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Environmental Health perspectives
Journal of the National Institute of Environmental Health Sciences

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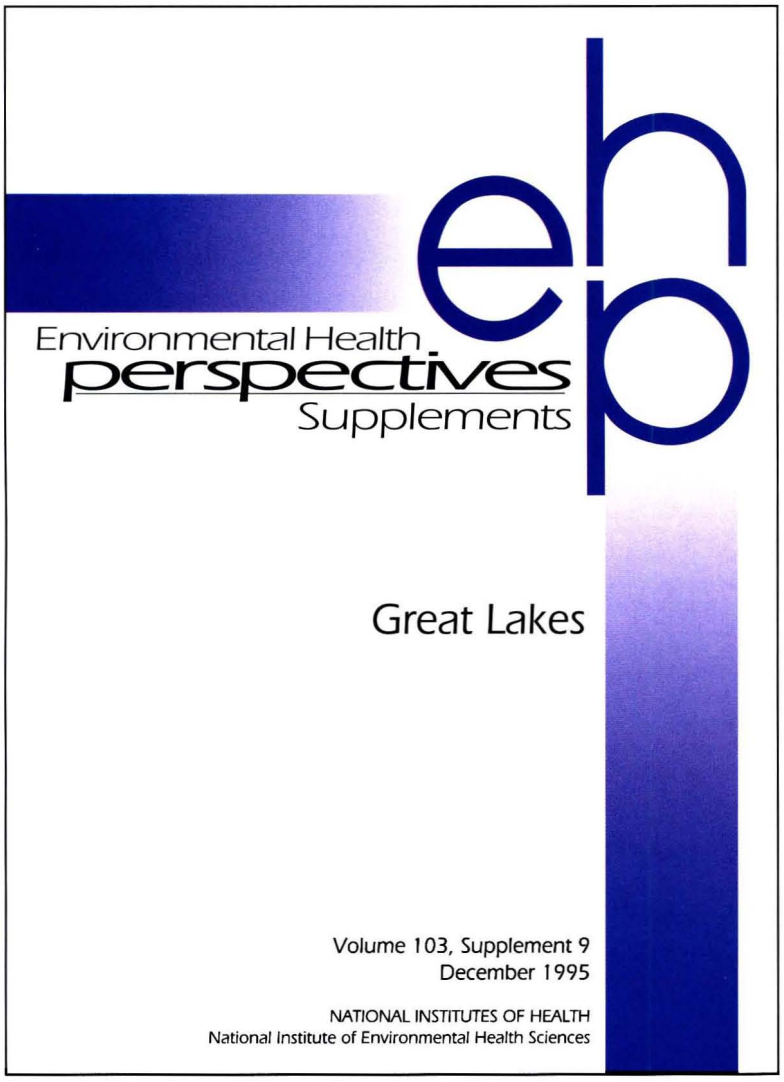

A Tradition of Progress

EHP Supplements continues its 23-year tradition of publishing monographs based on current environmental issues and conference proceedings, as well as an annual review of environmental health. The Great Lakes issue focuses on the health effects of pollutants in the Great Lakes and contains original research and reviews.

For subscription information, see p. 1164.

In This Issue

The Population Equation

The earth is currently home to 5.7 billion inhabitants, an increase of almost 5 billion people over the last 200 years. At current rates, 1 billion people will be added to the world population every 11 years. Some say it is only a matter of time before the world's limited resources are exhausted, as trends already show there is less land available for agriculture, less water fit to drink, and less protective ozone as increasing numbers of people struggle for survival and human endeavors destroy the environment. Others remain confident that human innovation and technology will compensate for environmental losses and shortages of resources. In the first **Focus** article (p. 1092), ecologists, demographers, sociologists, and others take a look at what more of us means.

Living with Livestock

The summer of 1995 may be remembered as the summer of livestock waste spills, in North Carolina and Iowa, at least. As waste flowed into streams, ponds, and rivers during a total of nine waste lagoon spills, the public was awakened to the problems of disposing of animal waste in the fast-growing but little-regulated livestock industry. The second **Focus** article (p. 1096) examines the problems of people living near cattle, hog, and chicken operations, the impact on the environment of livestock waste accidents, and the measures being taken to address these problems.

Nature's Kidneys

A new field of research is showing that sometimes nature provides the solutions for the problems humans create. Scientists are now investigating the use of plants to clean up soil and water contaminated by heavy metals, organic compounds, explosives, and radioactive materials (**Innovations**, p. 1106). Phytoremediation is a growing area of research, and, although researchers must still address issues like insect pests, animal predators (through which toxicants could be passed into the food chain), and the need for additional chemicals such as chelating agents, plants such as poplar trees, Indian mustard, turnips, and grasses are already showing promise as a cleaner, faster, and cheaper way to remediate waste sites.

Good Health around a Coal-Fired Power Plant

The potential short-term health effects of living near a coal-fired power plant in Israel were studied by collecting personal and environmental data over 10 years from clinics and adjacent air pollution monitoring stations. Goren et al. (p. 1110) found that there was no discernable relationship between sulfur dioxide levels emitted from the power plant and respiratory complaints or flu epidemics; air pollution levels were low and did not cause adverse health effects.

Genetic Control of Cadmium Tolerance in Fruitflies

A study by Maroni and Theodore (p. 1116) with a transgenic line of fruitflies showed that strains with two copies of the metallothionein allele Mtn^3 were more tolerant to cadmium than those with one copy of the gene. However, comparison of gene expression in a strain with one Mtn^3 allele to a strain with the Mtn^1 allele did not change cadmium tolerance, even though the latter is threefold more expressive. The authors propose that this unexpected finding was due to changes in amino acid substitution in the genes.

Fetotoxic Effects of Wood Preservatives

A cohort of pregnant teachers exposed to wood preservatives in daycare centers was evaluated for potential adverse health effects on the fetus. Karmaus and Wolf (p. 1120) conducted interviews and evaluated reproductive histories and report that exposure to pentachlorophenol, hexachlorocyclohexane, and polychlorinated dibenzo-*p*-dioxins and -furans from wood paneling and indoor air was associated with a reduction in birth weight and length of newborns.

Pesticides around Farm Homes

Even when not directly involved in farm work, children of agricultural families are more likely to be exposed to agricultural chemicals. Simcox et al. (p. 1126) sampled household dust and soil from farm homes around Washington State fruit orchards. The authors report residues of four commonly used organophosphate pesticides in 62% of the household dust sample, and greater than 1000 ng/g of at least one organophosphorus chemical in two-thirds of the farm homes. There was no indication of risk of acute intoxication, but the study suggests that exposure to low-level residues of azinphosmethyl occurred throughout the fruit-growing region.

Xenoestrogens Reduce Size of Rat Testes and Sperm Count

Female rats were exposed to low doses of octylphenol or butyl benzyl phthalate during gestation, as were male offspring during the first 21 days of postnatal life. Sharpe et al. (p. 1136) report that low doses of these environmental estrogens resulted in reduced testicular size and reduced daily sperm production in rats when they were examined at adulthood 70 days later. The authors hypothesize that these responses are due to a reduction in Sertoli cell numbers and suggest that falling sperm counts recently reported in humans might possibly be associated with similar exposure to xenoestrogens.

Radon Risks Lower in Transients

Warner et al. (p. 1144) report that estimates of radon-related lung cancer risks are lower than originally thought when residential mobility is taken into account. Because most people move about 10 times during their lives, potential exposure in the 7% of homes with elevated radon is actually well below levels that would result in elevated risks for lung cancer. As a result, current suggestions for remediating homes with elevated radon levels will benefit future rather than present occupants, and knowledge of current exposure to radon is not necessarily a useful indicator of risk.

