxin reductase interacts) play important roles in regulating cell growth and genetically programmed cell death. These processes are critically important to the growth and survival of tumor cells. Further studies of the influence of arsenic on the thioredoxin reductase-thioredoxin system may shed light on the role of arsenic in cancer development.

New genotoxic mode of action discovered

Also in 2001, the researchers reported that trivalent methylated arsenic compounds caused direct damage to free DNA and to DNA in cultured cells. This is significant because

- no interactions of an arsenic compound with DNA had been reported previously;
- the mode of action of arsenic in carcinogenicity had been thought to be through indirect mechanisms, rather than by direct damage to DNA;
- an implicit assumption underlying arsenic risk assessment had been that the methylation of arsenic is a detoxification process. This assumption is now in question; and
- these findings imply that people who methylate arsenic efficiently may be at increased risk of cancer when exposed to arsenic in drinking water or food.

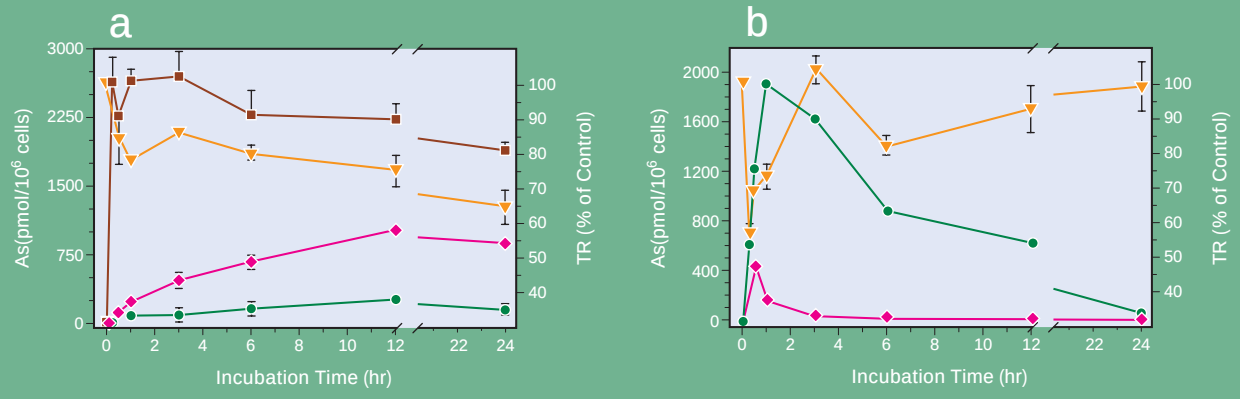


FIGURE 1. Relationship between intracellular concentration of arsenic compounds and thioredoxin reductase activity when cultured cells were exposed to arsenic compounds for 24 hours. (a) 10 µM inorganic arsenic As^{III}. (b) 1 µM methyl arsenic As^{III}. Both inorganic and organic As^{III} inhibited thioredoxin reductase. (At the concentrations used, none of the arsenic compounds killed cells.) Symbols: ■ = inorganic arsenic; ◆ = methyl arsenic; ● = dimethyl arsenic; ▼ = thioredoxin reductase.

The research team conducted two series of laboratory studies involving the trivalent (As^{III}) and pentavalent (As^{V}) forms of inorganic arsenic, methyl arsenic, and dimethyl arsenic. In a DNA nicking assay, only free DNA that had been exposed to trivalent methylated arsenic (methyl As^{III} and dimethyl As^{III}) showed evidence of breaks—"nicks"—in either one or both strands of DNA. Further, DNA damage was dose-dependent; the more extensive DNA damage occurred at higher concentrations. In a single-cell gel (SCG) assay using human lymph cells, the trivalent methylated forms (methyl As^{III} and dimethyl As^{III}) caused appreciable damage to cellular DNA, whereas both trivalent and pentavalent inorganic arsenic (As^{III} and As^{V}) caused only slight DNA damage. Pentavalent methylated forms (methyl As^{V} and dimethyl As^{V}) were basically inactive.

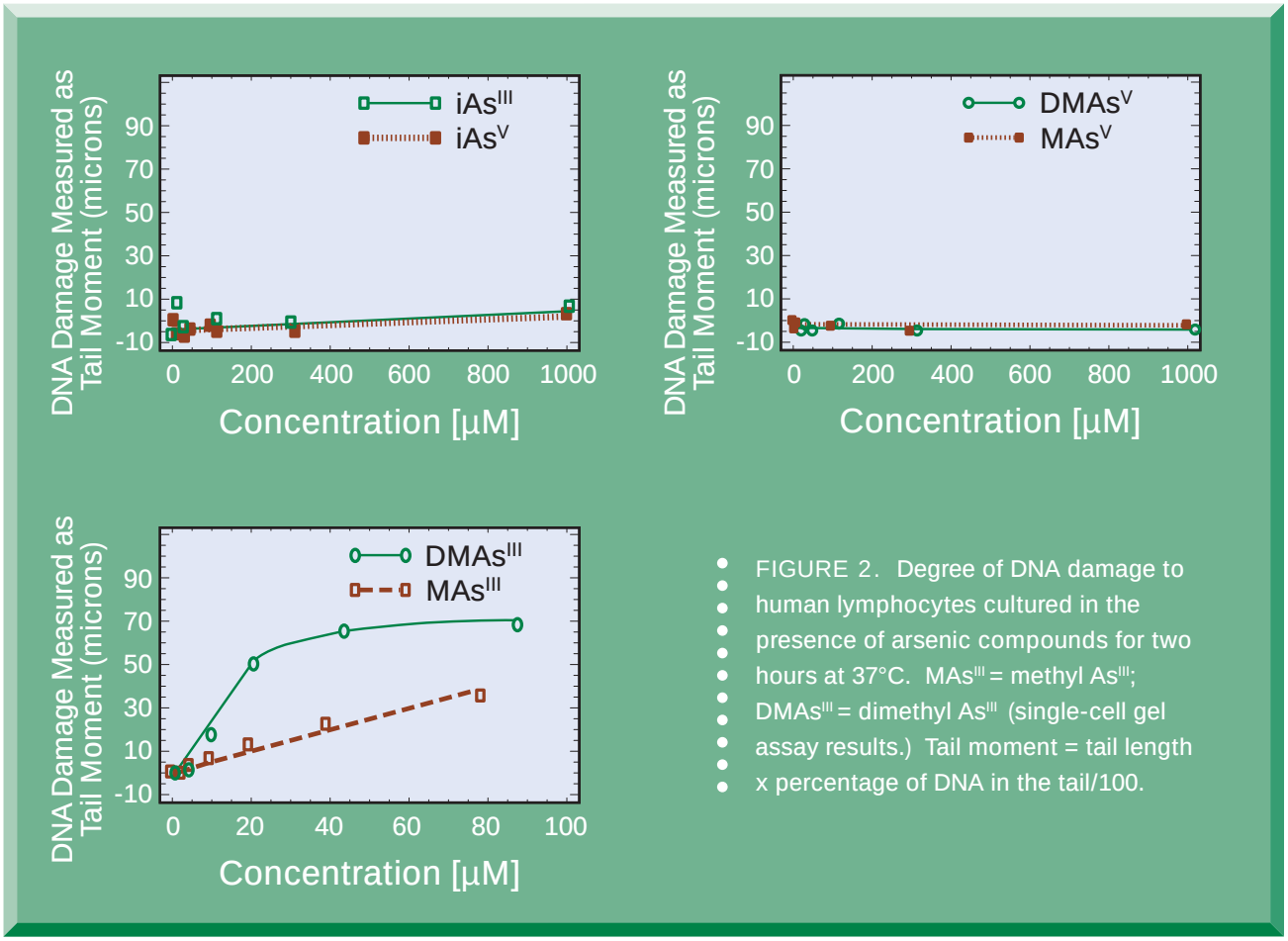


FIGURE 2. Degree of DNA damage to human lymphocytes cultured in the presence of arsenic compounds for two hours at 37°C. MAs^{III} = methyl As^{III}; $DMAs^{III}$ = dimethyl As^{III} (single-cell gel assay results.) Tail moment = tail length x percentage of DNA in the tail/100.

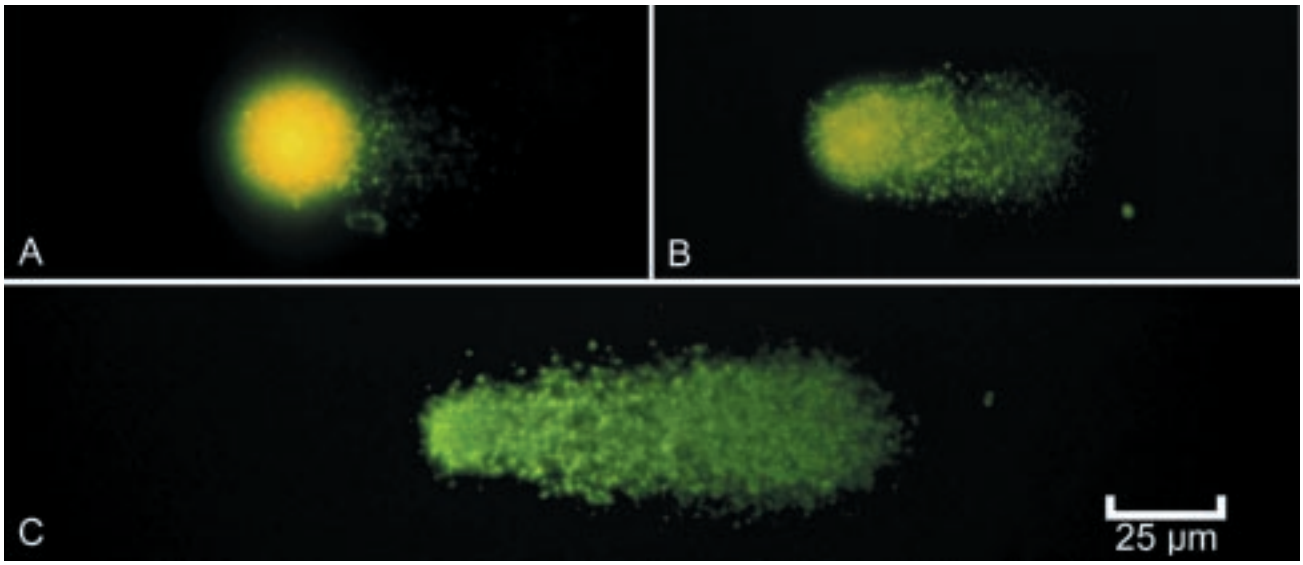


FIGURE 3. In the alkaline single cell gel (SCG) assay, as fragments of damaged DNA migrate in the electric field, they produce the appearance of the tail of a comet. These figures are representative of those seen in (A) control, up to 1 mM methyl As^{V} or dimethyl As^{V} (no DNA damage); (B) 1 mM inorganic As^{III} or inorganic As^{V}, or 10 mM methyl As^{III} (some DNA damage); and (C) exposures as low as 23 mM dimethyl As^{III} (appreciable DNA damage).

While the study results do not rule out the possibility that inorganic arsenic compounds may be genotoxic, the findings suggest that methylated trivalent arsenic compounds, produced in the body during the metabolism of inorganic arsenic, may cause direct damage to cellular DNA. The researchers plan further studies examining the genotoxicity of arsenic compounds and the possibility of a link between arsenic-induced DNA damage and the development of cancer following exposure to inorganic arsenic.

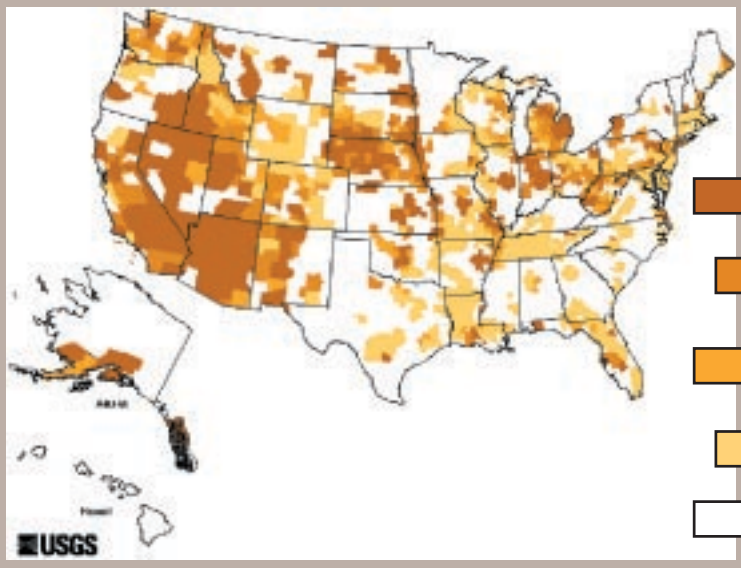
ARSENIC EXPOSURE IN A COMMUNITY

After a previous study suggested that locally harvested shellfish may contain large amounts of arsenic, a Native American Tribe requested EPA's assistance in determining the extent of Tribal members' exposure to arsenic in their food and drinking water. Scientists from NHEERL, National Exposure Research Laboratory (NERL), and the EPA Regional Office are working with the Tribal council to conduct a study of families that have resided in the community of interest for at least 12 months. (The Tribe has requested anonymity.)

All members of each participating household went to the community medical center for enrollment in the study. (Children in diapers are the only family members not eligible to participate.) Information about each individual's diet in the previous four days, water consumption habits, exposure to substances that affect arsenic metabolism, and underlying health conditions was obtained via questionnaire. Participants provided blood and urine samples at enrollment and samples of urine from the first morning void for the two days following enrollment. Also, participants were asked to record every food item

Gathering clams at low tide.

Arsenic Concentrations in Groundwater



- Counties with arsenic concentrations exceeding 10 µg/L in 10 percent or more of samples.
- Counties with arsenic concentrations exceeding 5 µg/L in 10 percent or more of samples.
- Counties with arsenic concentrations exceeding 3 µg/L in 10 percent or more of samples.
- Counties with fewer than 10 percent of samples exceeding 3 µg/L, representing areas of lowest concentration.
- Counties with insufficient data in the USGS data base to make estimates.

Citation:
Welch, A.H., Watkins, S.A., Helsel, D.R., and Focazio, M.F., 2000, Arsenic in ground-water resources of the United States: U.S. Geological Survey Fact Sheet 063-00, 4p.

consumed for a 24-hour period that coincided with the morning urine samples. Each household also collected a sample of water from the kitchen tap. One household member from each participating family was asked to provide a sample of all seafood, fish, potatoes, and rice consumed during the study period. The data collection phase of the project was completed in 2001.

Fish and shellfish samples will be analyzed for the different forms of arsenic using techniques recently developed by EPA researchers. Water, blood, and urine samples will be analyzed for arsenic and selenium, which is believed to affect the way the body metabolizes arsenic. Urine from the oldest member of each household will be analyzed for a series of other metals in addition to arsenic.

This project is the first EPA study to examine the association between different forms of arsenic in seafood and water and the forms of arsenic circulating in blood and eliminated in urine.

photo courtesy of University of Michigan's
Natural Resources Research Institute

The Clean Water Act requires states, territories, and tribes to report the condition of surface waters to EPA every two years. EPA then reports to Congress on the condition of the Nation's waters. States, territories, or tribes set water quality standards and designate the uses (e.g., drinking water supply, swimming, fishing) of each water body. In the reports to EPA, surface waters that do not meet water quality standards are listed as impaired in their ability to support the designated uses.