Hormonal Reduction of Bone-to-Blood Lead Transfer

Webber et al. (p. 1150) examined postmenopausal women to determine if hormonal replacement therapy would help retain lead stored in bone. After calcium supplementation and hormone replacement therapy for 4 years, lead content of bone was higher in treated women than in women not receiving therapy. The authors suggest that women more likely to release lead from bone into the circulation and suffer potential toxicity include those that have previously accumulated high lead concentrations, have undergone significant perimenopause increase in bone turnover and mineral exhange, and that have an insufficient calcium intake.

NIEHS Superfund

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BASIC RESEARCH PROGRAM

The National Institute of Environmental Health Sciences

Editorial

Education: A First Step in Solving the Planet's Pollution Problems

Pollution of the planet, practically speaking, begins and ends with people. Pollution is produced not only by the industrialized nations, but also by developing countries rushing toward progress and its rewards. In fact, some of the worst environmental problems begin in developing countries and then may affect their neighbors and even distant nations. Regardless of how well one country may deal with its own sources of pollution, environmental problems may remain until a neighbor institutes effective cleanup procedures as well. Professional diplomats, as well as others, often must deal with the question, How do I get my neighbor to do unto me as I have done for myself?

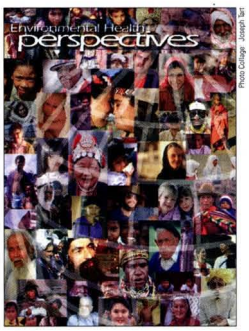
Toxic chemicals used for agricultural purposes wash into rivers that may cross national boundaries. The poisoning of aquifers may affect more than one nation. Atmospheric pollutants such as sulfur dioxide may be carried halfway around the world before descending in rain water to pollute lakes and kill trees. Depletion of the ozone layer by hydrofluorocarbons is an international problem of immediate concern. Volatile chemicals such as benzene hexachloride may be carried from warmer to colder regions of the world through a process called global distillation (*1*). Clearly, pollution is an international problem and needs to be addressed from an international perspective.

The earth can absorb the environmental impact of humans to a degree, but not indefinitely. We may have reached a limit where changes begin to influence climate and destruction of the environment could become permanent. With the lifting of the Iron Curtain, enormous degradation has been revealed in Russia and other former Soviet bloc countries. Exploitation of natural resources has occurred without regard for the environment or for the people whose lives were affected by the development of those resources. Slovakia, Poland, the Czech Republic, and Russia will take many years to recover from the pollution of rivers and lakes. In Poland, poor air quality, contaminated drinking water, and absence of treatment of industrial and municipal wastes are of major concern. As much as 30% of the effluent is discharged directly into the rivers and eventually into the sea.

In Brazil, gold mining has resulted in massive contamination of the environment with mercury, which is used in extraction of gold, especially around the Serra Pelada mine in the state of Pará. The burning of mercury-gold amalgam to release the bound gold releases mercury fumes, which then injure workers and settle on vegetation, where it is eventually washed by rain into the soil and into rivers. Studies of reservoirs downstream of gold mining demonstrate mercury contamination of soil, water, and fish.

China is a rapidly developing country with over 1.2 billion people. Environmental problems have arisen from overpopulation and rapid industrialization and overuse of natural resources. Air pollution is a major problem. Much of China's surface water is contaminated with heavy metals including lead, cadmium, and arsenic from industrial pollution.

The cover of this month's *EHP* and the first Focus article (p. 1092) focus on the people of the world and their role in both degrading and protecting the environment. The world's population in the next 50



years may exceed 9 billion, whi
the global economic output is est
mated to increase possibly b
about 5-fold (*2*). Clearly, renew
able resources in the future ma
become severely strained c
depleted. Destruction of the env
ronment leads to cultural and ecc
nomic impoverishment (*2*) and a
environmental problems becom
more severe, they may trigger civ
and international strife (*3*). It
extremely important that we begi
to address the issues. Somehow we have to build a future that is sustain
able, that does not destroy the environment.

A hope for the permanent resolution of pollution problems aroun
the world lies in the education of its people. Pollution occurs becaus
of the activities of people, and those activities may continue until thos
who engage in polluting the environment fully understand the conse
quences of their actions. Government legislation and policing is neces
sary, but information and education can influence the choices tha
people make.

Quite often those who are in greatest need of education are thos
that can least afford to invest. Because of this lack of discretionar
funds, especially U.S. currency, NIEHS offers subscriptions to *EHI*
free of charge, to any educational or research institution in any devel
oping nation. Our only condition is that the journal reside in a librar
and be freely available to all who have need of its information. In thi
way, we at NIEHS try to contribute to and influence environmenta
health programs around the world. At the present time we provid
over 2,000 subscriptions to developing nations. Of these, 249 sub
scriptions are sent to Russia, 102 to Poland, 9 to Slovakia, 65 to th
Czech Republic, 174 to China, and 73 to Brazil. I consider this pro
gram an investment in the future of humanity.

Kenneth Olden, PhD
Director, National Institute o
Environmental Health Science

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Nobody Said It Would Be Easy

Cooperation at the international level is one of the most important ingredients for making progress toward improving the global environment. During the last 20 years environmental treaties have proliferated, with nearly 200 being drafted and placed into law (*1*). One example is the International Climate Change Treaty, which became legally binding in 1994 and requires that countries submit detailed lists of their greenhouse gas emissions and implement programs to curb such emissions. A second example is the Montreal Protocol, which was drafted in 1987 and established a time table for the prohibition of production of chlorofluorocarbons (CFCs) to halt the destruction of the ozone layer.

In 1909, Canada and the United States signed the Boundary Waters Treaty and established the International Joint Commission (IJC). The main premise of the treaty was that neither party may use the water on its side of the lakes to the detriment of the water, health, and property of the other side. The role of the IJC was to investigate and recommend remedial actions for problems identified under the Boundary Waters Treaty. The first referral to the IJC dealing with the Great Lakes occurred in 1912 and involved binational concern about waterborne diseases. Actions emanating from that referral included the building of water and sewage treatment plants in urban areas. Over the intervening years many Great Lakes environmental problems have been addressed by the IJC. In 1972, the Great Lakes Water Quality Agreement (GLWQA) was signed to address the issue of eutrophication of the Great Lakes by reducing the phosphorus loading of the lakes. The agreement was subsequently renegotiated in 1978 and its scope broadened to include control of persistent, toxic substances. Further expansions of the agreement were made in 1987, including management plans and ecosystem indicators.

How important is the Great Lakes basin to Canada and the United States? One-fifth of all the fresh surface water of the earth is contained in the Great Lakes. The basin is rich in agricultural land, mineral deposits, extensive forests, and diverse wildlife. Because of these attributes, many people have been attracted to the basin, so that today there are over 32 million people living there. Employment in the basin represents nearly 11% of the total binational workforce and 15% of all manufacturing jobs (*2*). Considering these figures, it is not hard to understand why there is severe environmental stress on this ecosystem and why the protection of this ecosystem is important to these neighboring nations.

Effectiveness of the GLWQA can be judged in many ways. Significant reductions of the phosphorus loading have been achieved. Ambient concentrations of most toxic inorganic and organic chemicals contaminants have declined to below the goals set by the agreement (*4*). Concentrations of toxicants in fish and bird species have also declined on a lakewide basis, and increases in the reproductive success and in the populations of bald eagles, double-breasted cormorants, herring gulls, and other predatory birds in the Great Lakes basin have been observed (*5*). In addition, the concentrations of PCB and organochlorine pesticide residues in breast milk have declined and are lower than those reported in European countries (*6*). Although much needs to be done, it is doubtful that this degree of success would have been possible without the cooperation of both the United States and Canada (*7*).

One of the programs that Canada has established under the GLWQA is the Great Lakes Action Plan (GLAP), which focuses on accelerated cleanup of the contaminated areas as well as prevention of future pollution. The most recent United States' initiative is the issuance of the final Great Lakes Water Quality Guidance by the U.S. Environmental Protection Agency. The ecosystem-wide guidance is intended to provide a framework and consistent policy for the eight states surrounding the lakes for further reduction of the toxic chemicals that have contaminated the Great Lakes basin.