Maintaining ecological integrity in aquatic ecosystems involves protecting these natural resources from degradation of habitat, reduction in diversity of plant and animal species, and disruption of ecosystem functions. To this end, environmental managers must be able to

- assess the condition of an aquatic resource,
- determine the degree of impairment,
- diagnose the cause(s) of impairment (the stressors),
- forecast the effects of changing stressor levels, and
- design and implement restoration and maintenance strategies.

AQUATIC STRESSORS IMPLEMENTATION PLAN

Within NHEERL, a research implementation plan is a mechanism for prioritizing research efforts to ensure that projects are relevant and responsive to EPA's needs. (See the Air Toxics chapter for a discussion of implementation plans.) In 2001, the NHEERL Aquatic Stressors Implementation Plan was completed and readied for external peer review. This Plan outlines research goals and a multi-year timetable for research projects on stressor diagnostics and four types of stressors (habitat alteration, nutrients, toxic chemicals, and suspended/bedded sediments). NHEERL's aquatic stressors research is focused on (1) diagnosing the causes of aquatic ecosystem impairment and (2) investigating stressor-response relationships. Knowledge of stressor-response relationships will provide the scientific foundation to guide remediation and restoration activities and will enable resource managers to forecast the benefits and/or consequences of changing stressor levels. Specific projects are being developed to investigate the influence of the four types of stressors on fish and shellfish populations. The

Nationwide, 44% of stream or river miles; 49% of lakes, reservoirs, and ponds; 98% of Great Lakes shoreline miles; and 42% of estuaries have been designated as impaired. (National Coastal Condition Report, 2001)

photo courtesy of Dr. Vera Trainer, NOAA/NWFSC Seattle

A harmful algal bloom (white and tan areas in the middle of the water body) off the central California coast in the late spring of 1998.

steering committee for the Plan included representatives from each of ORD's laboratories and centers, EPA Regional Offices, and EPA's Office of Water.

STRESSOR DIAGNOSTICS

One research area identified in the Aquatic Stressors Implementation Plan is development of diagnostic tools to identify chemical and nonchemical stressors over a range of geographic scales. In addition to information about individual water bodies, information about stressors at region and watershed levels is vital to planning integrated restoration and remediation programs. In 2001, NHEERL drafted the Stressor Diagnostics portion of the Aquatic Stressors Implementation Plan to guide this research. Working with other ORD Labs and Centers, the tools developed under this Plan will enable resource managers to

- define the primary causes of impairment,
- assign responsibility for the observed effects among the various stressors, and
- assess potential interactions among stressors.

In addition to providing the foundation for ecosystem maintenance and restoration programs, the information gained by using these tools will support EPA's Total Maximum Daily Load Program, Superfund, and other regulatory activities.

The goal of EPA's Total Maximum Daily Load (TMDL) Program is attainment of water quality standards. The TMDL is a written, quantitative assessment of water quality problems and contributing pollutant sources. It is prepared by the state or local water quality manager. The TMDL

- identifies the need for point and nonpoint source controls,
- provides a basis for action to restore a water body,
- specifies the quantity by which a pollutant needs to be reduced, and
- allocates pollutant load reductions within a watershed.

The Lake Michigan eutrophication modeling study addressed questions that included:

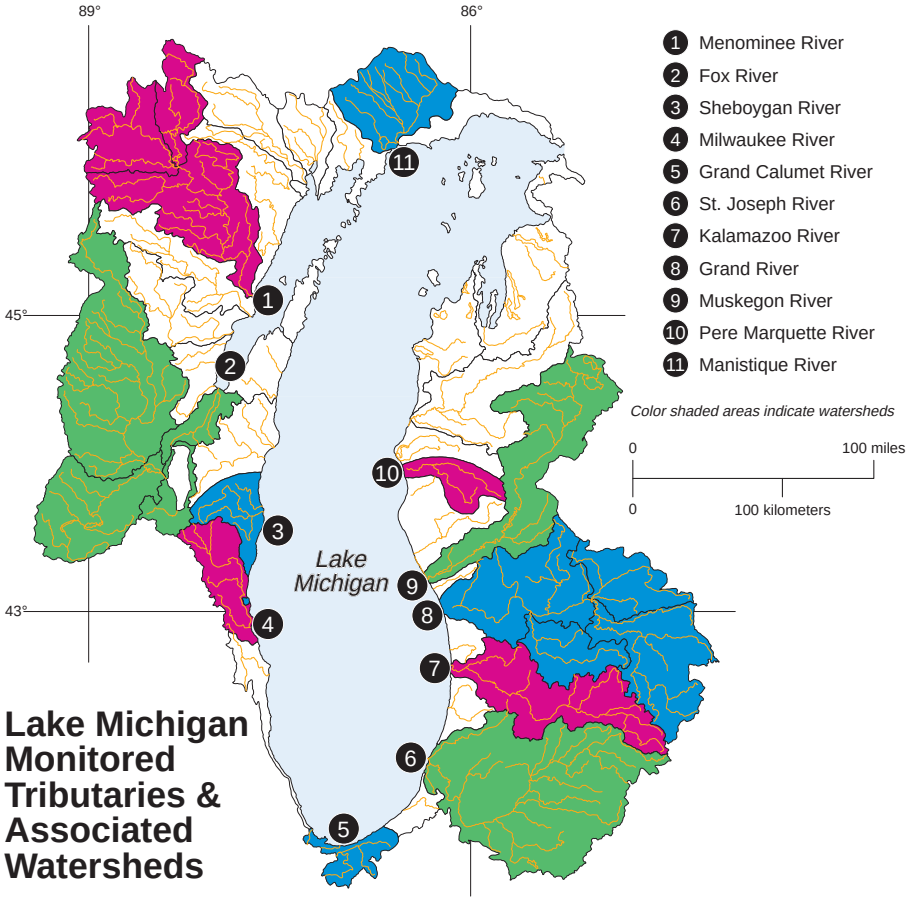
- Which media (atmospheric deposition, tributaries, or sediment resuspension) are the major sources of phosphorus to Lake Michigan?
- Are specific tributaries major contributors? What are the nearshore zone effects of these tributaries?
- What is the history of phosphorus loads to Lake Michigan?
- Do total phosphorus loads and ambient concentrations of phosphorus and chlorophyll a (measure of phytoplankton numbers) meet the Canada-U.S. Water Quality Agreement?
- If increases or decreases in phosphorus loads occur in the future, what are the forecasted consequences or benefits?

EUTROPHICATION MODELING

One type of aquatic stressor is eutrophication due to human activities. In this context, eutrophication refers to high levels of nutrients in water bodies from sewage discharge or agricultural or urban runoff. Eutrophication may produce a number of effects, including algal blooms, which may shade out submerged vegetation and ultimately cause fish kills due to reduced dissolved oxygen levels. Nitrogen and phosphorus are nutrients that may be found in excess in surface waters as a consequence of fertilizer-rich agricultural and urban runoff.

The Lake Michigan Mass Balance Study is a collaboration among EPA's Great Lakes National Program Office and a number of EPA, federal, state, academic, and private partners. As part of this larger study, NHEERL and other ORD scientists developed three computer models to examine the relationship between phosphorus and phytoplankton (microscopic plant-like aquatic organisms, including many types of algae) in Lake Michigan. In 2001, the eutrophication databases were completed, the models were calibrated, and simulation studies were conducted.

The model simulations showed good agreement with observed data. The simulation results indicated that monitored tributaries emptying



Lake Michigan Monitored Tributaries & Associated Watersheds

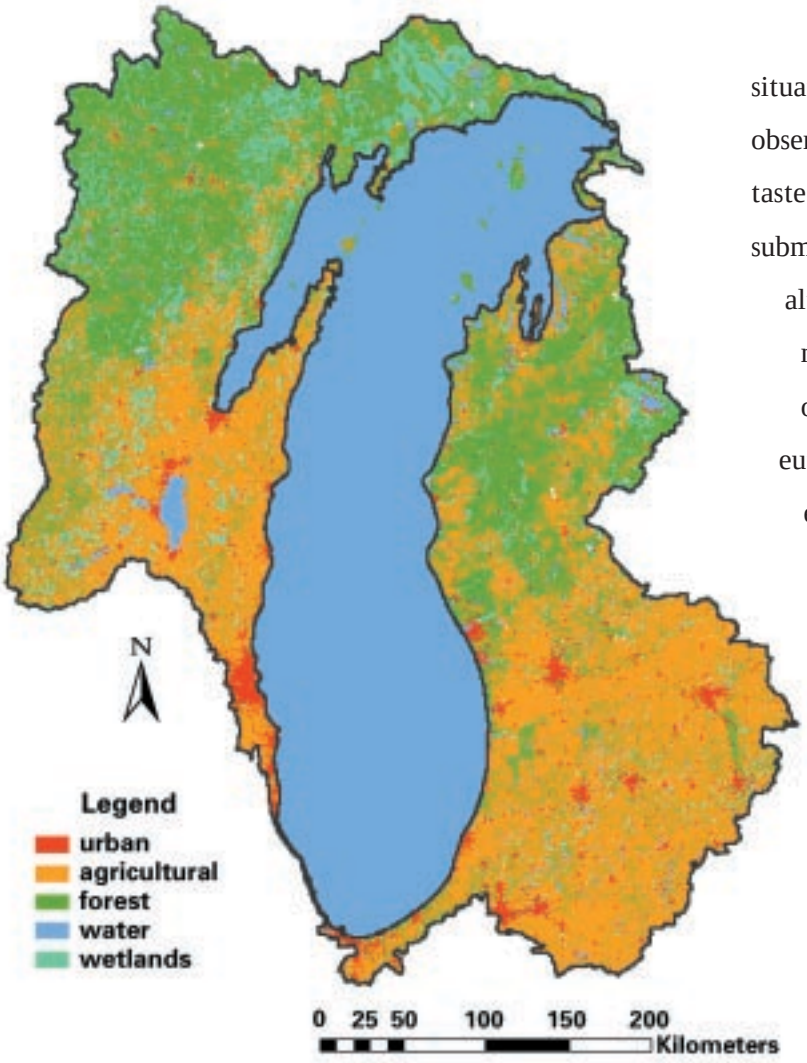
into Lake Michigan were the largest contributors of phosphorus to the lake. Also, substantial amounts of phosphorus were present in Lake sediments. Sediment resuspension contributed appreciably to phosphorus levels in the water. Both phosphorus loading data and model simulations indicated that phosphorus loadings have been stable for the last decade.

Targets for phosphorus loading and ambient water concentrations established by the Canada-U.S. Water Quality Agreement were being met on a lakewide basis. However, localized phosphorus concentrations varied. Model applications using the finest resolution indicated that nearshore and coastal zones were being degraded due to localized phosphorus inputs even though lakewide target levels were met. The Mass Balance Study scientists concluded that target nutrient levels for nearshore and coastal environments, especially near tributary inputs, may need to be re-evaluated to provide adequate ecosystem protection for these areas.

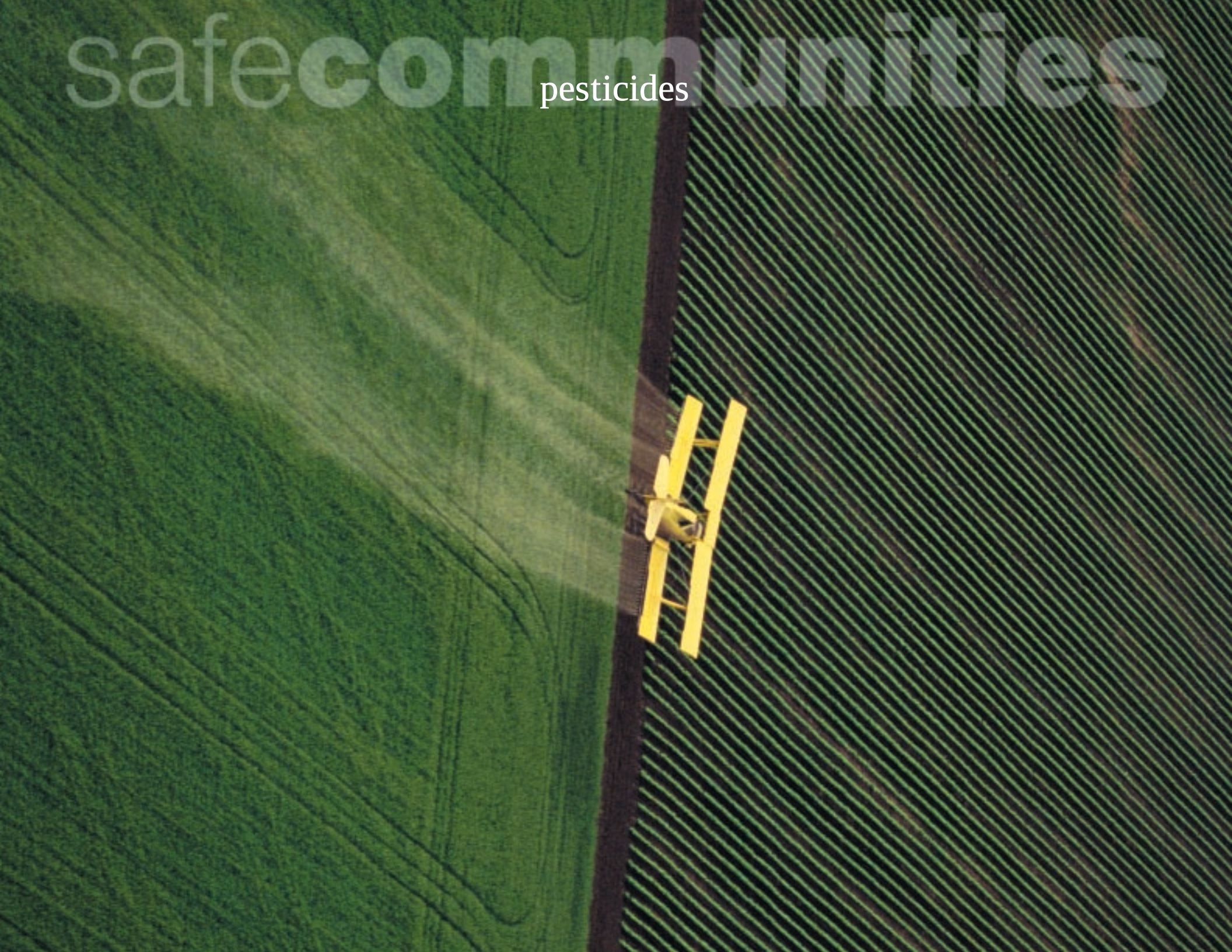
Environmental managers have expressed concern that phosphorus loadings to Lake Michigan will increase in the future due to human population increases and land use changes. The eutrophication model forecasted that a phosphorus loading increase of 20 percent or more would challenge targets for lakewide phosphorus loading, ambient phosphorus concentration, and steady-state phytoplankton population. This

Zebra mussels and *Bythotrephes*, a zooplankton, are two non-native species that are influencing Great Lakes nutrient dynamics, the lower levels of the aquatic food chain, and fish populations. Zebra mussels filter phytoplankton (microscopic plant-like algae) for food. Because of their tremendous filtration capacities, they remove phytoplankton, particles, and nutrients from the water column and deposit them in sediment. By filtering large amounts of phytoplankton, the zebra mussels compete with zooplankton, some larval fish, and foraging fish for the same food source. Similarly, *Bythotrephes* consumes other zooplankton and competes with zooplankton, larval fish, and foraging fish for this food source. In both cases, selectivity and competition for the same food sources appear to be adversely affecting both foraging and predatory fish populations, especially when these food sources are required at critical life stages.

zebra mussel photo courtesy of University of Michigan's Center for Great Lakes and Aquatic Sciences



situation could produce symptoms commonly observed in eutrophic water bodies: algal blooms, taste and odor problems in drinking water, submerged vegetation loss, food chain structure alterations, and water clarity reductions. The model predicted that reduced dissolved oxygen, another frequent consequence of eutrophication, was unlikely to be a problem even if phosphorus loadings increased by 20 percent. (Dissolved oxygen in Lake Michigan is considered to be saturated or supersaturated, which is characteristic of cold, deep lakes with low productivity. Even if productivity increased substantially, dissolved oxygen would not decrease greatly.)


safecommunities
pesticides

Many of NHEERL's research projects directly support EPA's decisions to regulate pesticides and toxic chemicals under the Federal Insecticide, Fungicide, and Rodenticide Act and the Toxic Substances Control Act. In order to develop regulations that protect public and environmental health, policymakers need scientific information about a chemical's persistence in the environment and its toxicity to humans and other animals.