In this month's issue of the *Environmental Health Perspective Supplements*, 11 articles focus on the health effects of pollutants in the Great Lakes. The articles grew out of the Canadian Great Lakes Health Effects Program (GLHEP), which is one of the major initiatives of the GLAP. The GLHEP looks at the human health effects from Great Lakes pollutants and supports research and dissemination of health information. The articles contain both original research and reviews that reflect some of the accomplishments of the first five years of the GLHEP. The areas covered include immunotoxicity, neurotoxicity, and reproductive toxicity. Readers will find these papers to be a valuable resource on the human health impact of environmental contaminants in the Great Lakes basin.

Although international cooperation to address environmental issues is complicated by limited funding, trading concerns, financial limitations, international sovereignty, etc., innovative approaches must be developed. Changing in voting mechanisms have been used successfully to allow for amendments to strengthen existing treaties, as has the use of "soft laws"; i.e., nonbinding agreements that depend on moral persuasion and public embarrassment (*8*). Although the negotiations for such treaties are extremely difficult and their success is not assured, there are no other alternatives. As the great baseball player and tongue-in-cheek philosopher, Yogi Berra, said: "When you get to a fork in the road, take it!"

Thomas J. Goehl
Science Editor

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Correspondence

What's in a Name?

Kathryn Rosica of the Chemical Manufacturers Association raised an interesting point in her letter (*EHP* vol. 102, p. 1006). Neither the term "glycol ethers" nor the term "ethylene glycol ethers" strictly identifies a class of chemicals whose members all share a common distinctive toxicological profile. As Rosica correctly noted, "Higher molecular weight ethylene glycol monoethers that have been tested have not been associated with significant adverse developmental and reproductive effects." For these compounds, the most sensitive toxic endpoint is usually the destruction of red blood cells.

However, Rosica's carefully limited language may have created a misimpression of its own. The class of "higher molecular weight ethylene glycol monoethers that have been tested" is limited, so far as I am aware, to just four chemicals: ethylene glycol propyl ether, ethylene glycol butyl ether, ethylene glycol hexyl ether, and ethylene glycol phenyl ether. Most glycol ethers are excluded by this careful description. Many of the excluded compounds have been shown to cause fetal malformations, embryofetal death, and testicular atrophy, by the same mechanisms and in some cases with the same potency as the more notorious ethylene glycol methyl ether (EGME) and ethylene glycol ethyl ether (EGEE). These similarly toxic but less frequently discussed glycol ethers include ethylene glycol dimethyl ether (*1–6*), ethylene glycol diethyl ether (*3,7*), diethylene glycol methyl ether (*3,8*), diethylene glycol dimethyl ether (*3–5,9–13*), diethylene glycol diethyl ether (*3,4*), and triethylene glycol dimethyl ether (*3,4,10,14,15*). In fact, most of the ethylene glycol ether derivatives tested do share a common toxicological profile. Nor is the teratogenicity of the glycol ethers limited solely to ethylene glycol ether derivatives; the beta isomer of propylene glycol methyl ether is also a powerful teratogen (*16,17*).

Clear terminology is always desirable. To be strictly accurate, one might properly use the phrase "the teratogenic, embryolethal, and spermatotoxic glycol ethers," to differentiate these compounds from other glycol ethers such as the highly hematotoxic ethylene glycol butyl ether. However, as a practical matter, most ethylene glycol ethers are teratogens and testicular toxins. The fact that research and discussion have focused heavily on EGME and EGEE, the toxicological archetypes of the series, may have fostered an erroneous belief on the part of chemical manufacturers, users, and product formulators that the less frequently cited compounds are "safe" substitutes. Clearly, many of them are not. To continue that narrow focus and to suggest that glycol ethers other than the "classic" EGME and EGEE and their acetates do not share a similar toxicological profile would be a great disservice to those people who may be exposed to these compounds.

Will Forest
Hazard Evaluation System and Information Service
California Department of Health Services/ Department of Industrial Relations
Berkeley, California

What's Your Perspective?

EHP welcomes letters about articles or topics appearing in the journal. Send letters to: Editor-in-Chief, *Environmental Health Perspectives*, National Institute of Environmental Health Sciences, PO Box 12233, Research Triangle Park, NC 27709 USA.

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Intriguing Innovation

I was delighted to read "An ECOLOGICal Way to Dispose of Waste" in the September **Innovations** (*EHP* vol. 103, pp. 808–810). For many years I have been concerned about the human health effects from minute doses of persistent synthetic chemicals. It seemed that there was no way to get them out of our environment. Now the organic compounds can be changed back into harmless substances and can be separated out, contained, and then perhaps reused.

One doctor who would have been glad to know of any elimination of man-made chemicals was Theron G. Randolph, who died September 29, 1995, at age 89. In the 1950s he discovered that many of his patients were made ill by minute doses of chemicals; for example, the pesticides on

food. He was honored for his discoveries that benefited so many patients. Dr. Randolph continued in practice until last year.

Again, thank you for the excellent article on an innovation that can reduce exposure to man-made toxins.

Marjorie Fisher
NOHA (Nutrition for Optimal Health Association) News
Winnetka, Illinois

Malaria Carriers

In regard to the article, "Potential Impact of Global Climate Change on Malaria Risk," by Martens et al. in the May issue of *EHP* (vol. 103, pp. 458–464), I do not pretend to discuss the matter of climate change and malaria and the approaches used by Martens and colleagues. I am not convinced there is a real risk of the hypothetical global climate change on the spread of malaria. In my opinion, there are many other important factors to take into account, mainly concerning the so-called Third World.

What I cannot accept is the cover photograph of a mosquito species that has nothing to do with the *Anopheles* genus. It looks much more like an *Aedes*. I felt compelled to bring this to your attention in the hope it will contribute to the improvement of *EHP*.

Oswaldo Paulo Forattini
Universidade de Sao Paulo
Sao Paulo, Brazil

Erratum

We apologize for our error in identifying the mosquito on the cover of the May issue as *Anopheles stephensi*. Dr. Forattini is correct: the mosquito is of the genus *Aedes*. The photograph came to us from the World Health Organization. Following is

an excerpt from the letter from WHO correctly identifying the mosquito.

Dear Dr. Hook:
As you will have seen from the data sheet that was sent with the [mosquito] image, it was supplied to the TDR Image Library [of WHO] by the Medical Illustration department of the Liverpool School of Tropical Medicine as part of a group depicting *Anopheles stephensi*.

I have now checked with the department concerned. They apologise for the mix up and do, indeed, confirm that the picture is of an *Aedes* species.

I echo the apology over the misidentification, and I sincerely hope this has not caused too great a problem.

Andy Crump
TDR Image Library Coordinator
World Health Organization

We all worry about the population explosion—but we don't worry about it at the right time.

Arthur Hoppe, attributed

Forum

Pesticides in Baby Food

Sixteen pesticides have been detected in eight brand-name baby foods, according to a study by the Environmental Working Group and the National Campaign for Pesticide Policy Reform, two public interest groups based in Washington, DC.

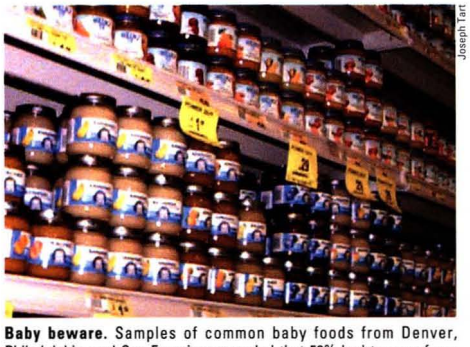
In their study, the EWG and the NCPPR collected a random sampling of 76 jars of baby food from grocery store shelves in Denver, Philadelphia, and San Francisco. The group chose fruits and vegetables babies most commonly eat during their first year of life. Of these, 53% harbored traces of one pesticide, and 18% had two or more pesticides. Plums contained the highest amounts at 46 parts per billion and peaches contained 29 parts per billion. Pears had the highest number of multiple pesticides overall (five).

The report, *Pesticides in Baby Food,* was published in July by the EWG and the Tides Foundation (available on the World Wide Web at: http://www.ewg.org) and indicates that all levels of pesticides found were below federal standards. While these standards are set with safety considerations for infants, children, and other sensitive populations, the actual risk assessments are based on an average adult. According to the EWG, the concern is that pesticides are not currently "tested for safety in the way babies are exposed to them," and that babies and young children "react differently than adults to many drugs and toxic substances."

These findings are consistent with the conclusions of a five-year investigation by the National Academy of Sciences, published in 1993 in the report *Pesticides in the Diets of Infants and Children,* which suggests that federal standards embodied in the Delaney Clause may not adequately account for the special vulnerability of infants and growing children to chemical substances. Nor do these standards account for the total doses of pesticides that babies receive from many other sources, including fresh produce, drinking water, possibly breast milk, or from the additive effects of these pesticides.

Some groups believe government standards are outdated and should be strengthened to provide broader protection to children. Other groups agree that federal standards are flawed, but see them as antiquated for other reasons. Scientists can measure one-billionth of the amount of a chemical now, for example, as compared with the 1950s when standards were set.

For almost four decades, the Delaney Clause, which bans the use of food additives shown to cause cancer in people or animals, has provided at least some measure of protection to the public food supply. The clause was passed in 1958 as an amendment to the Food, Drug and Cosmetic Act. However, this law is being challenged as obsolete. The current debate centers on whether Delaney should be repealed and, if so, what should take its place. Two bills are currently pending that would revise legislation governing food safety. HR 1627 would relax regulations by repealing the Delaney Clause and amending the Federal Insecticide, Fungicide and Rodenticide Act by bringing the separate standards for pesticide products under one umbrella. This bill calls for the EPA to apply a negligible risk standard to both raw and processed foods. HR 1771 also allows a negligible risk standard, but would require the EPA to determine whether a pesticide causes cancer, damages developing neurological, immune, or reproductive systems, or has other serious, adverse health effects in children before the agency could set tolerance levels. The bill would also require warning labels on foods that have been sprayed with a known or probable carcinogen.



Joseph Tart

Baby beware. Samples of common baby foods from Denver, Philadelphia, and San Francisco revealed that 53% had traces of one pesticide and 18% had two or more pesticides.

Philip Landrigan, professor of pediatrics at the Mount Sinai School of Medicine in New York City, and chair of the committee that prepared the 1993 NAS report, believes the pesticide regulatory system in the United States is flawed because of its approach for setting food tolerances. Instead of being based on health considerations, says Landrigan, they are based on field trials conducted by pesticide manufacturers and are "a balancing process in which health considerations are weighed against economic factors and agricultural practices." Landrigan believes that HR 1627 is inconsistent with the recommendations of the NAS report because it "will perpetuate the current inadequately controlled exposure of children to pesticides in their diets."

Richard Wiles, vice president for research of the EWG, argues that Congress is moving toward weakening what he sees as already weak laws governing food safety for kids. For example, in describing the EWG study, he notes that a fungicide called iprodione was found in more samples and at higher levels than any other pesticide. EPA studies identify it as a probable human carcinogen, yet it slips through the regulatory cracks in the Delaney Clause. Language within the clause prohibits any amount of a cancer-causing pesticide "that concentrates during food processing." Iprodione levels do not concentrate in baby foods; thus, it passes the safety test. "There are no standards out there that are specifically designed to protect infants from pesticides in their diet, or from the environment in general," says Wiles.

Physicians for Social Responsibility, another Washington-based group, has also studied these issues. *Beyond Delaney—Preventing Exposures to Hazardous Pesticides,* a 1995 report by the group, updates the NAS study. The group supports the recommendations of the NAS, and likewise favors stronger regulations to safeguard young children from exposures to toxic chemicals.

Joseph Schwartz, associate director for policy of Physicians for Social Responsibility, says the debate over the Delaney

Clause is important because it's time to begin phasing out the most unsafe pesticides on the market and provide the right kinds of incentives for safer biological alternatives. "We are looking for an opportunity to influence pesticide legislation reform, so five or ten or twenty years from now, farmers are not limited to the same pesticides their parents and grandparents were using," he says. "We can do better than that."

The agricultural community believes it is doing better than that. Will Carpenter, former vice president and general manager of Monsanto Agricultural Company, says that advances in biotechnology, coupled with integrated pest management strategies, are allowing great strides in reducing pesticide usage. "Within 5 to 10 years, the two biggest uses of insecticides in this country will drop precipitously," he says. "This is due to our ability to genetically vaccinate cotton and corn plants with a gene from a disease microbe. The insect eats the plant leaves, gets sick, and stops feeding." Carpenter continues, "Also, farmers are shifting to no-till/conservation tillage in greatly increasing numbers. This practice reduces soil erosion to 1% of what it once was, so pesticides stay in the soil on site, where they belong, instead of running into our reservoirs."

Meanwhile, the worldwide agricultural industry shoulders the responsibility of providing an adequate supply of food for millions of people in the world each day. Carpenter says pesticides are necessary if these goals are to be met. Carpenter opposes strict government regulations—especially when they are as old as Delaney. "I like to think we can do without them," he says.

At present, the Delaney Clause stands while alternative legislation is stalled at various stages in Congress. Whatever decision Congress reaches regarding the Delaney Clause, most participants in the debate agree that the major objective must continue to be protection of public health, especially children's health.

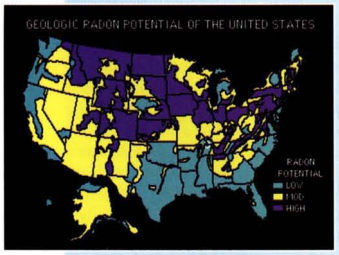
EHPnet

Recent reports in both the scientific and the popular press have focused attention on the potential hazards of radon exposure in homes. In the June 7 issue of the *Journal of the National Cancer Institute*, Jay Lubin and colleagues published an extensive analysis of 11 studies of radon-exposed mine workers that demonstrates that radon exposure in homes may account for as many as 14,400 lung cancer deaths per year. A research article in this issue of *EHP* suggests that EPA figures may overestimate radon risks by failing to account for residential mobility (see p. 1144).

The Radon Home Page (http://sedwww.cr.usgs.gov:8080/radon/radonhome.html), created by the United States Geological Survey (USGS), part of the Department of the Interior, provides information for citizens and scientists on basic and applied research on geology and geochemistry of radon in rocks, soils, and water.

Radon gas results from the natural breakdown of uranium in soil, rock, and water. Although levels of radon vary from place to place, high levels of indoor radon are found in every state. People may feel especially vulnerable to radon exposure because humans are unable, via their own senses, to detect radon. The Radon Home Page provides a means of becoming better educated about radon and of learning how to reduce potential radon risks. In addition to basic information on radon in the geologic environment, the site provides a hyperlink to *The Citizen's Guide to Radon* and other consumer publications on radon from the EPA, as well as a list of radon-related publications.

The Radon Home Page contains a hyperlink to two maps describing radon potential by state and by EPA region. More detailed information may be obtained from USGS geologic radon potential books (which can be ordered from the USGS or obtained from a local library). These reports describe the geology, soils, radioactivity, generalized housing construction characteristics, and other relevant information, and include discussions of the geologic factors controlling radon potential in each state. The site also describes the High Radon Homes Project, which is a cooperative effort of the USGS, the EPA, the Department of Energy, and Lawrence Berkeley National Laboratory that aims to develop a model for identifying the proportion of homes in an area where occupants are suffering high exposures (occupant exposures exceed the occupational radiation limit of 20 picocuries per liter) to radon decay products. Information from this project will be used to convey information to the public on high radon areas, assist homeowners in testing and remediation, develop procedures and codes for new construction, and confirm scientific investigations in high radon areas. Such information, coupled with epidemiologic studies, may also be useful in determining the cancer risk associated with high residential exposures to radon.