Researchers at several NHEERL divisions are investigating the means by which pesticides may cause health problems, including immune system suppression, cancer, nervous system dysfunction, and endocrine disruption. These studies examine the role of variables such as the dose and duration of exposure to the pesticide and the developmental stage — fetus, newborn, immature young, mature adult — of the exposed individual. Another area of research is development of computer models that can predict the toxicity of new chemicals based on their similarity to other chemicals whose toxic activity has been determined in laboratory tests.

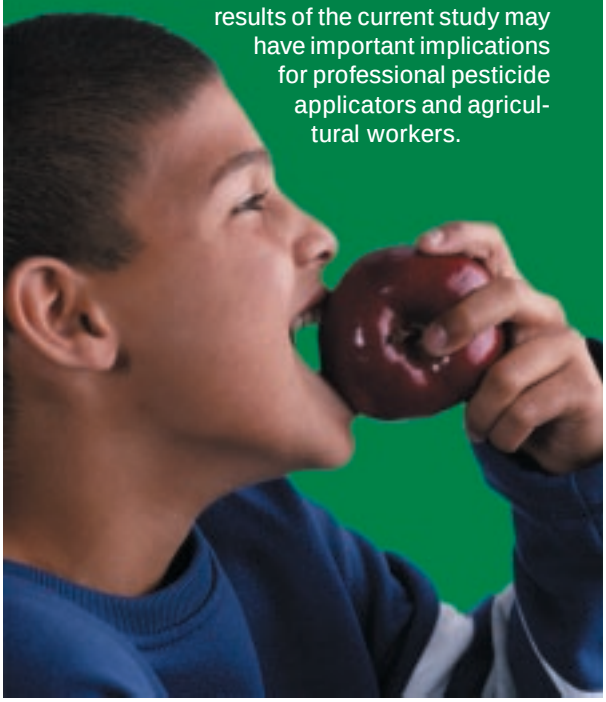
HEPTACHLOR

Heptachlor is an organochlorine compound that was used as an agricultural and domestic pesticide from the mid-1960s to the early 1980s. In 1974, EPA canceled its registration for all uses except subterranean termite and fire ant control, treatment of field corn, seeds and bulbs, citrus, and pineapple. By 1983, EPA had phased out

Safe Food

While the health effects of short-term exposure to high doses of pesticides have been studied fairly well, the potential adverse consequence of chronic exposure to lower pesticide levels has not been thoroughly investigated. Researchers at NHEERL have been collaborating with scientists from North Carolina State University to study long-term exposure of rats to the organophosphorous pesticide chlorpyrifos. The primary purpose of the study is to determine what, if any, adverse health effects may be caused by chronic exposure and what pattern of exposure is most harmful. The scientists also hope to learn if chlorpyrifos must enter the brain and spinal cord to cause problems or if a body burden limited to the organs and peripheral nerves can cause adverse health effects. The scientists assessed a large variety of endpoints including visual, neurophysiological, nervous system, and liver function; learning and memory; overt toxicity; pathology of all major organs; and tissue distribution of chlorpyrifos and its metabolites. In 2001, the exposure phase of the study was completed and end-of-exposure assessments were conducted.

Based on previous studies, EPA recently revised the risk assessment and risk mitigation measures for chlorpyrifos. Sale of products containing chlorpyrifos for use by homeowners and in schools, parks, and other settings where children may be exposed ended December 31, 2001. Some uses of chlorpyrifos are still allowed; results of the current study may have important implications for professional pesticide applicators and agricultural workers.



many of the remaining registered uses, including that for pineapples. The U.S. manufacturer voluntarily discontinued heptachlor production in 1988. The chemical is still of interest because heptachlor and its major metabolite, heptachlor epoxide, are stored in fat and persist in the environment. In fact, heptachlor epoxide is more toxic and more biologically persistent than heptachlor.

In a cooperative venture with the Hawaii Heptachlor Research and Education Foundation and the National Institute of Environmental Health Sciences, NHEERL scientists studied the effects of perinatal-plus-juvenile exposure to heptachlor on the developing immune systems of rats. Researchers exposed pregnant rats to heptachlor from midgestation through post-natal day 7. Pups were exposed to heptachlor from 8 to 42 days of age. In addition to litter size, pup growth rate, pup survival, reproductive system characteristics, reproductive capacity, and several other indicators of health, several immune system functional endpoints were evaluated in the pups. The most significant immune system finding was a dose-dependent suppression of the antibody response at 8 weeks and 26 weeks of age (Figure 1).

These findings are consistent with other studies that point toward a predisposition for the male

When most uses of heptachlor were canceled in 1974, the pineapple producers in Hawaii were granted an extension until December 1982 that enabled them to use up existing stocks of the pesticide. During the same time period, green chop—the chopped leaves of pineapple plants after the fruit has been harvested—was promoted as an economical feed for dairy cattle. In early 1982, high levels of heptachlor epoxide were found in a routine milk test at a state Health Department laboratory. This finding eventually resulted in a series of 11 recalls of milk, other dairy products, and meat from dairy cattle.

The University of Hawaii had founded a Pesticide Hazard Assessment Project (PHAP) in the 1960s. Shortly after the recalls were begun, the director of PHAP started testing breast milk that was donated to a milk bank during 1982 through 1984. In 350 samples, the average concentration of heptachlor epoxide in human milk fat was 100 ppb. The maximum was 438 ppb. By comparison, the federal standard for heptachlor epoxide in drinking water is 0.2 ppb.

Two class action lawsuits were filed by two local environmental groups and approximately 100 mothers and children. In 1986, the Hawaii Heptachlor Research and Education Foundation, whose purpose is medical monitoring, scientific research, and education regarding the potential health effects of heptachlor exposure, was founded as part of the settlement of these lawsuits.

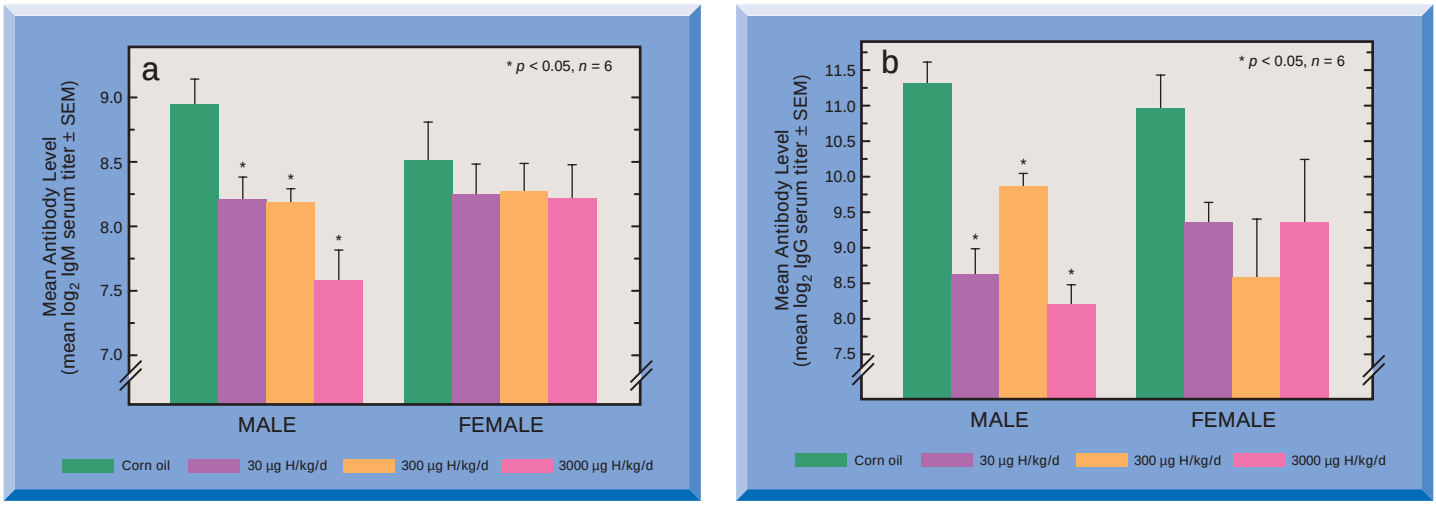


FIGURE 1. The immunosupressive effect of perinatal-plus-juvenile exposure to heptachlor (H). Sheep red blood cells were used as an antigen to induce a specific, measurable, antibody response. The production of antibodies after contact with an antigen requires the coordinated function of several different immune cells. The lowest dose was selected to produce heptachlor epoxide levels in the rats' milk comparable to levels that had been found previously in human milk. (a) Primary IgM antibody response at 8 weeks of age. (b) Secondary IgG antibody response at 26 weeks of age.

immune system to be suppressed by certain organochlorine chemicals. However, these studies are preliminary in nature, and it is too early to draw any conclusions regarding potential implications for humans exposed to organochlorine pesticides. Future research will determine if exposure to heptachlor influences susceptibility to infectious disease in rats. Additional studies will define the critical time periods for exposure and the cellular and molecular mechanisms by which heptachlor influences immune system development.

TRIAZINE HERBICIDES

Atrazine is a widely used triazine herbicide. An estimated 68 to 72 million pounds of atrazine were used in the United States in 1995, primarily to control annual grasses and broadleaf weeds in the cultivation of food crops and conifers. In the heavily farmed Midwest, many drinking water sources, including groundwater, contain triazine herbicides. Because atrazine has induced mammary tumors in female laboratory rats and is so widely used, concerns about potential adverse health effects in humans have arisen.

Many compounds that cause cancer damage the DNA of cells; that is, they are genotoxic. Previous *in vitro* and *in vivo* studies of atrazine's potential for genotoxic activity produced conflicting results. In an attempt to determine if three triazine herbicides — atrazine, cyanazine, and simazine — are genotoxic, NHEERL scientists conducted a series of three experiments. Only one study found small to negligible amounts of DNA damage in the white blood cells of mice

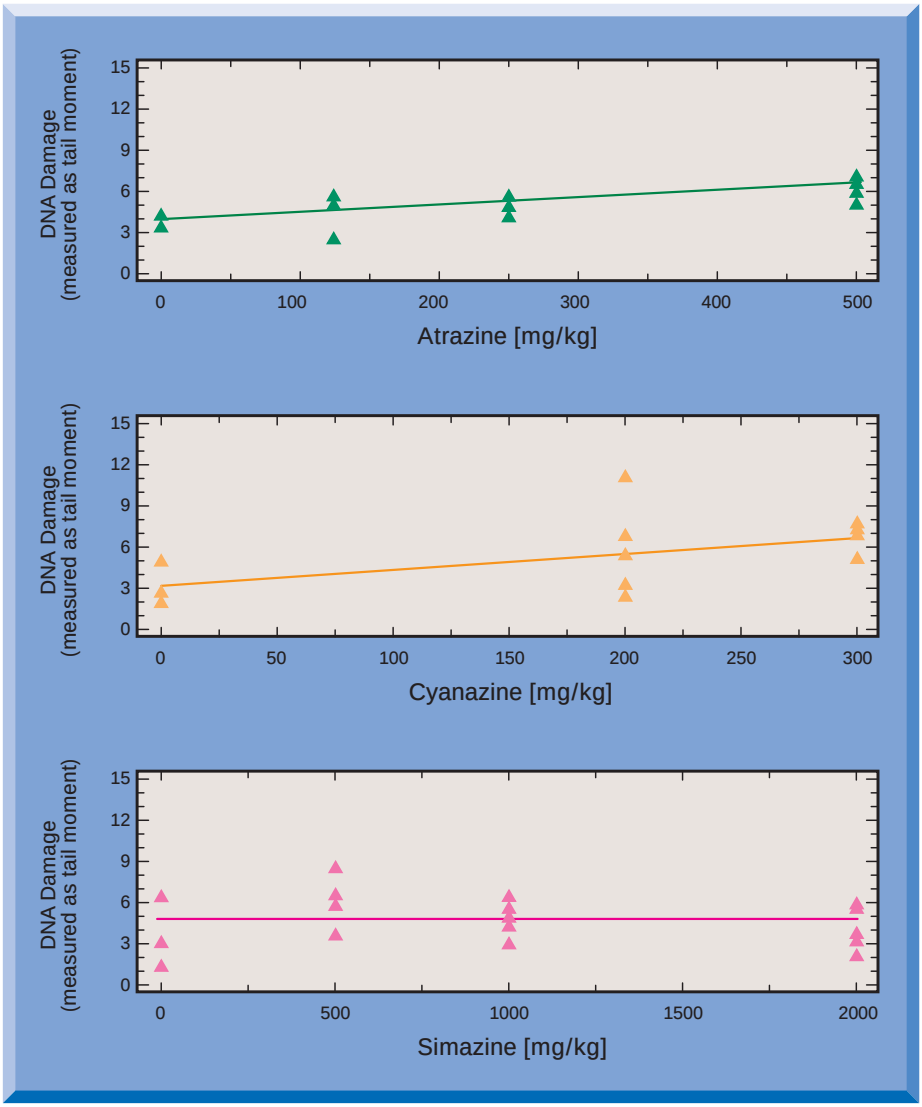


FIGURE 2. Relationship between DNA damage and dose of each herbicide. A = atrazine; B = cyanazine; C = simazine. Note: different doses were used for each herbicide because of differences in acute toxicity.

exposed to atrazine, cyanazine, and simazine. In this study, atrazine caused the most appreciable amount of damage, but only at doses that were highly toxic to the animal. Previous studies found that atrazine caused mammary tumors in one strain of intact female laboratory rats, but not in others. Evidence from those earlier studies pointed toward a hormonal mechanism that is unique to that strain of rats. Because these recent studies by NHEERL scientists found that atrazine damaged DNA only at high doses and that cyanazine and simazine did not cause significant DNA damage even at toxic doses, the scientists concluded that these three triazine herbicides do not pose a genotoxic threat to humans at the levels currently found in the environment. (See the Endocrine Disrupting Chemicals chapter for more on atrazine risk assessment.)