For research scientists, The Radon Home Page includes a section on basic and applied geographic radon research. Hyperlinks in this section take the user to abstracts of current work in fault and shear zones in the eastern United States, glacial deposits of the upper Midwest, and terrestrial gamma radioactivity of the contiguous United States.

Milk May Do a Body Good

Researchers in Sweden have isolated a substance from human milk that destroys cancer cells while leaving healthy cells unaffected. The research, published in the August 15 issue of the *Proceedings of the National Academy of Sciences*, may stimulate a new approach to developing antitumor drugs and suggests that breastfeeding may play yet another important role in infant health.

The discovery grew out of a serendipitous observation made by Anders Håkansson, a graduate student in clinical immunology at Lund University in Sweden. Håkansson originally set out to study how milk affects the attachment of pathogenic bacteria to lung cells, an important step in the disease process. Like many investigators, he used malignant, or transformed, lung cells in his experiment because these can be cultured more easily *in vitro* than normal cells. When the malignant cells were exposed to milk, the cells died. This observation stimulated further research by Håkansson and collaborators at Lund University and at the Karolinska Institute in Stockholm.

The investigators isolated the active component of milk, the common milk protein lactalbumin. For reasons not understood, the cytotoxic effect was not

produced by single protein molecules but by aggregates of two or more molecules, called multimeric lactalbumin (MAL). Research on other cell types demonstrated that the effect of MAL was selective—in addition to tumor cells, lymphoid and embryonic cells were also killed, while mature normal cells of lung epithelium and solid organs were left unharmed. Moreover, the investigators noted that the antitumor property of human milk is not shared by bovine milk, which lacks the ability to form aggregates of the protein.

Although animal experiments using MAL are planned, Håkansson emphasizes that MAL is not a cancer cure. "We don't know anything about the *in vivo* effects of the protein, or if this will work as an anticancer drug," he said. In fact, Håkansson feels the real significance of his observation may be another line of research it has stimulated—the elucidation of the mechanism by which MAL destroys tumor cells.

The researchers found that MAL destroys tumor cells by inducing the process of apoptosis, the sequence of genetically programmed steps by which cells self-destruct. Apoptosis can occur naturally at the end of a cell's life or when a cell is diseased and "sacrifices itself" to prevent the spread of disease. In many diseases, however, the mechanism that initiates apoptosis malfunctions. "In about 50% of tumors, the cancer cells cannot undergo apoptosis," Håkansson observes. "The cells have inactivated the pathway [for apoptosis], so they live longer than they're supposed to. This is a mechanism of cancer. The interesting part of this study is that we can perhaps get past this inactivation and still induce apoptosis in these cells," he said.

"This is a unique observation that could have good potential in the design of new antitumor agents," comments Kathryn Rich, assistant professor of pathology and ophthalmology at the University of Southern California, whose research focuses on apoptosis in neurons. One of the goals of apoptosis research, Rich observes, is to "use this normal physiological process as a weapon against the cancer cell." Unraveling the apoptotic pathways in cancer cells could lead to design of antitumor agents that would switch on apoptosis, thereby killing cancer cells. An important finding of the Swedish study, Rich adds, is the selectivity of MAL's effects. "Most other external agents tend to kill both normal and tumor cells. Understanding why tumor cells are sensitive [to MAL] and why normal gut epithelial cells are resistant . . . could be very promising."

According to Charles Isaacs, director of the Laboratory of Protein Chemistry at the New York State Institute for Basic Research, the new study is also important because it suggests that breastfeeding plays a previously unrecognized role in infant development. He notes that while it has long been known that human milk provides excellent nutrition and protection against infection, a possible role in regulating cell growth has been hypothesized but never demonstrated. "This study suggests that yes, maybe some of the baby's cells can be affected because there are compounds in milk that induce apoptosis," Isaacs said. "It's possible that MAL has an effect on cells in the baby's gastrointestinal tract and also on the baby's own lymphocytes." Cautioning that further testing is needed to see if the same effects can be observed *in vivo*, Isaccs adds that the study could stimulate research to find other substances in milk that regulate cell growth.

Why would milk contain a substance that destroys tumor cells? The answer is uncertain, but may lie in another of MAL's effects—it destroys not only tumor cells, but immature cells as well. Håkansson speculates that this selective cytotoxicity may play a role in controlling populations of maturing cells in the infant's gastrointestinal tract. Controlling the growth of immature cells may, in fact, be the primary function of MAL, and its effects on tumor cells may be secondary. Håkansson notes, "Cancer cells also have a very immature cell phenotype, so [that may be the reason] they are also affected." He adds, "It is not strange that milk has effects other than just nutrition. We see now that it has some control over cell proliferation and cell death."

TRI Troubles

In a move to prevent Republican lawmakers from weakening environmental laws through amendments tacked onto the budget bills, President Clinton signed an executive order August 8 requiring companies that contract with the federal government to report their releases of toxic chemicals.

The impetus for the order was 1 of 18 amendments to the House appropriations bill for the EPA. This rider would prohibit the EPA from requiring manufacturing plants to report new data to the Toxics Release Inventory (TRI) of the 1986 Emergency Planning and Community Right-to-Know Act for any reason not already in the law. The TRI contains emission reports of 23,000 companies on 651 chemicals. EPA Administrator Carol Browner said that the rider "essentially limits my ability to guarantee that the people of this country will know about toxic chemicals in their communities."

Although the executive order applies only to those firms doing business with the federal government and would not exempt any company already reporting to the TRI, it would effectively limit the EPA's proposed toxic use inventory—a system that would include not only toxic emissions, but also toxic chemicals entering a plant's process. Many industry groups, including chemical manufacturers, already question the effectiveness of the TRI and are lobbying against the new inventory.

And there may be some evidence to support industry claims that TRI data do not provide the most relevant information in terms of risk from chemical exposures. Researcher Reid Lea and colleagues at the University of New Orleans developed a system for weighing the risk of toxic exposure to humans and the environment and used it to analyze TRI data for Louisiana. They found that the amount of a chemical released into the environment does not necessarily correlate with the extent of the danger it poses. In their paper, "Comparative Risk Analysis of TRI Data as an Environmental Indicator—A Louisiana Case Study," presented at the Air & Waste Management Association's 88th Annual Meeting, the researchers wrote, "The traditional method of analyzing the TRI data fails to address the true concerns about chemical emission to the environment."

New Clues to Cell Death

Two groups of scientists have independently identified an important trigger in apoptosis, the complex sequence of events that causes cells to self-destruct. The finding raises the possibility of developing drugs that could selectively induce or inhibit apoptosis.

The trigger, a protease, or enzyme that cleaves other proteins, was recently identified by Donald W. Nicholson at the Merck Frosst Centre for Therapeutic Research in Quebec, Muneesh Tewari at the University of Michigan Medical School, and their collaborators. In the July 6 issue of *Nature*, Nicholson and colleagues named the protease apopain. In the June 2 issue of *Cell*, Tewari's group named it "Yama" after the Hindu god of death. The authors believe Yama/apopain is a cru-

cial component of the mammalian cell-death pathway. "It is something that we suspect will be activated in a variety of different instances of apoptosis," Tewari said.

Apoptosis is a sequential, programmed series of steps by which cells destroy themselves. Some scientists suggest that inappropriate apoptosis may be responsible for damage seen in autoimmune disorders, immune deficiencies, Alzheimer's, Huntington's and Parkinson's diseases, cancers, stroke, and heart attack.

The recent discovery was made using mammalian systems. It underscores the similarities between apoptosis in the nematode worm, *Caenorhabditis elegans* (which has provided much of our understanding about apoptosis) and mammals. In the nematode, apoptosis is controlled by sets of genes including *ced*-3 and *ced*-9. Expression of *ced*-3 is required for cell death to occur. Expression of *ced*-9 blocks cell death. The *ced*-9 gene is homologous to a set of mammalian oncogenes.