TOXICITY MODELS

An important component of EPA's mandate to protect public health and the environment is to assess the potential risks that new or existing pollutants may pose to humans and ecosystems. A number of groups within EPA use structure-activity relationship (SAR) concepts to establish toxicity testing requirements and to support regulatory actions. A central assumption of SAR methods is that structurally similar chemicals likely act through a common mechanism of action.

To date, a large amount of SAR research has focused on developing predictive SAR models for rodent carcinogenicity. This is due to the regulatory importance of carcinogenicity in assessing the risk of environmental chemicals and the tremendous investment of time and money required for two-year rodent carcinogenicity studies. Collaborating with an international SAR expert from the Instituto Superiore in Rome, Italy, an NHEERL scientist recently coauthored a review and critique of available SAR and artificial intelligence models for predicting rodent

Within a group of chemicals, if differences in chemical properties or structural features can be related to changes in biological activity, this knowledge may be used to predict the activities of new chemicals with similar characteristics.

carcinogenicity. The more complex models supplemented chemical structure information with biological information derived from cell and tissue culture studies and other sources. The review described the various types of models in use, their limitations, and their relative success in recent prediction "contests." The review also indicated potential directions for model improvement.

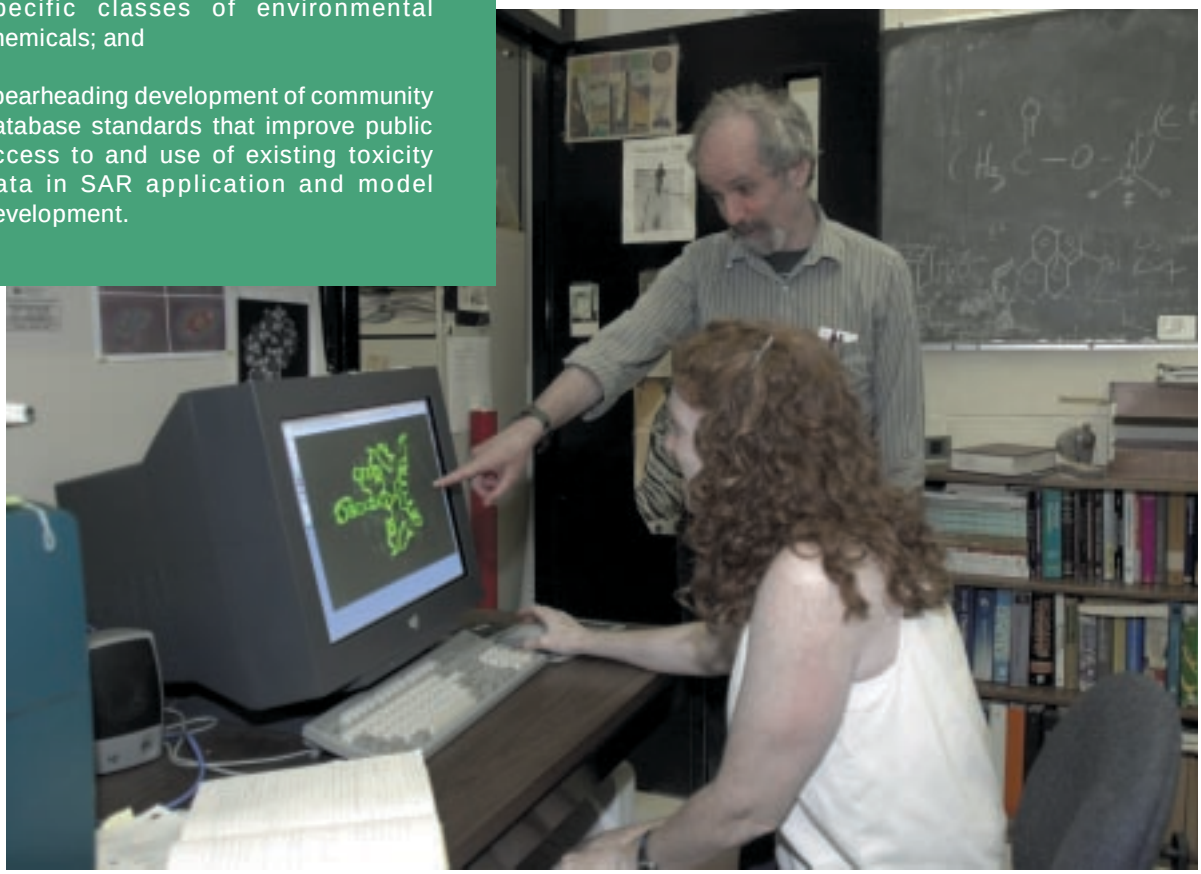
In more direct SAR applications, NHEERL researchers have modeled key metabolic steps and identified molecular mechanisms for well-defined chemical classes and biological endpoints. For example, scientists from NHEERL, CIIT Centers for Health Research, Meijo University in Japan, and the University of Missouri at St. Louis collaborated to study a series of organophosphate pesticides and related compounds capable of interacting with the male hormone (androgen) receptor in cells. The project yielded a theoretical model that linked specific structural features of the chemicals with differences in androgen receptor activity. This knowledge can be a useful component of an SAR strategy to screen similar types of chemicals for androgenic activity. As SAR technology develops, it is likely to become a very important tool when screening chemicals for endocrine-disrupting activity. (See the chapter, Endocrine Disrupting Chemicals, for information on NHEERL's work to develop laboratory-based screening tests.)

In an SAR project that has potentially much broader scope and impact, NHEERL investigators

are developing and promoting a database standard for public toxicity databases that includes chemical structures. This standard format will enable scientists and others to search across and within these databases by defining specific structural characteristics in the search parameters. The goal of this effort is to enlist the toxicology and modeling research communities in creating a decentralized network of Web-accessible, standard toxicity databases that can be downloaded and used in an unrestricted manner by persons in government, academia, public interest organizations, and industry. These databases will span multiple toxicity endpoints (e.g., cancer, nervous system dysfunction, and immune suppression) and will be located at widely distributed sources such as EPA, other federal agencies, and selected academic sites. This project has the potential to greatly improve the ability to explore and model public toxicity information from a chemical structure perspective.

EPA and ORD are continually working to improve the efficiency of toxicological assessment. In this context, NHEERL's SAR researchers have been:

- evaluating and providing guidance to the scientific community in the use of SAR methodologies for toxicity screening;
- using computational and SAR approaches to study mechanisms of toxicity for specific classes of environmental chemicals; and
- spearheading development of community database standards that improve public access to and use of existing toxicity data in SAR application and model development.




climatechange
global change

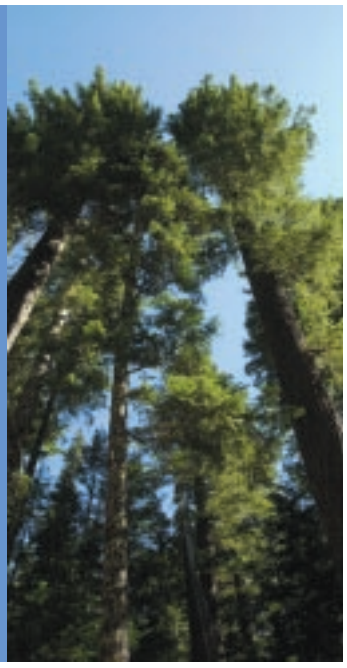
As a participant in the U.S. Global Change Research Program, EPA's role is to assess the impact of global climate change on ecological and human health and to assess strategies for adapting to climate changes. During these assessments, climate change is viewed as one of many stressors that may interact to cause adverse effects. Several specific questions are being addressed in EPA's Global Change Research Strategy.

- What are the potential consequences of climate change and climate variability on human health, ecosystems, and social well-being in the United States?
- What are the indicators of climate change at population, community, and ecosystem levels of organization?
- How can one identify future ecological vulnerabilities on a range of spatial scales resulting from the joint effects of changes in climate, sea level, and other stressors such as pollutants and land use?
- How do climate-induced changes like temperature, moisture, and atmospheric composition affect the biology of ecosystems?
- How are human and ecosystem exposures to UVB radiation changing and what are the effects of these exposures?

NHEERL scientists have been studying the effects of global climate change in a variety of ecosystems across the nation, including coral reefs, wetlands, and forests. They have investigated the impact of climate change on a variety of plant and animal species including birds, fish, mammals, and amphibians. Recently completed projects examined the impact of global climate changes on terrestrial ecosystems. Specifically, scientists investigated the qualitative and quantitative effects of elevated carbon dioxide, temperature, and ozone on tree growth and on biogeochemical processes in forests. Field

This coral shows evidence of the bleaching that occurs when symbiotic algae are lost, which is one consequence of environmental stress associated with global climate change.

Carbon dioxide and tropospheric ozone are two major pollutants associated with industrialization and urbanization. Carbon dioxide is necessary for plant growth. However, at elevated levels, it is a major greenhouse gas that contributes to global warming. Elevated levels of ozone in the troposphere (the atmospheric layer nearest the ground) have been associated with damage to forests and health problems in humans. NHEERL scientists studied the separate and combined effects of these two stressors on forest ecosystems in the Pacific Northwest.



studies were conducted across a transect in the Pacific Northwest that included coastal and Cascade Range Douglas fir-hemlock forests and western juniper forests. Multi-year experimental studies were conducted in controlled-environment chambers where climatic factors and chemical and physical characteristics of the soil were monitored. Two research efforts combined experimental and computer modeling methods to determine the effects of climate change on plant and soil processes. One study examined the effects of elevated carbon dioxide and temperature on a Douglas fir seedling ecosystem. A second study investigated the effects of increased carbon dioxide and ozone on a Ponderosa pine seedling ecosystem. The studies measured carbon and nitrogen inputs, reservoirs, fluxes, and losses. Data collected in these experiments were used in the computer models, which provide a consistent analytic framework and a conceptual basis for (1) integrating diverse measures into an internally consistent framework, (2) relating stressors to probable effects, and (3) making meaningful extrapolations across scales of time, space, and biological organization.

In Douglas fir seedlings, increased temperature caused several physiological changes that affected growth. However, there was no net influence on seedling biomass because the increases in growth that occurred in response to some physiologic changes were offset by the growth-stunting effects of other physiologic changes. Elevated carbon dioxide increased water use efficiency but did not alter plant growth or carbon uptake and distribution through the test ecosystems. Subsequent modeling studies confirmed that low soil nitrogen limited the response of Douglas fir seedlings to elevated carbon dioxide levels.

Nutrient Cycling and Global Change

Ecosystem stability depends on the regular cycling of nutrients, water, and energy through different components and levels of the system. As key indicators of ecosystem function, the cycling of carbon, nitrogen, and water through living systems can be used to study the impact of environmental stressors. Previous studies found that elevated levels of carbon dioxide in the atmosphere stimulated cycling of carbon and nitrogen but inhibited cycling of water; elevated ozone inhibited cycling of all three substances.

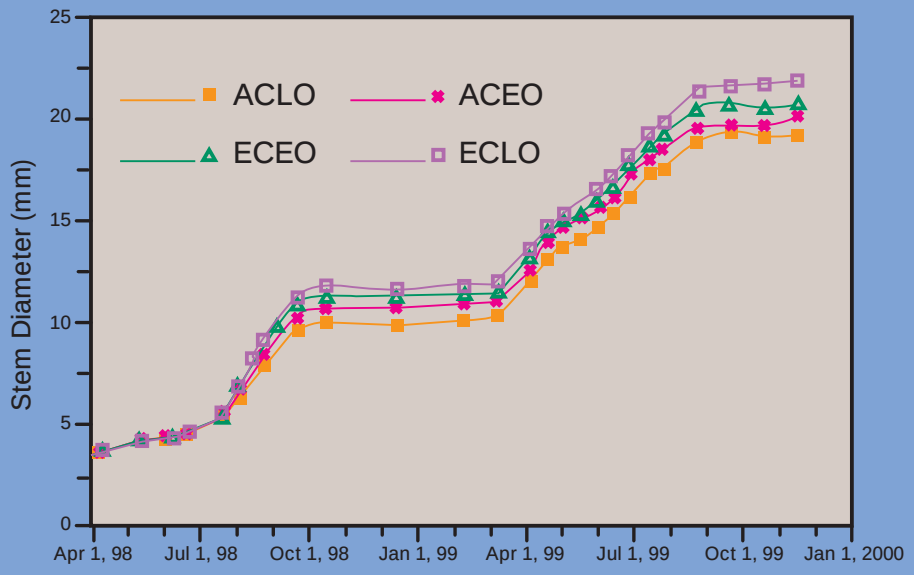
Stem diameters for ponderosa pine seedlings exposed to elevated levels of carbon dioxide and/or ozone.

ACLO = ambient carbon dioxide, low ozone.

ACEO = ambient carbon dioxide, elevated ozone.

ECEO = elevated carbon dioxide, elevated ozone.

ECLO = elevated carbon dioxide, low ozone.



In Ponderosa pine seedlings, elevated carbon dioxide caused an increase in growth even though soil nitrogen was low, suggesting that different plant species vary in their response to changing climatic conditions. Elevated ozone levels decreased both shoot and root growth.

These NHEERL projects support EPA's global climate change research program by providing (1) biological data on basic ecosystem processes, the biogeochemistry of terrestrial systems, and the effects of climate change factors on these processes and systems; (2) a parameterized model (TREGRO) for plant growth simulations; and (3) a parameterized model (GEM) for biogeochemical simulations. These models will be available for public and private groups to use in future assessments of the effects of global climate change.

An NHEERL scientist climbs to great heights to monitor the health of a forest.


soundscience
ecosystems protection

As part of its mandate to protect the environment, EPA conducts and sponsors ecosystems research. The goal of this research is to provide scientific leadership and the knowledge necessary to assess, improve, and restore–at multiple geographic scales–the integrity and sustainability of various types of ecosystems. This research program has four fundamental areas: monitoring, processes and modeling, risk assessment, and risk management and restoration.

CONDITION OF THE NATION'S ESTUARIES

The National Coastal Condition Report, finalized in November 2001, results from a cooperative venture among many offices and agencies. The Office of Wetlands, Oceans, and Watersheds (EPA Office of Water) and the ecology divisions of NHEERL were the lead organizations in producing this report. The report is based largely on data collected during ongoing federal and state coastal monitoring programs. Information was obtained from numerous sources, including EPA's Environmental Monitoring and Assessment Program (EMAP), the National Oceanic and Atmospheric Administration (NOAA), the U.S. Geological Survey (USGS), the U.S. Fish and Wildlife Service (FWS), and state and tribal agencies. Most of the data upon which this report is based were collected in estuaries, the highly productive regions where freshwater rivers and streams meet the ocean. Adequate information was available to fully assess only the estuaries of the Northeast, Southeast, and Gulf of Mexico. Conclusions drawn about West Coast estuaries and the Great Lakes represent partial assessments based on available data. The estuaries of Alaska, Hawaii, and island territories were not evaluated due to lack of data. The National Coastal Condition report may be viewed on EPA's Web site at http://www.epa.gov/owow/oceans/cwap/downloads.html.

Seven primary indicators were used to evaluate the condition of estuarine waters: water clarity, dissolved oxygen, eutrophic condition, fish tissue contaminants, benthic condition, sediment contamination, and coastal wetlands (Figure 1). Although additional ecological indicators were used by some monitoring programs, these seven were the ones used most widely and consistently. Based on the monitoring data, each indicator was assigned a value of good (=5), fair (=3), or poor (=1) for each coastal region—northeastern,

What is an Estuary?