Mammals also have a set of genes that function like the nematode's *ced*-3 gene. One such gene encodes an enzyme called ICE, interleukin-1ß converting enzyme. ICE is implicated in some forms of apoptosis but is not the trigger for all forms of mammalian apoptosis. Scientists concluded that a different, unidentified enzyme played that role. Both Tewari's and Nicholson's groups identified this enzyme as CPP32ß, a previously uncharacterized protein related to ICE, which they named Yama/apopain. As apoptosis begins, enzymes, including one called PARP [poly(ADP-ribose) polymerase], are cleaved into smaller fragments. PARP maintains and repairs DNA. The researchers used PARP cleavage to identify Yama/apopain.

"I am sort of surprised that this discovery hasn't received more coverage in the scientific lay press. It really is a pivotal finding," said Tewari's co-author Vishva M. Dixit of the University of Michigan Medical School. "It allows for the first time a toe in the door of mammalian cell death. We can enlarge on the knowledge and identify the other components. I think the entire story, the essential components of the pathway, will probably fall in place the next couple of years."

Gloria Preston, an apoptosis researcher at the NIEHS, notes that this is an important finding because proteolytic cleavage does appear to play a role in apoptosis. "Apparently, other proteases exist that have some similarity to Yama/apopain, and whether this particular protease is pivotal to the process is not clear. At this time it is difficult to determine what cleavage events are essential and what ones are merely part of the degradation process."

Yama. University of Michigan researchers named the new-found apoptosis enzyme after the Hindu god of death.

Yama/apopain does appear to be the key protease in the apoptosis studied in the mammalian osteosarcoma cells described by Nicholson et al., according to Shai Shahan, who studies apoptosis in nematodes at MIT. But since Yama/apopain is part of a family of proteases, it is possible there is redundancy. More than one protease might be responsible for triggering cell death in a given cell. Also, one protease might be responsible for activating cell death in a given tissue and another protease of the same class will activate it in yet another tissue, Shahan said.

That would please pharmaceutical companies. The question from a pharmaceutical point of view is whether or not there are certain proteases that are present in only certain cells. "I guess the ideal thing would be if in a neurodegenerative disease there were an ICE homologue or close relative that caused cell death. If one could identify a specific inhibitor, we would be able to prevent cell death," said Douglas K. Miller, senior investigator at the Merck Research Laboratories in New Jersey, and a co-author of the *Cell* paper. In applying the finding to cancer therapy, however, it would be necessary to induce, not inhibit, apoptosis under controlled conditions.

While Dixit and colleagues are now concentrating on identifying the enzyme that activates Yama, the Merck researchers are concentrating on characterizing the five other mammalian homologues of ICE. They will then look at them to see if they are associated at all with disease, Miller said.

Consequences of Climate Change

Intense summer heat waves could kill thousands more people each year in New York, St. Louis, and other U.S. cities, epidemics of infectious diseases could continue sweeping into temperate climates from the tropics, and numbers of skin cancers will probably rise in mid-latitude regions of North America, Europe, and Australia, all during the next century. These impacts of climate change were predicted by experts at the Conference on Human Health and Global Climate Change, September 11–12, at the National Academy of Sciences in Washington, DC.

Until recently, scientists and policymakers had primarily focused on how the earth's physical systems would be affected by global warming, examining the potential for increased sea level rises and bigger hurricanes. But the Intergovernmental Panel on Climate Change, in a forthcoming update of its 1990 study, will for the first time include a chapter on the possible human health effects of climate change. In turn, Vice President Al Gore asked the Office of Science and Technology Policy and the Council on Environmental Quality to organize a conference on this theme. Co-sponsored by the National Science and Technology Council and the Institute of Medicine, the conference gathered a diverse group of experts from academia, government, and nongovernmental organizations. Conference participants discussed how climate change might damage human health, then made recommendations for improving global health surveillance systems, research, education of health professionals, and international cooperation.

In industrialized nations, the greatest impacts of climate change probably will be experienced through heat waves. Global warming of 1°–3°C would probably increase the frequency of intensely hot days in temperate regions, resulting in several thousand extra deaths annually due to heat stress in the United States, said Laurence S. Kalkstein of the Center for Climatic Research at the University of Delaware. More than 500 Chicagoans died during a July 1995 heat wave, a type of disaster that could occur more often in the future. "Living in heat-trapping brick tenements with black-tar roofs, many poor, elderly, or frail residents of New York, Chicago, and other cities will be unable to adjust to a warming climate," said Kalkstein.

Faced with hotter summers, those who can afford air conditioning will use it more frequently, resulting in more air pollution, predicted Joel Schwartz, epidemiologist at the Harvard University School of Public

An uncertain future. Manufacturing and deforestation contribute to climate change effects such as global warming and spread of disease. (*Ebola: Frederick A. Murphy, Aedes aegypti: Leonard Munstermann.*)

Health. Fine particulates from power plants, he noted, are clearly associated with hospital emissions for respiratory disease and heart disease. "Power plants are the biggest source of air pollution concentrations along the East Coast, and we think this pollution will get worse."

Over the next 50 years, depletion of stratospheric ozone is expected to lead to greater numbers of skin cancers, especially among fair-skinned people in temperate climates, where the ozone layer is thinner. Although the ozone layer will reach its lowest level around the year 2000, it will gradually thicken because of international regulations on the use of chlorofluorocarbons and other chlorine-containing chemicals. But skin cancers usually do not appear until decades after exposure. "Even under the Montreal Protocol, the numbers of nonmelanoma skin cancers will be about 25% higher in 2050 than in 1980 in mid-latitude cities such as Vancouver, Paris, and Prague," said Margaret Kripke, an immunologist with the M.D. Anderson Cancer Center in Houston. "Melanoma will also increase, but nobody knows by how much."

A warming planet will continue to bring more extreme and erratic weather, with greater incidence of droughts, floods, and hurricanes, which in turn will increase rates of death, injury, and infectious diseases due to proliferations of pests, experts said. For example, during each of the past five years, El Niño, an ocean warming system, has spread very warm, wet spells around the world. As a result, the mosquitoes that carry malaria, yellow fever, and dengue have moved into some temperate climates and higher altitudes, infecting people who lack immunity to these diseases, said Paul Epstein, specialist in tropical public health at Harvard University. Other major diseases likely to spread with global warming are cholera, filariasis, and sleeping sickness.

Deforestation and urbanization in some developing countries contribute to this dangerous mix. As tropical forests are cut down for timber and agriculture, people are more likely to come into contact with previously remote disease carriers. Meanwhile, growing numbers of poor in developing nations are leaving rural areas for burgeoning, unsanitary cities, which are "veritable incubators for disease," said J. Brian Atwood, administrator for the U.S. Agency for International Development. The recent outbreak of the Ebola virus in Africa is an example of the movement of a very rare disease from a rural area into a city, where it could spread rapidly.

To address these issues, health professionals in the field must be better trained to monitor and report emerging diseases. "Local health-care workers need training to increase their sensitivity to new health problems, and they need to know who to tell about the problems," said William Bancroft, director of the Military Infectious Disease Research Program at the Army Medical Research and Materiel Command. A global network for disease surveillance exists informally, but it must be strengthed, with improved links to health professionals. "Health-care workers need to know how to get into the surveillance system so they can get a response," Bancroft added.

Vulnerable populations, including children, elderly people, and those in transitional areas where disease epidemics could spread, should be targets of an improved global surveillance system. Vulnerable people must be better educated about growing health risks, with access to vaccines and information about their exposure.

The United States has taken an international leadership role in disease surveillance, control, and research, a role that should be continued, said conference participants. "We're going to lead the situation," said Bancroft. "No other nation can do what we're suggesting ought to be done." But the nation's current funding for research on infectious diseases, other than AIDS and tuberculosis, is limited. Now the United States must better support the work of epidemiologists, laboratory scientists, entomologists, behavioral scientists, public health experts, and others so they can find new solutions for emerging disease threats, according to conference speakers. Conference participants also agreed that more bridges should be built between environmental science and the public health communities. In particular, physicians and other health professionals need improved environmental science education.

Finally, pilot research projects should study El Niño as a possible signal that we are entering a new climate pattern. "We should look at El Niño as an analogy for climate change," Epstein said. "El Niño, as a warming event in the ocean and atmosphere, gives us an idea of what future warming events might be like. [The current] El Niño has persisted for five years in a row, yet no El Niño has persisted for more than three years. So studying El Niño can help us understand the trends and variability of climate, and the effects that climate changes can have on disease carriers such as mosquitoes."

What's News to You?

Environmental Health Perspectives welcomes topic suggestions for upcoming "Environews" articles. Send suggestions to: News Editor, *Environmental Health Perspectives*, National Institutes of Environmental Health Sciences, PO Box 12233, 111 Alexander Drive, Research Triangle Park, NC 27709 USA. Fax: (919) 541-0273 or E-mail: Thigpen_K@niehs. nih.gov.

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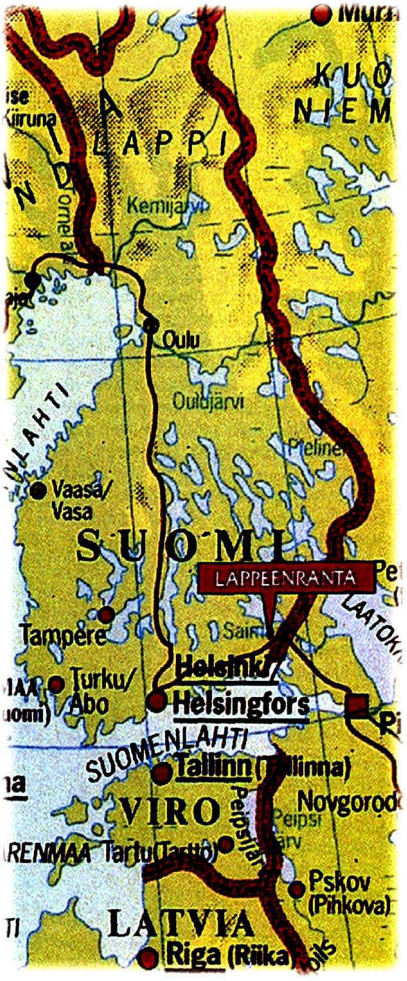

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Molecules and Mechanisms

After 28 years, environmental health scientists at the NIEHS Environmental Health Sciences Center at Oregon State University (OSU) are still trendsetters. The research agenda has matured with the times to a focus on the mechanisms by which toxic substances affect living tissues. Today researchers observe events that were barely discernible in the center's infancy, coupling the latest mass spectrometry techniques with cell culture and DNA gene splicing to peer deep into subcellular workings. "In molecular biology, and particularly in nucleic acid sequencing, something that had taken you two months to do in '65 now takes a day," points out center Director Donald Reed, a distinguished professor of biochemistry. "You can ask questions at a level we would've once thought impossible."

Early Years

For almost as long as chemical companies have promoted pesticides as the means to gain miraculous increases in crop yields, researchers at OSU have carefully investigated the unexpected side effects and unintended distribution of these chemicals. The concern began well before World War II, when arsenic and other inorganic chemicals were the main members of the farmer's arsenal. After the deluge of synthetic pesticides in the mid-1940s, scientists from OSU's agricultural chemistry department began investigating incidents like fish kills in the wake of improper use of pesticides, the appearance of chlorinated hydrocarbon and organic phosphorous-containing insecticides in fish and soil sediments, and pesticide residues in fruits and vegetables.

By 1964, concerned researchers at the university had amassed sufficient evidence of the problems with pesticides to receive an NIH program grant to study them. The program, "Toxicology of Pesticides in the Environment," ultimately led to OSU earning one of the first Environmental Health Sciences Center research core grants from the National Cancer Institute in 1967. When the NIEHS was founded in 1969, it took over these grants. The current research program grant, "Toxicology of Environmental Chemicals," funds areas of research such as the biochemical and molecular mechanisms through which exposure to dioxins affects T-lymphocytes and how this is related to specific kinds of immunosuppression. It also supports research in which scientists expose *E. coli* bacteria to ultraviolet light and sulfur dioxide and look for mutations to investigate how UV radiation and sulfur dioxide, which associated with respiratory disease, cause damage to DNA. Humans are exposed to sulfur dioxide through automobile exhaust and food and wine additives.

Under Reed's leadership since 1981, the center has shifted its focus toward molecular biology and toxicology, making it one of the first centers to focus on mechanisms of action. The center's researchers were recently reorganized into groups reflecting the center's major areas of inquiry. These research core units include carcinogenesis, cell biology and immunotoxicology, molecular and genetic toxicology, and structural and environmental chemistry.

Two Centers in One Campus

Across from OSU's Environmental Health Sciences Center in Corvallis is the university's Marine/Freshwater Biomedical Sciences Center. One of the five specialty centers funded by the NIEHS, the MFBS center's close proximity allows for a rich mix of information and faculty. In addition to directing the MFBS Center, George Bailey, distinguished professor of food toxicology, is also the co-leader of the main environmental health sciences center's carcinogenesis research core. (The OSU center also has ties to the Oregon Health Sciences University in Portland which includes a medical school.)

Even before the adjunct center for studies of aquatic environmental issues was formed, the OSU center was respected for its interdisciplinary pursuits. When the center received its first university funding in 1966, Virgil Freed, director of the Department of Agricultural Chemistry, solicited help from scientists in the departments of psychology, fisheries and wildlife, engineering, nuclear chemistry, veterinary medicine, and agricultural economics. This collection of researchers from varied disciplines was indisputably effective in helping achieve some of the center's major research breakthroughs.

For example, researchers at the center generated much of the data that ultimately resulted in DDT being banned in the United States and have led the field in dioxin toxicity investigations. Center research into the physical basis for widespread distribution of man-made chemicals into the environment helped explain how pesticides made their way in the polar ice caps. Researchers at the OSU center conducted the earliest investigations into the mechanisms behind the preferential effects of pesticides on certain species and have made major contributions to understanding how protective systems aid in coping with exposure to toxicants. Research at the center has also contributed to the understanding of the mechanisms of action of aflatoxin, a widely recognized food carcinogen, the heart's muscarinic receptors, changes in which are associated with Alzheimer's disease, cardiac arrhythmias, and pesticide poisoning, and the cytochrome P450s affected by disruption of enzyme regulation.

Extending Expertise

The OSU center consists of an integrated staff of researchers from departments such as agricultural chemistry, biochemistry and biophysics, food science and technology, statistics, veterinary medicine, and zoology, whose expertise complements each other.

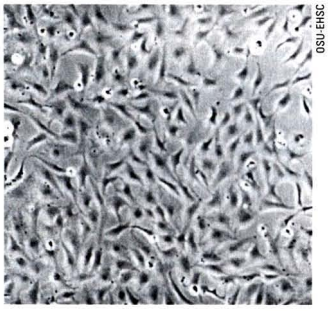
For example, the specialized knowledge of mass spectrometry represented by the structural and environmental chemistry research core has helped Dale Mosbaugh, a professor of agricultural chemistry and the leader of the molecular and genetic toxicology research core investigating DNA repair,

OSU-EHSC

Partners in progress (left to right): George Bailey, director of the MFB Center; Ken Olden, director of NIEHS; Anne Sassaman, director of extramural research and training, NIEHS; and Donald Reed, director of the OSU EHS Center.

perform analyses. Recalling a project that attempted to define the domains of the uracil DNA glycosylase protein that interacted and bound to DNA, Mosbaugh said, "Our approach was to cross-link the enzyme to the DNA and isolate for the cross-linked peptide fragments. The peptides would define what parts of the protein were actually contacting the DNA. We ran into a bit of a roadblock, in that we had difficulty resolving all of the cross-linked peptides because the DNA that we'd cross-linked it to dominated their properties." Mosbaugh learned that another center researcher, Doug Barofsky, professor of agricultural chemistry and leader of the structural and environmental chemistry research core, was cross-linking proteins with DNA, then analyzing by mass spectrometry the size of those cross-linked products. Because of this research, Barofsky was able to suggest a different approach. Said Mosbaugh, "I'm not sure I would've known [the answer] as soon had we not both been in the center . . . Working at the center means I'm able to interact with other members and feel that their expertise and equipment are an extension of my own."