An estuary is a coastal region where a river or stream empties into the ocean, mixing fresh water with salt water. Estuaries are influenced by tides but are protected from the full force of ocean currents and storms by reefs, barrier islands, and/or projections of land, mud, or sand. Because the rivers and streams carry nutrients from the land to the sea, and ocean tides keep the nutrients from settling out, estuaries are among the most productive ecosystems on earth. An estuary generates more organic matter each year than a comparable area of forest, grassland, or farmland. Estuaries and the associated wetlands support a great diversity of living organisms and are the nurseries of numerous marine animals, including most of the commercially important fish and shellfish species. The protected coastal waters characteristic of estuaries also serve as ports and harbors for shipping, commercial fishing operations, and recreational use.

southeastern, Gulf of Mexico, West Coast, and Great Lakes. The seven indicator values were then averaged to assign an overall rating for a region. In calculating the national scores, each region's score was weighted by its area.

Based on available data, the overall condition of the Nation's coastal waters was fair to poor.

Although about 56 percent of the U.S. estuaries evaluated were in good condition to support human use and aquatic life, about 33 percent were impaired for human use, about 34 percent were impaired for aquatic life, and about 23 percent were impaired in their ability to support both human use and aquatic life.

Icon	Poor Condition	Ranking
 Water Clarity	Water clarity is considered poor if less than 10% of surface light reaches a depth of 1 meter.	*Good:* Less than 10% of the coastal waters have poor light penetration. *Fair:* 10% to 25% of the coastal waters have poor light penetration. *Poor:* More than 25% of the coastal waters have poor light penetration.
 Dissolved Oxygen	Dissolved oxygen levels are considered poor when concentrations are less than 2 ppm.	*Good:* Less than 5% of the coastal waters have poor dissolved oxygen. *Fair:* 5% to 15% of the coastal waters have poor dissolved oxygen. *Poor:* More than 15% of the coastal waters have poor dissolved oxygen.
 Coastal Wetland Loss	Areas with a greater than 40% decline in wetland acreage from 1780 to 1980 and/or a greater than 10% decline from the mid-1970s to the mid-1980s are considered to be in poor condition.	*Good:* Less than 25% decline in wetlands' acreage from 1780 to 1980 and/or less than 5% decline from the mid-1970s to the mid-1980s. *Fair:* Between 25% and 40% decline from 1780 to 1980 and/or between 5% and 10% decline from the mid-1970s to the mid-1980s. *Poor:* Greater than 40% decline from 1780 to 1980 and/or greater than 10% decline from the mid-1970s to the mid-1980s.
 Eutrophic Condition	Eutrophic condition is a measure developed by NOAA that examines six different eutrophication symptoms and assigns a value of low, moderate, or high. High eutrophic condition is equivalent to poor condition for this indicator.	*Good:* Less than 10% of the coastal waters have high eutrophic condition. *Fair:* 10% to 20% of the coastal waters have high eutrophic condition. *Poor:* More than 20% of the coastal waters have high eutrophic condition.
 Sediment Contamination	Sediment contamination is evaluated using ERM and ERL criteria. ERM is the concentration of contaminant that will result in ecological effects 50% of the time. ERL is the concentration of contaminant that will result in ecological effects 10% of the time. An estuary is in poor condition if it exceeds one ERM criterion or five ERL criteria.	*Good:* Less than 5% of the coastal waters exceed one ERM criterion or five ERL criteria. *Fair:* 5% to 15% of the coastal waters exceed one ERM criterion or five ERL criteria. *Poor:* More than 15% of the coastal waters exceed one ERM criterion or five ERL criteria.
 Benthic Index	A poor benthic index score indicates that benthic communities are less diverse than expected, populated by greater than expected pollution-tolerant species, and contain fewer than expected pollution-sensitive species.	*Good:* Less than 10% of the coastal waters have a low benthic index score. *Fair:* 10% to 20% of the coastal waters have a low benthic index score. *Poor:* More than 20% of the coastal waters have a low benthic index score.
 Fish Tissue Contaminants	An estuary is in poor condition for fish tissue contaminants if more than 10% of fish sampled have tissue residues greater than FDA and international criteria, or more than 20% of fish sampled have tissue residues greater than EPA Guidance Values.	*Good:* Less than 2% of the coastal waters have poor fish tissue condition. *Fair:* 2% to 10% of the coastal waters have poor fish tissue condition. *Poor:* More than 10% of the coastal waters have poor fish tissue condition.

FIGURE 1. Indicators used to assess coastal condition.

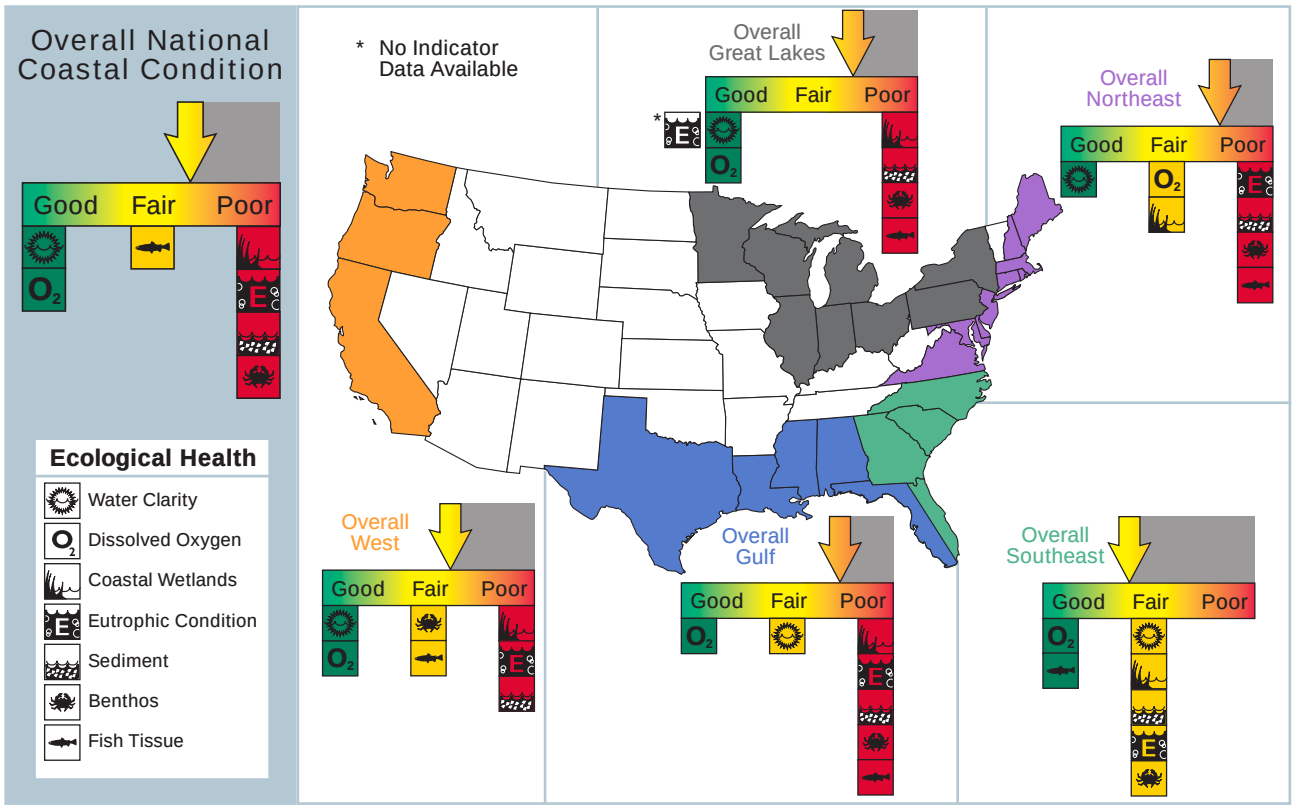


FIGURE 2. Overall national coastal condition and coastal condition by region.

Of the seven ecological indicators, only water clarity and dissolved oxygen levels were rated good overall. Poor light penetration was a problem primarily in the western Gulf of Mexico and western tributaries of the Chesapeake Bay. In the Southeast, naturally high productivity and strong sediment transport and resuspension processes contributed to poor water clarity. The water clarity indicator does not distinguish between human-induced and naturally occurring causes of poor clarity (Figure 2).

Dissolved oxygen is essential to support aquatic life. Low dissolved oxygen levels are often associated with large algal blooms. As large amounts of algae die and sink to the bottom, oxygen is consumed during the decay process.

Shellfish Growing Waters

Between 1990 and 1995, an increasing number of states classified estuarine and nonestuarine waters according to their suitability for growing edible shellfish. In 1995, 60% of the shellfish growing waters were classified as approved. The most common pollution sources that limited shellfish harvests were urban runoff, upstream sources, wildlife, and wastewater treatment systems. At the time of the report, 19 of 21 shellfish growing states were involved in at least one restoration project that either improved water quality, restored habitat, or enhanced shellfish stocks.

Fortunately, low dissolved oxygen levels were a problem only in a few specific areas. Low dissolved oxygen levels are one potential consequence of eutrophication, an increase in nutrient levels. The overall rating for eutrophication was poor and scientists expect eutrophic conditions to become worse in 70 percent of U.S. estuaries by 2020.

Based on data from the East Coast and Gulf of Mexico, the overall rating for fish tissue contamination was fair. Of the fish sampled, 26 percent had elevated levels of contaminants in edible tissues. However, 22 percent were contaminated with organic arsenic compounds not considered to be toxic to humans. Therefore, only 4 percent of sampled fish contained nonarsenical compounds of concern to humans. Fish sampled in the EMAP program were examined for signs of disease and external abnormalities. Bottom-feeding fish had the highest frequency of disease. The number of fish with multiple abnormalities was highest in areas where sediments contained high levels of multiple contaminants.

Benthic condition (as measured by quantity and diversity of bottom-dwelling organisms) and sediment contamination were poor overall. These two indicators were related: 62 percent of the estuaries that scored poor on benthic condition also had contaminated sediments. Benthic

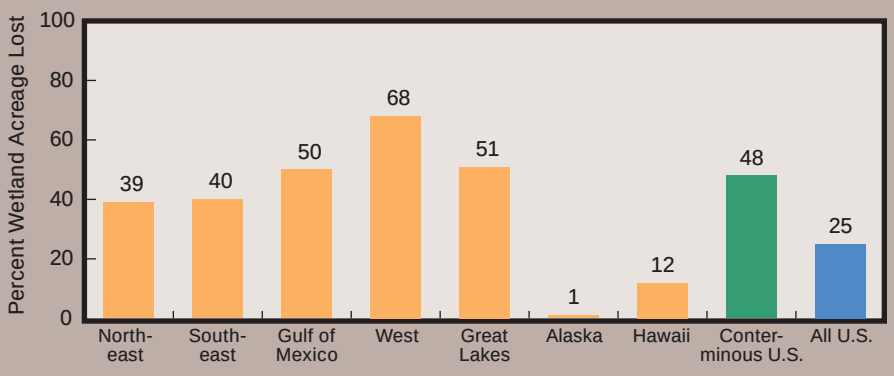


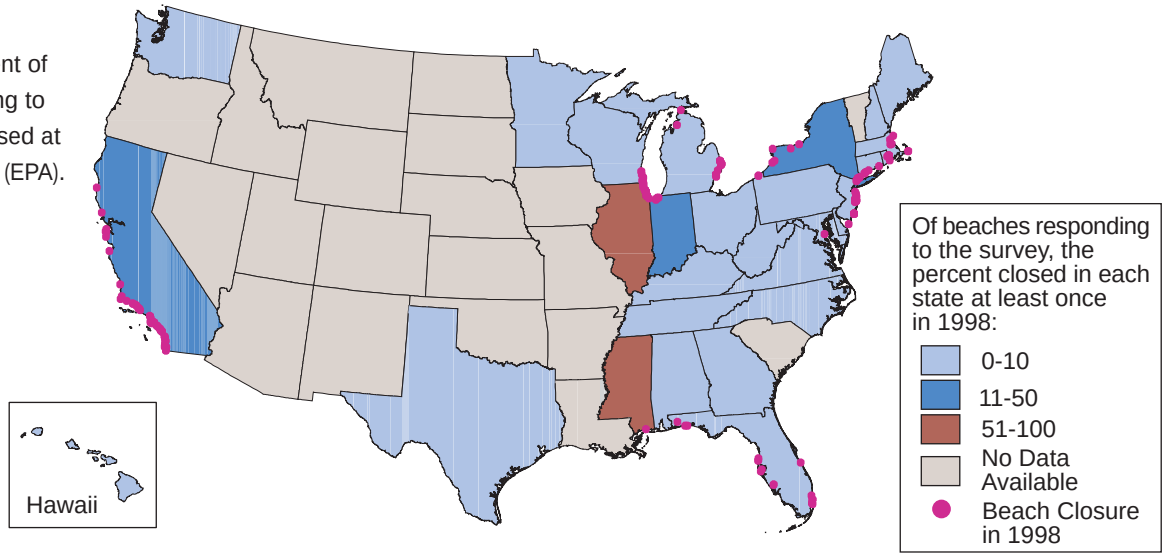
FIGURE 3. Proportion of total wetland acres existing in 1780 lost by 1980 by region of the United States (Turner and Boesch, 1988; Dahl, 1990).

communities in poor condition were characterized by less diversity or abundance of organisms than expected, greater than expected pollution-tolerant species, and/or fewer than expected pollution-sensitive species. The most common sediment contaminants were pesticides, PCBs, and metals. The most heavily contaminated sediments were in the Northeast.

The coastal wetlands indicator earned a poor rating overall. Scientists estimate that nearly 50 percent of the coastal wetlands in the lower 48 states have been lost in the 200-year period between 1780 and 1980. During the mid- to late-1990s, coastal wetland losses in the Southeast and Gulf of Mexico continued at a high rate of more than 1 percent per year (Figure 3).

State water quality assessments (required under the Clean Water Act) and state advisories were examined in preparing the National Coastal Condition report. Although states used different monitoring techniques and methodologies, these data provided important information about coastal condition. In 1998, state water quality reports suggested that 44 percent of assessed estuaries in the continental U.S. were impaired by some type of pollution or habitat degradation. The most frequent impairments were for aquatic life support, swimming, and fish consumption. The major factors causing impairment were pathogens, oxygen-depleting substances, metals, and nutrients. The primary sources of impairing pollutants were municipal point sources, urban runoff or storm sewers, atmospheric deposition, industrial discharges, and agriculture (Figure 5).

FIGURE 4. Percent of beaches responding to the survey that closed at least once in 1998 (EPA).



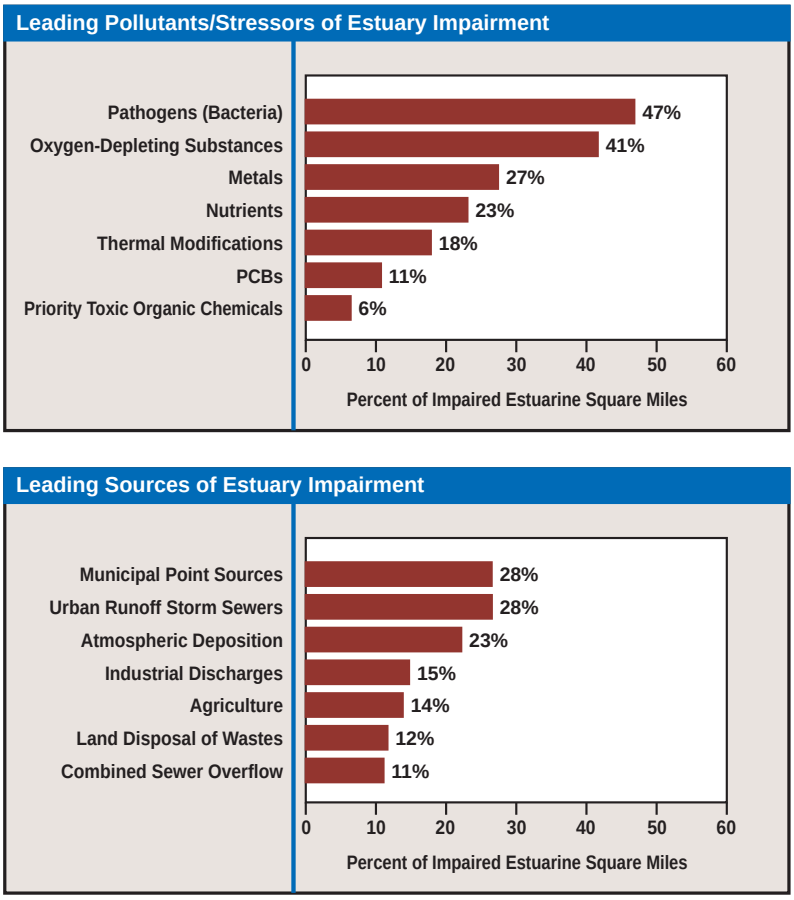


FIGURE 5. Leading factors responsible for estuary impairment in 1998 and sources of those factors.

EPA conducted a voluntary survey of beaches in 1998 (Figure 4). Of the 1,062 coastal beaches that responded, 33 percent had an advisory or closing at least once during 1998. Approximately 16 percent experienced at least one closing. The major causes of beach closure included stormwater runoff, pipeline breaks, and combined sewer overflows (due to storm water and sewage being transported in the same system).

CONDITION OF THE GREAT LAKES

NHEERL ecologists and other scientists are collaborators in a study to assess the condition of the Great Lakes. The lead organization is the Natural Resources Research Institute (NRRI) of the University of Minnesota-Duluth, which received a $6 million grant through EPA's Science to Achieve Results (STAR) program in January 2001. Other collaborators include scientists at the University of Minnesota-Twin Cities, Minnesota Sea Grant, University of Wisconsin-Green Bay, University of Wisconsin-Madison, Cornell University (New York), University of Windsor (Ontario, Canada), John Carroll University (Ohio), and University of Michigan. EPA's Great Lakes National Program Office has a representative on the steering committee (Figure 6).

Environmental indicators are measurable biological, physical, or chemical characteristics that reflect the overall health of the ecosystem or the area being studied.

The purpose of the study is to determine what environmental indicators will most efficiently, economically, and effectively measure the condition, integrity, and sustainability of the Great Lakes basin. The Great Lakes basin covers 200,000 square miles and contains approximately 18 percent of the world's surface fresh water. Because the lakes are interconnected, environmental changes in one area of the basin have a ripple effect on other areas. The environmental health of the basin affects, and is influenced by the activities of, 36 million residents. In addition to providing in-depth information on the condition of the Great Lakes,

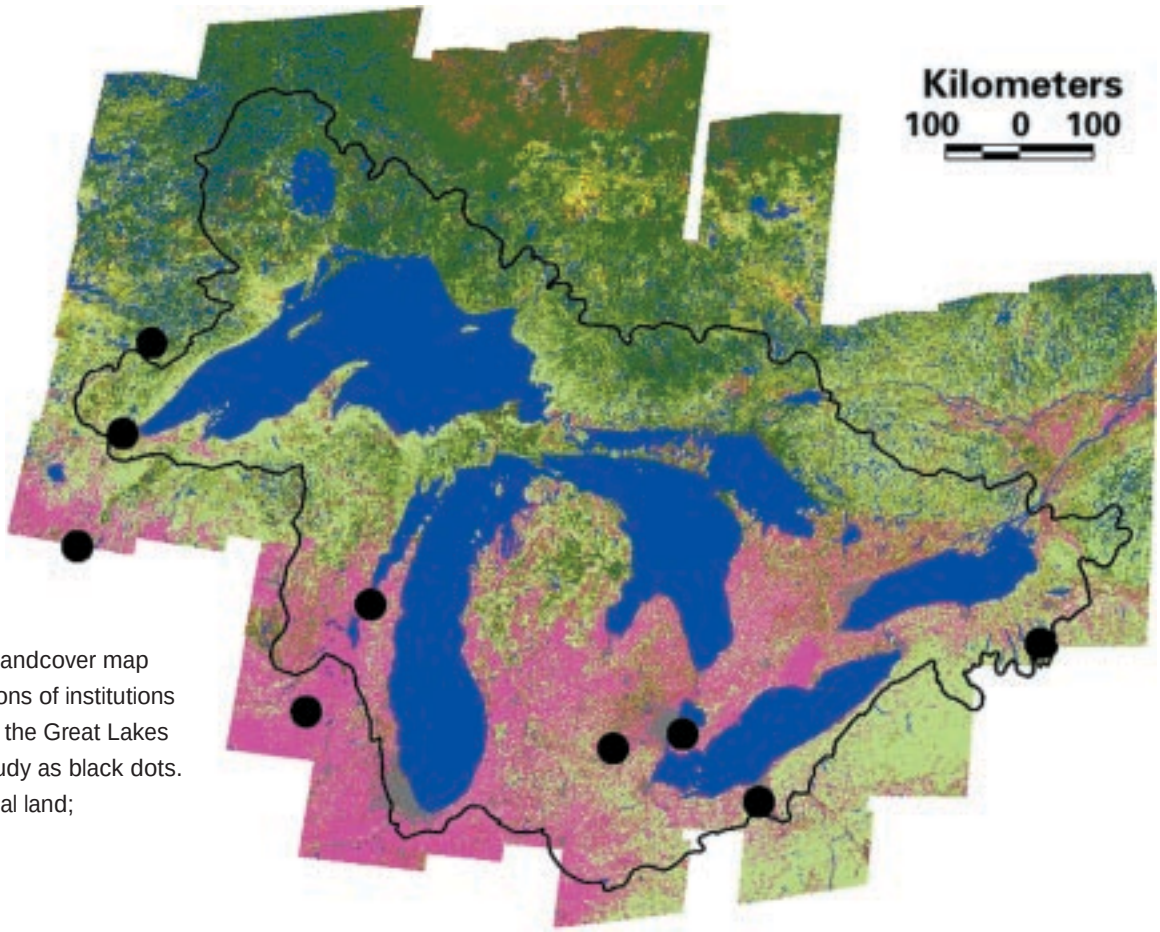


FIGURE 6. Landcover map showing locations of institutions participating in the Great Lakes cooperative study as black dots. Pink: agricultural land; green: forest.

this study will serve as a model for studying and monitoring other critical watersheds globally. Close monitoring enables rapid recognition of changing conditions, in turn enabling quick action to correct adverse situations.

Based on previous research, EPA has identified more than 80 environmental indicators to be evaluated in the study. New indicators may also be identified and assessed. The overall project is organized into five major focus areas. Teams of scientists from NHEERL and the participating universities will investigate indicators of environmental stress and ecosystem responses to stress in each focus area:

- water quality and diatoms (one type of microscopic algae),
- fish and macroinvertebrates (aquatic insects, crustaceans, and worms),
- wetland vegetation,
- birds and amphibians, and
- chemical contaminants.

Environmental Indicators

Two types of indicators will be evaluated, those indicative of stressors and those indicative of the environment's response to the stressors. Examples of stressors include land use change, climate change, point and nonpoint discharges, nonnative species, atmospheric deposition (e.g., acid rain), and hydrological modifications. Indicators of response to stress include changes in land cover, water quality (contaminants and nutrients), and biological populations and communities (amphibians, birds, diatoms, fish, macroinvertebrates, and aquatic plants).

The project spans four years and includes three phases: examination of existing data, a pilot study, and a comprehensive field study. To date, the cross-organization teams have been sharing GIS-landscape coverages, existing datasets, and methods. In the fall of 2001, the research teams met in Duluth to analyze the summer's pilot study and establish the sampling frames for the comprehensive field study. These sampling frames will be the basis for evaluating the ability of the different classes of indicators, either alone or in combination, to detect changes in environmental condition. While assisting the university researchers in establishing the most efficient study design, the initial pilot effort and future studies will expand and extend NHEERL's on-going investigations. These projects include studies of the coastal wetlands in Lakes Superior and Michigan, research on persistent bioaccumulative toxicants in the Great Lakes, and development of fish and macroinvertebrate indicators for nearshore and coastal zones. At the end of the project, the investigators will recommend a portfolio of indicators that is cost-effective and that accurately reflects the condition of the Great Lakes. These indicators will be used to further study and monitor the Great Lakes basin.

In 2001, preliminary assessments were conducted of Lake Superior, Lake Michigan, and Lake Ontario. These initial assessments estimated variability of the proposed indicators, verified landscape classifications previously determined by remote sensing technology, and determined logistic constraints of access to sampling sites. The data obtained will be used to develop the sampling design for the more extensive studies to be conducted in subsequent years.

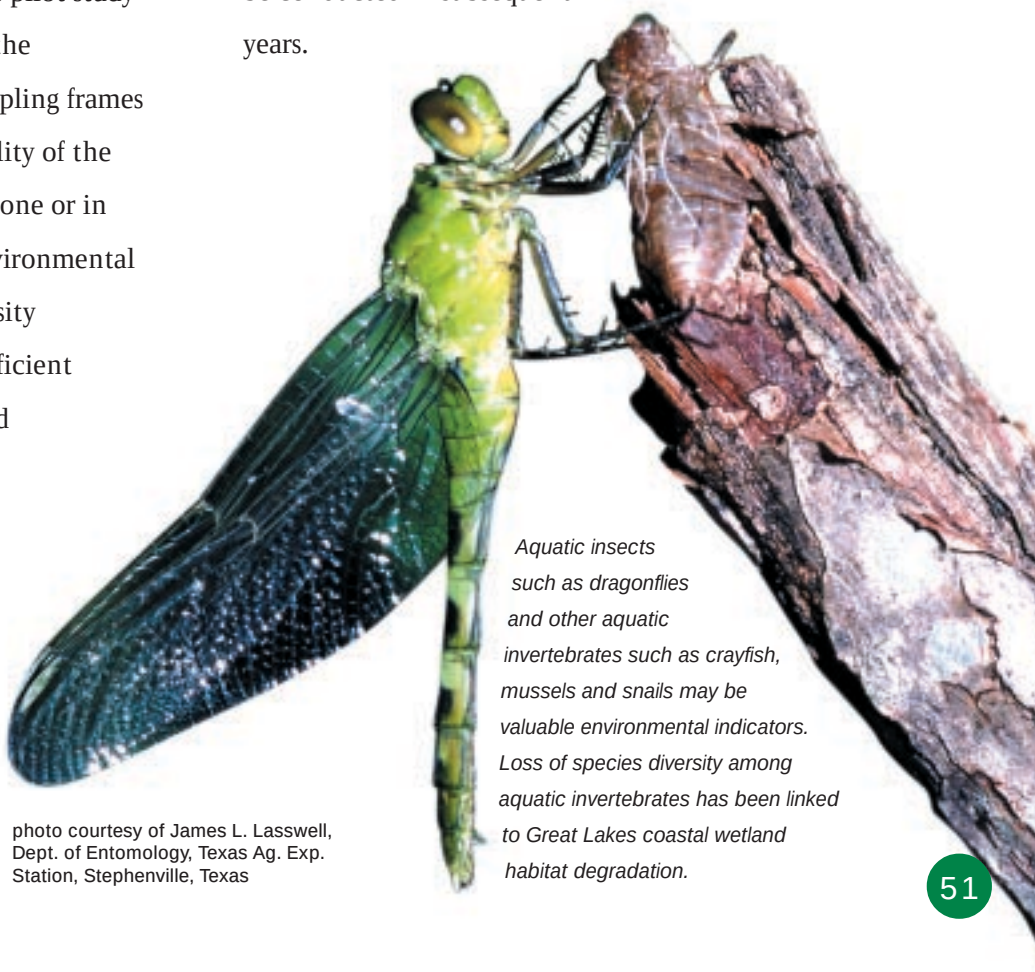


photo courtesy of James L. Lasswell, Dept. of Entomology, Texas Ag. Exp. Station, Stephenville, Texas

Aquatic insects such as dragonflies and other aquatic invertebrates such as crayfish, mussels and snails may be valuable environmental indicators. Loss of species diversity among aquatic invertebrates has been linked to Great Lakes coastal wetland habitat degradation.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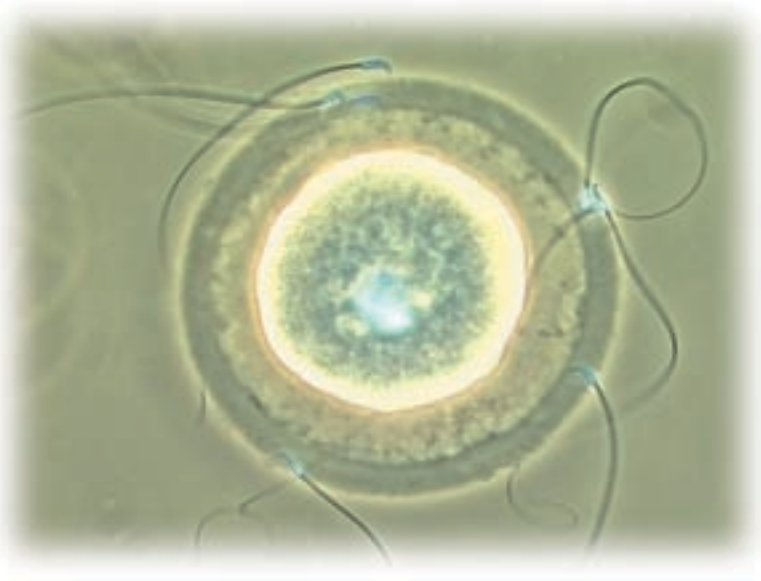
human health protection

In addition to protecting our environment, EPA is charged with protecting human health. This task is fulfilled by conducting research on priority environmental chemicals, assessing risks to humans and the environment associated with environmental chemicals, and establishing regulations and management actions based on those risk assessments. A major emphasis at NHEERL is to introduce new technologies that expand the set of tools available to evaluate human health risk following exposure to environmental chemicals. New technologies developed at NHEERL have important applications in this area. Assays using a sperm protein patented by NHEERL have the potential to identify when environmental chemicals impair male fertility. Also, a new Genomics Program will coordinate NHEERL research on how environmental chemicals affect the human genome—the body's genetic blueprint.

FIRST EPA BIOTECHNOLOGY PATENT

In 2001, an NHEERL scientist became the first EPA researcher to obtain a biotechnology patent on behalf of EPA. The patent is on the sperm protein SP22, the nucleotide (DNA) and amino acid sequence of SP22, and all recombinant fragments thereof. A second patent is pending. It covers the use of SP22 in fertility diagnostics of humans and animals and other reproductive technologies including contraception, artificial insemination, and *in vitro* fertilization. NHEERL will receive any royalties derived from commercial licensing of the patents. The scientist-inventor is entitled to one-third of the royalties, up to a maximum of $150,000 per year.

SP22 was discovered during *in vivo* rodent studies designed to identify molecular factors associated with infertility. Male rats were exposed to four chemicals known to reduce fertility. To improve the likelihood that the study would detect molecular influences on fertility, the chemical doses used were low enough that sperm shape and motility were either unaffected or only slightly affected. (Abnormalities of sperm shape and motility are also often associated with reduced fertility.) Mature sperm were collected and surgically inseminated into female rats. Sperm from the same collections were examined for changes in shape, motility, and the type and amount of specific proteins present on the sperm membrane. Male fertility was measured as the number of embryos implanted in the uterus divided by the number of eggs ovulated (determined by counting *corpora lutea* of



In this photomicrograph, sperm are attached to the *zona pellucida* surrounding the egg. Only one sperm will penetrate the zona pellucida and fertilize the egg.

pregnancy on the ovaries) expressed as a percentage. All four test chemicals used in the study caused a decrease in fertility. Importantly, levels of one sperm protein were also diminished and were highly correlated with fertility (Figure 1). Because this sperm protein was 22 kilodaltons in size, it was named SP22. Further analysis of the data showed that the amount of SP22 in sperm could be used to predict male fertility.

In other NHEERL studies, antibodies to SP22 have identified the location of SP22 on sperm from the rat, hamster, rabbit, bull, and human. In addition, these antibodies have inhibited fertilization of hamster eggs *in vitro* (Figure 2). Similar results have been obtained with human eggs and with rats (*in vitro* and *in vivo* studies). Collectively, the data indicate that SP22 plays a critical role in the initial interaction of the sperm

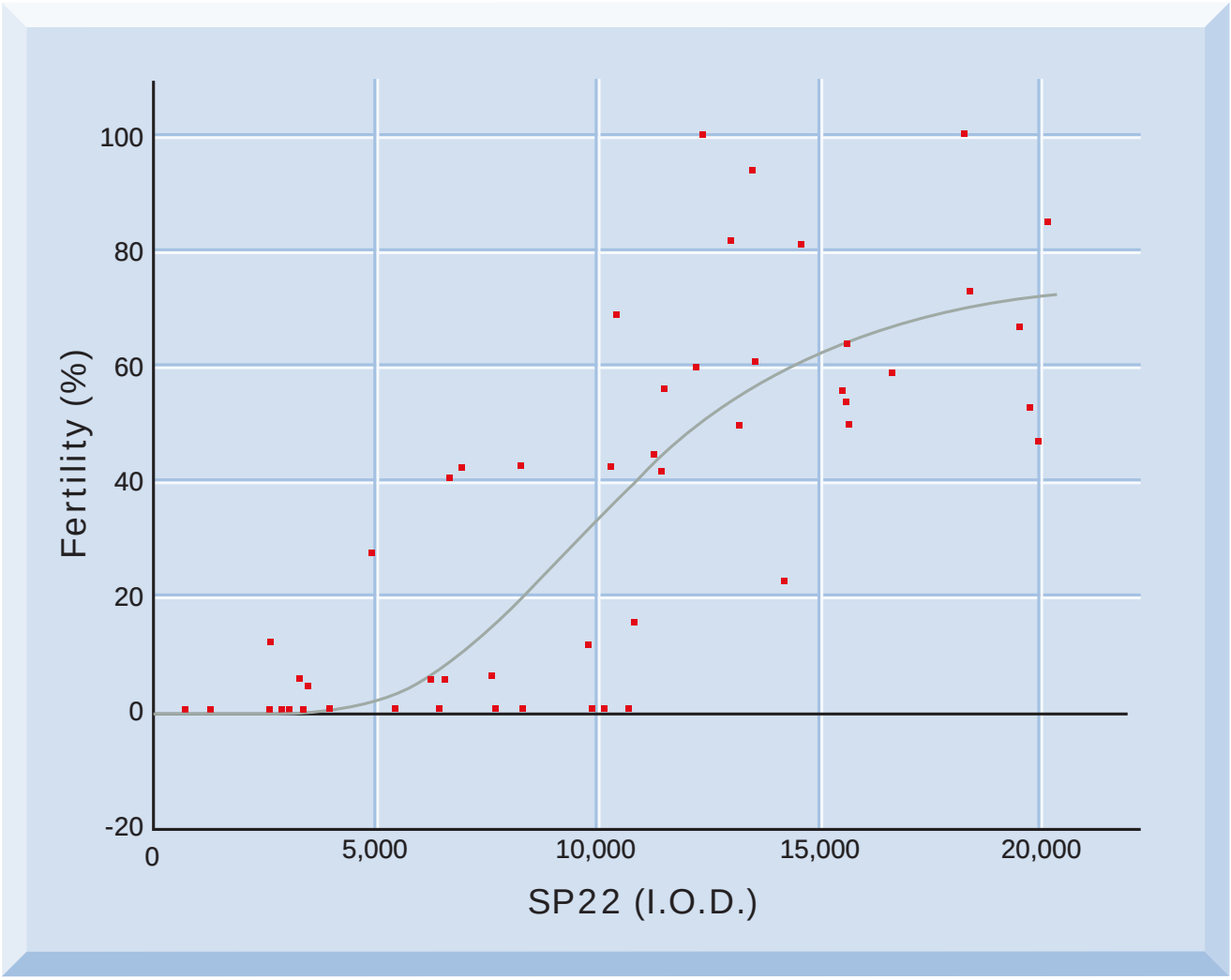


FIGURE 1. The relationship between fertility and SP22 levels. SP22 is measured in integrated optical density units (I.O.D.).

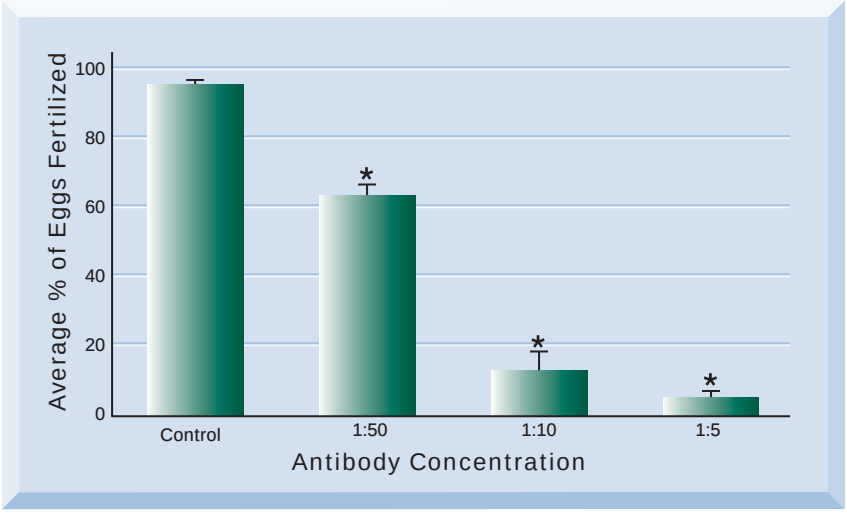


FIGURE 2. Inhibition of *in vitro* fertilization in the hamster by antibodies to SP22. Bars represent the average percentage of eggs fertilized. * indicates a statistically significant difference compared to controls. The antibody concentration is expressed as a ratio representing the dilution of n parts of antibody-containing serum with n parts of diluent.

and egg during fertilization. Moreover, preliminary data obtained from men with infertility of unknown cause show reduced SP22 levels. These results indicate that SP22 levels may be used as a biomarker of male reproductive capability. An epidemiologic study is underway to determine if SP22 levels in human sperm are affected by environmental exposure to varying levels of by-products formed by drinking water disinfection.

NEW GENOMICS PROGRAM

The U.S. Human Genome Project began in 1990 with the primary goals of identifying all of the estimated 30,000 genes in human DNA and determining the sequences of the three billion chemical bases that constitute human DNA. As the Human Genome Project continues, scientists are building on the knowledge gained and expanding research into new areas. The emphasis is shifting from sequencing and mapping to studying gene patterns of expression and the factors that influence gene expression. In addition, a new, but related, field of study has emerged. Proteomics is the study of all the protein products that result from a cell's gene expression.

What do genes do?

In the simplest sense, genes are blueprints for the production of proteins. This includes proteins that regulate cell functions in addition to proteins that form the structure of cells.

Genes associated with cancer provide examples of the type of topics that might be studied under a genomics-proteomics program. Two categories of cancer-associated genes are tumor suppressor genes and oncogenes. When a cell's tumor suppressor genes are active, the resulting proteins regulate the cell's life cycle so that the cell grows, matures, ages, and dies at a normal pace. If an event inactivates tumor suppressor genes, the cell no longer produces these regulating proteins. Absence of growth-regulating proteins can result in abnormal growth and reproduction, and a cell that eventually develops into a tumor unless other body defenses destroy the growing mass first. On the other hand, when oncogenes are activated, protein products resulting from their expression may cause abnormal cell growth and maturation, with the same outcome—the development of a tumor.

When chemicals damage DNA, tumor suppressor genes may be deactivated and/or oncogenes may be activated. The mechanisms by which chemicals interact with genes, influence the activity of these genes, and alter the protein products of gene expression are topics to be studied in future genomics-proteomics research endeavors.

In 2001, NHEERL established a Genomics Steering Committee, which will initially propose an in-house program for coordinating genomics and proteomics research. An example of interdivisional cooperation, the committee consists of one representative from each health division and two ad hoc members. NHEERL researchers have already conducted a number of individual projects applying genomics and proteomics knowledge to environmental health risk assessment. The new Genomics Program will coordinate NHEERL research efforts in these areas and will formalize NHEERL's role in this important field. In addition to research on gene expression patterns and the resulting protein products, the NHEERL Genomics Program will also include studies of how environmental chemicals interact with genes and influence gene expression. This is consistent with NHEERL's Strategic Plan and EPA's role of assessing the risk that environmental pollutants pose to human health.

Also in 2001, NHEERL joined the North Carolina Biotechnology Center Consortium for Genomics and Bioinformatics. This affiliation will facilitate collaborative research between NHEERL scientists and researchers at other Consortium institutions.

Example Genomics Projects at NHEERL

- Use gene array data in rats to identify and examine suspected mechanisms of toxicity for inhaled environmental pollutants, with the goal of distinguishing carcinogenic from noncarcinogenic air toxicants.

- Identify molecular alterations in cells lining the urinary bladder of rats after exposure to disinfection by-products in drinking water. The urinary bladder is one of the principal sites of cancer in humans exposed to drinking water disinfection by-products.

- Describe the effect of two dietary antimutagens (substances that prevent genetic mutation), vanillin and cinnamaldehyde, on gene expression in *Salmonella*, *E. coli*, and cultured human liver cells.

- Examine the effects of dietary folate deficiency on arsenic-induced genotoxicity in mice. This work includes analyses of altered gene expression resulting from folate deficiency, from arsenic exposure, and from the combination of folate deficiency and arsenic exposure.

- Identify genetic or molecular changes (biomarkers) that occur in genes in response to environmental chemicals. The purpose of this research is to eventually assess potential sensitivity of children to adverse outcomes following exposure to environmental chemicals.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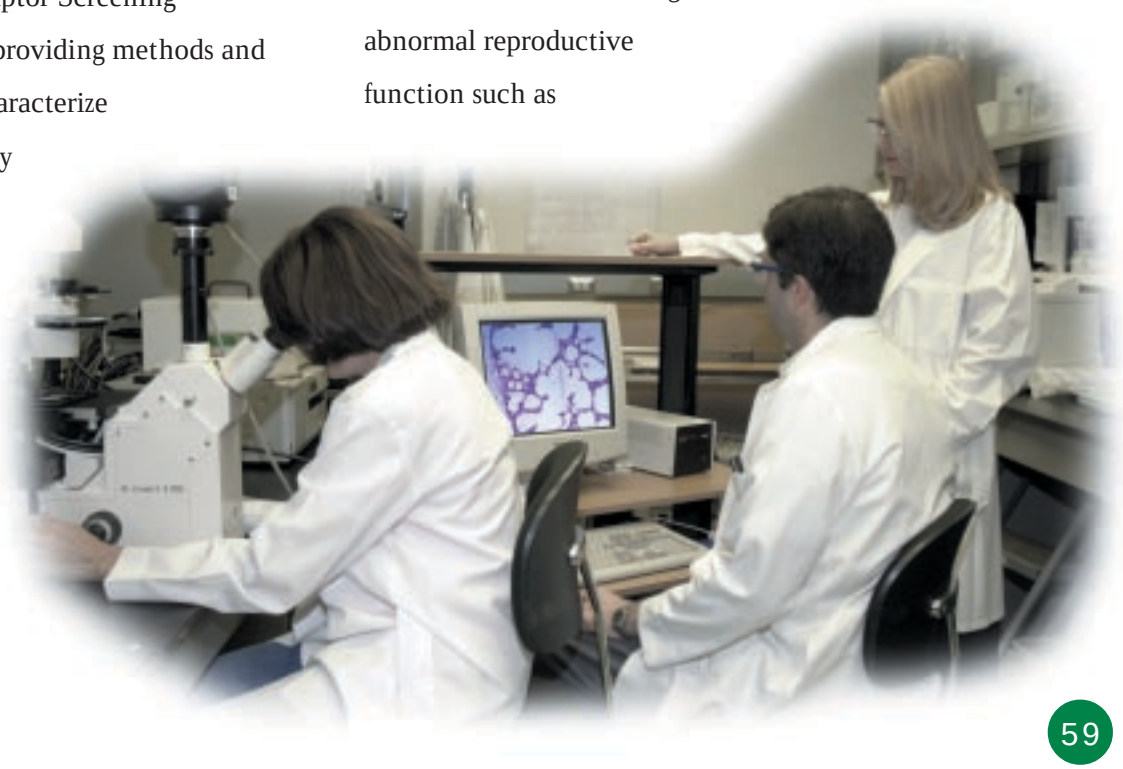
endocrine-disrupting chemicals

EPA defines an endocrine-disrupting chemical (EDC) as an exogenous chemical substance or mixture that alters the structure or function(s) of the endocrine system and causes adverse effects at the level of the organism, its progeny, populations, or subpopulations. The Food Quality Protection Act and Amendments to the Safe Drinking Water Act of 1996 reflected growing concern about the presence of potential endocrine-disrupting chemicals in food, water, and the environment. Passage of these laws required EPA to develop a screening program to determine whether individual substances may cause endocrine disruption in humans.

EPA established the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) and charged the committee to provide recommendations for a screening and testing program. Based on these recommendations, EPA created the Endocrine Disruptor Screening Program, which focuses on providing methods and procedures to detect and characterize endocrine-disrupting activity in pesticides, commercial chemicals, and environmental contaminants. The use of these standardized protocols will help EPA and industry efficiently gather information regarding endocrine-disrupting activity of the estimated 87,000 chemicals in commercial use and the many thousands of chemicals under development. Based on this information, EPA will be able to take appropriate action. NHEERL scientists are playing a major role in the development of these testing protocols.

PUBERTAL RAT PROTOCOLS

Puberty, the onset of sexual maturity, is a developmental stage characterized by extensive interactions among many hormones, organs, and tissues. This time period is also distinguished by increased sensitivity to environmental chemicals. NHEERL scientists, in collaboration with program representatives from EPA's Office of Prevention, Pesticides and Toxic Substances (OPPTS), are developing testing protocols to detect potential effects of EDCs on puberty in the male and female rat. These protocols are designed to detect when a chemical exposure causes structural malformation of sexual organs, abnormal reproductive function such as



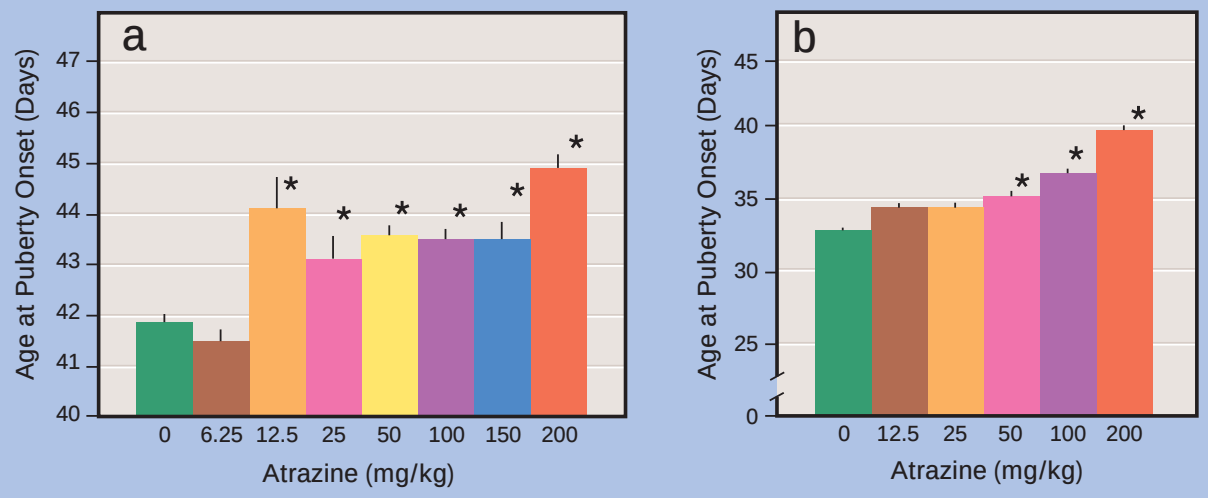


Figure 1. Effect of atrazine on puberty onset in (a) male and (b) female rats. Age is measured in days. Puberty onset is determined by the age of preputial separation in male rats and the age of vaginal opening in female rats. * indicates a statistically significant difference compared to controls.

delayed onset of puberty, or abnormal function of other endocrine organs such as the thyroid and hypothalamus. (The hypothalamus is an area of the brain that influences all hormonal activity in the body.)

NHEERL scientists published background papers describing the attributes and weaknesses of the male and female pubertal rat protocols in 2000. These papers raised several issues that are important to the successful implementation of these two protocols for EDC screening.

Research activities at NHEERL in 2001 addressed these issues. As part of the standardization and validation process, NHEERL scientists assisted OPPTS in coordinating the evaluation of these protocols by an independent contractor. This step is important because contractor laboratories will likely be the major sites conducting the tests after the protocols are finalized and implemented. One key element in this process is the use of test chemicals whose identity and endocrine-disrupting activity are unknown to the contractor. To date, compounds representing various classes of endocrine-disrupting chemicals (e.g., estrogens, anti-androgens, thyroid toxicants) have been readily detected by the contractor using the pubertal rat protocols. Future studies will use expanded dose ranges to investigate the sensitivity of these protocols by determining the lowest dose

that causes an effect. The results will indicate whether or not the pubertal rat protocols can detect weak EDCs as well as potent ones.

NHEERL scientists used the male and female pubertal rat protocols to determine if they could identify chemicals with a central nervous system mode of action. The protocols, conducted using the chlorotriazine herbicide atrazine, showed that puberty was delayed in both sexes (Figure 1). This work showed, for the first time, that atrazine alters male reproductive function during development. It also showed that these protocols are able to detect adverse effects of atrazine in the female following a much shorter duration of exposure than previously reported. (See the Pesticides chapter for more information on atrazine.)

IN VIVO ASSAYS

The Hershberger assay is a 10-day test to identify substances that act like male hormones (androgens) and substances that interfere with male hormones (antiandrogens). This assay is being developed as a cooperative venture between EPA and the Organisation for Economic Co-operation and Development (OECD), a European organization, with NHEERL as the lead laboratory. In 2001, the first phase of an interlaboratory standardization and validation study was conducted with 17 participating laboratories. NHEERL scientists provided information on optimum protocol procedures to the laboratories, analyzed the data, and wrote a report for OECD.

An *in utero*-lactational assay is being developed to identify chemicals that affect the developing fetus and the nursing newborn. NHEERL is developing the protocols, selecting chemicals, and analyzing results of tests that are conducted by a contractor.

Atrazine Risk Assessment

The human health risk assessment for a substance is a determination of the danger it poses to humans. It is based on health problems in humans and/or laboratory animals that have been associated with exposure to the substance. Three types of health conditions that carry great weight in the risk assessment process are cancer, reproductive problems, and birth defects. During risk assessment, different guidelines are followed for agents that cause different types of health problems. For agents that cause cancer, the mechanism by which the agent causes cancer is emphasized during the risk assessment process, particularly when extrapolating findings in laboratory animals to humans.

Over the years, laboratory studies conducted in different strains of rats produced conflicting results regarding atrazine's ability to cause cancer. Atrazine caused cancer in only one strain of laboratory rats, but not in others. Recent studies at NHEERL confirmed that atrazine does not cause cancer by damaging the DNA of cells, which is one mechanism by which some chemicals cause cancer in humans. These results suggest a hormonal mechanism of cancer production unique to one strain of rats. Therefore, the risk assessment guidelines for substances that cause cancer do not apply to atrazine. However, two NHEERL research efforts indicate that risk assessment guidelines for chemicals that cause reproductive problems and/or birth defects may be more appropriate for atrazine: (1) pubertal rat studies demonstrated atrazine's endocrine-disrupting effects on sexual maturation and (2) recent studies revealed adverse effects on prostate gland development following neonatal exposure.

Risk assessment is a dynamic, not static, process. As the studies on atrazine demonstrate, the risk assessment for a chemical is subject to change as new information emerges.

IN VITRO ASSAYS

In addition to assays in live rodents, NHEERL is developing a variety of screening tests using hormone receptors in cell and tissue cultures. Substances that are similar in size and shape to a natural hormone may bind to hormone receptors. If this happens, the chemical may mimic the hormone by stimulating the same response in the cell as the hormone or it may inhibit hormone activity by preventing the natural hormone from binding to the receptor. Thus, identifying chemicals that bind to hormone receptors is an efficient way to distinguish between chemicals that should be tested further (those that interact with hormone receptors) and chemicals that are unlikely to have endocrine-disrupting activity.

The *in vitro* assays under development at NHEERL involve

- androgen receptors,
- estrogen receptors,
- genes regulated by androgens, and
- synthesis of steroid hormones.

Two androgen-receptor binding assays, one that is cell-free and the other that uses whole cells, have been developed and are in use. Another whole-cell screening assay uses receptors tagged with fluorescent antibodies. Because androgen receptors move to the DNA in the cell nucleus after they have been activated, the distribution of fluorescence in the cells indicates whether or not the test substance bound to the androgen receptor (see Figure 3).

How Steroid Hormones Work

Steroid hormones travel in the blood from the tissue in which they are synthesized to specific organs and tissues where they exert their influence. Once inside a cell in the target organ or tissue, a steroid hormone binds to a specific receptor molecule. The receptor is activated and relocates to the nucleus near the DNA. The activated receptor then binds to a specific promoter sequence in the DNA of the target gene, which is either activated or inactivated. The result of gene activation is the production of specific proteins, which may be enzymes, structural proteins, growth factors, or other active substances, including other hormones. An endocrine-disrupting chemical that binds to a steroid hormone receptor may mimic the natural hormone or it may prevent the natural hormone from binding. In either situation, normal hormone-cell interaction is disrupted.

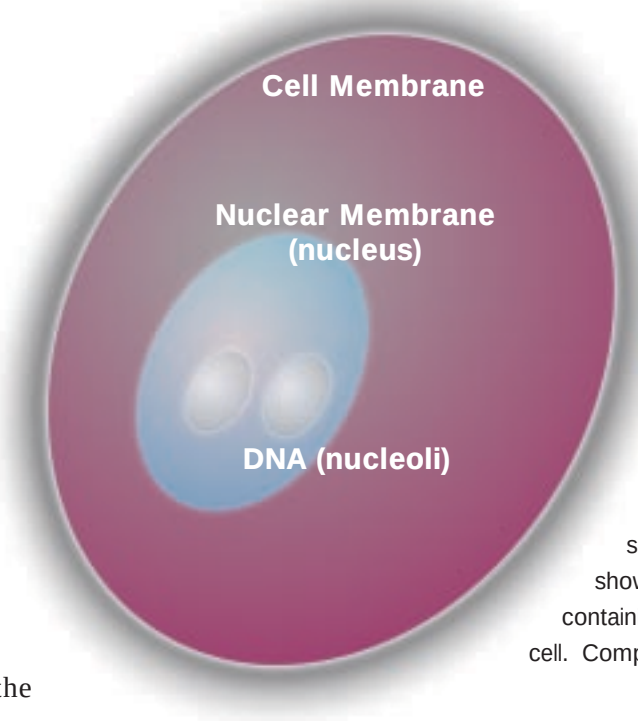


Figure 2. Simplified schematic diagram showing the DNA-containing nucleus in a cell. Compare to Figure 3.

Using a novel approach, one NHEERL researcher developed an assay that uses a virus to insert androgen receptors into the cultured cells. This process is efficient and the receptors remain very sensitive to androgens. However, inserting receptors into cells every time an assay is prepared creates quality control issues due to variability from one test to the next. In contrast to these assays, another NHEERL scientist developed a cell line that retains the receptors of interest through cell replication cycles. These androgen-responsive cell lines have been distributed to other laboratories for validation studies. NHEERL researchers are in the early stages of developing analogous estrogen-responsive cell lines. A different type of assay is being developed to detect inhibition of steroid hormone synthesis in tissue cultures.

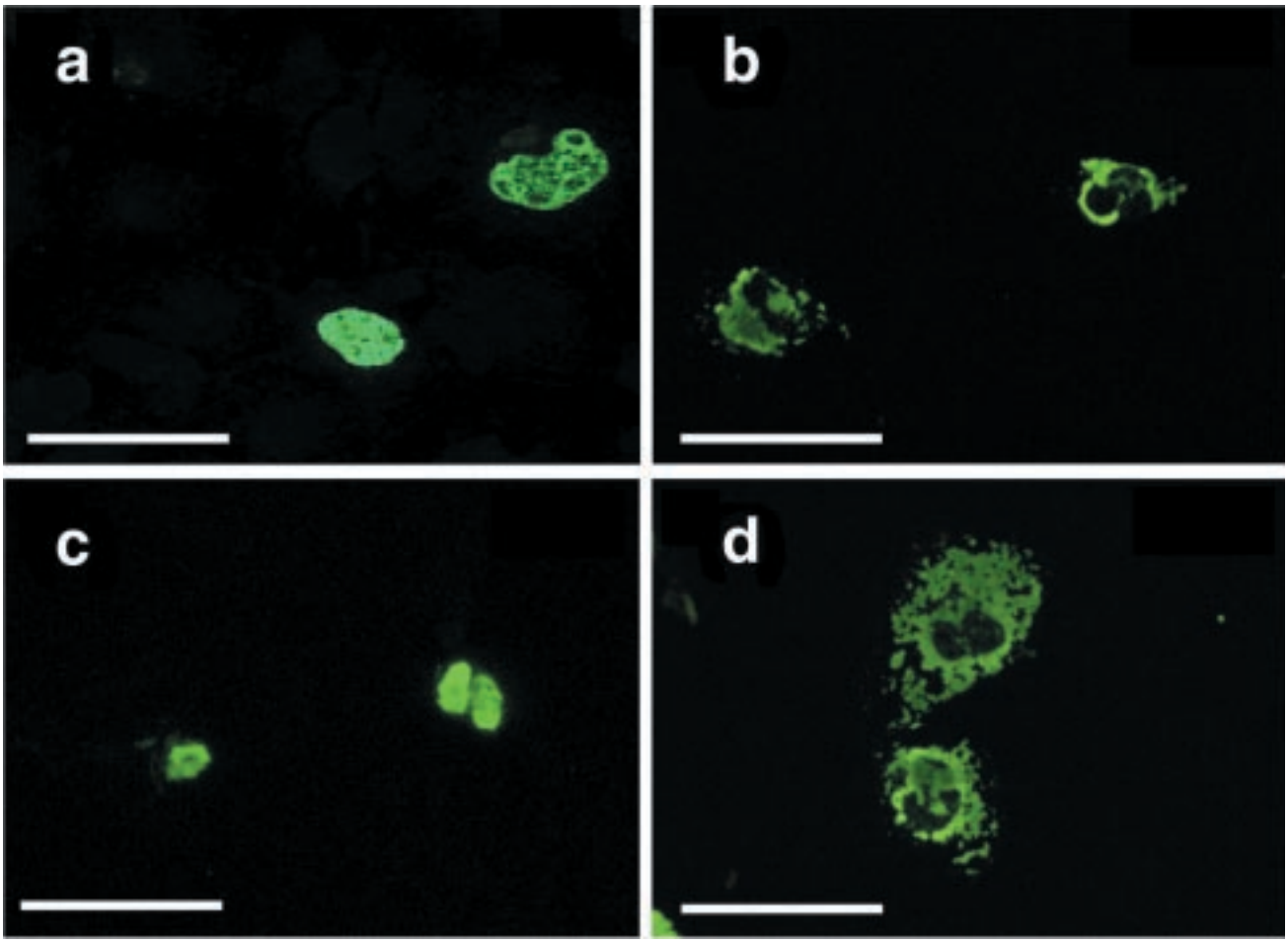


Figure 3. In (a) and (c), androgen receptors exposed to a positive control and a positive test substance migrated into the nucleus and attached to DNA, forming dense areas of fluorescence. In (b) and (d), the receptors remained scattered about outside the nucleus, forming a more diffuse pattern of fluorescence after being exposed to media alone and a negative control. These patterns help to distinguish between EDCs and non-EDCs.

Coming Soon...

- A report on the contamination levels of mercury in selected finfish and shellfish in the estuaries of the United States.
- A summary and comparison of multiple toxicities following developmental exposure to pesticides: neurotoxicity, immunotoxicity, and reproductive toxicity.
- A report on waterborne disease outbreaks in the United States in 1999-2000; this report will provide information on causative agents, health effects, water quality, and treatment issues.
- A searchable database platform to enable researchers and risk assessors to explore structure-activity associations across toxicity endpoints of regulatory interest.
- A report describing the significance for cancer risk assessment of a newly discovered mode of action of polycyclic aromatic hydrocarbons.
- The development and application of *in vivo* immuno-histochemical methods to verify that mechanisms of toxicity observed during *in vitro* studies can be extrapolated to the whole organism, enhancing the use of *in vitro* data in risk assessment.
- A report describing the influence of nutrient loadings and altered nutrient ratios on the occurrence of hazardous algal blooms.

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