A physicist by training, Barofsky built the center's two matrix-assisted desorption-ionization time-of-flight (MALDI-TOF) spectrometers used to conduct such research. In collaboration with Mosbaugh, he recently confirmed the formation of a uracil DNA glycosylase–DNA complex by using ultraviolet light to permanently bind the two together and then using MALDI-TOF mass spectometry to identify the peptide sequences defining the DNA binding domain. This was the first time this technique had been used to establish contact points in a DNA–protein complex. Barofsky is now focusing on the process by which DNA and proteins interact.



OSU-EHSC

Culture club. Center researchers use zebrafish embryo cell cultures to examine Ah receptor-mediated responses to toxicants.

OSU-EHSC

Experts in mass spec. Max Deinzer (left) and Douglas Barofsky discuss mass spectrometric approaches to center research.

Collaborations of this type have allowed the center to excel in DNA repair research. Building on previous research investigating the enzymatic nature involved in uracil–DNA repair, Mosbaugh is now involved in elucidating the biochemical and biological role of the uracil–DNA repair pathway in human fibroblasts.

Max Deinzer, professor of agricultural chemistry and deputy director of the center, and Michael Schimerlik, professor of biochemistry and biophysics and also a deputy director of the center, are using an electrospray-ionization mass spectrometer to investigate the folding kinetics of proteins, specifically the human recombinant macrophage colony-stimulating factor, by measuring the ratios of deuterium to hydrogen. "We're trying to determine how the proteins form and what environmental chemicals will do to upset the formation," Deinzer explained. Deinzer, Schimerlik, and Mosbaugh also hope to use the technique to observe how the uracil *N*-glycosylase inhibitor protein folds.

The cell biology and immunotoxicology research core is led by David Barnes, a professor of biochemistry and biophysics. A decade ago Barnes used an unorthodox approach to create a medium on which neural stem cells could grow normally without senescence; previously, biologists believed that cells could not continue to grow normally for an indefinite period. Currently Barnes is developing *in vitro* culture techniques suitable for introducing and expressing exogenous DNA in zebrafish and trout cells to examine them for aryl hydrocarbon (Ah) receptor-mediated responses.

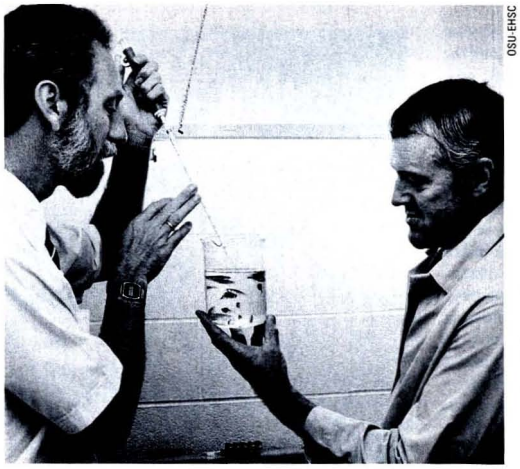
Nonmammalian Alternatives

Barnes's research is one example of a major focus of the OSU center on developing alternative models for research into chemical carcinogenesis. Another is the rainbow trout model, which established the value of nonmammalian alternatives for carcinogenesis studies. Trout, like humans, are resistant to peroxisome proliferation. Because peroxisome proliferation has been shown to cause liver cancer in rodents, but not in humans, the trout model allows scientists to explore these mechanisms. "Fish are incredibly economical in terms of space requirements and rearing constraints," explained Bailey. "We can focus attention on issues in cancer dose response that are really statistically quite challenging. Therefore, we are able to propose and carry out studies with from 10,000 to 30,000 animals at a fraction of the cost for rodents. We can establish a relationship between a carcinogen and/or an anticarcinogen and the amount of damage, then statistically quantify the relationship in a way not possible with rodent models."

A model currently under investigation by the cell biology and immunotoxicology research core uses zebrafish. Zebrafish are advantageous as a model because they are available year round and reach sexual maturity in three months. They are especially suitable for embryonic studies, Barnes points out, because fertilization

occurs outside the body. "Besides being observable under the microscope, it's easy to synchronize the mass fertilization of hundreds of embryos," says Barnes. "With a mouse, by comparison, you get maybe a dozen if you're lucky. We ultimately have our eyes on the dioxin [Ah] receptor. Some studies suggest that the Ah receptor has a key function in normal embryonic development."

The possibilities of the new fish model are especially exciting considering the breakthroughs achieved with the trout model. Bailey has used the trout model to study the mechanisms of inhibition of chemical carcinogenesis for more than a dozen years. Building on successes with the indole-3-carbinol from plants of the genus *Brassica,* which include broccoli, cauliflower, and cabbages and have shown promise against human breast cancer, Bailey recently researched the chemoprotective properties of chlorophyllin, a form of the chlorophyll found in all green plants. The studies were the first to prove in an animal model that chlorophyllin could inhibit experimental cancer, primarily by trapping carcinogens in the intestinal tract. Eighty percent tumor inhibition was achieved with only 1–2% of chlorophyll levels found in common spinach. Other scientists are following up with rodent studies and human trials are under consideration at The Johns Hopkins University.



OSU-EHSC

Earning their stripes. Fast-maturing zebrafish are used by center scientists George Bailey (left) and Jerry Hendricks as animal models in cell biology and immunotoxicology research.

Outreach

In addition to sharing their expertise among themselves, the center's scientists have long recognized the need to share their findings with researchers outside the center. The center's strong ties to the College of Agricultural Sciences at OSU and the state's Cooperative Extension service at OSU have greatly aided in this effort. Researcher Nancy Kerkvliet, professor of agricultural chemistry, now directs the outreach program. "Our mission is to bring the latest in university research to the public," said Kerkvliet. "We focus on presenting the relative risks of pesticides in general, as well as environmental chemicals like domoic acid [a shellfish contaminant] and dioxins."

At present, the center's focus remains man-made chemicals, with the exception of Bailey's research into chemoprotective materials. In the future the center may also investigate how naturally occurring substances fit into the toxicology picture. "As we understand more and more about the mechanisms of man-made chemicals, we wonder, are there things that are naturally existing that do the same thing in the same way?" said Reed. "We know that tobacco and alcohol make some impact in terms of outcomes of whatever environmental exposures we have. We need to do more to demonstrate the actual role of these components so that people have a better basis for making decisions about their individual lifestyles."

Kellyn Betts

1996 Keystone Symposia

on Molecular and Cellular Biology

Application/Abstract Deadline: Sept. 20, 1995

Integrins and Signaling Events in Cell Biology and Disease
Organizers: David A. Cheresh and Martin A. Schwartz
Molecular and Developmental Biology of the Extracellular Matrix
Organizers: Benoit de Crombrugghe, Clayton A. Buck and Francesco Ramirez
January 5-11, 1996; Keystone, Colorado

Small GTP-binding Proteins and Growth Factor Signaling Pathways
Organizers: Gary M. Bokoch and Richard A. Cerione
January 5-11, 1996; Tamarron, Colorado

Oxidant Stress: From Molecules to Man
Organizers: Mary E. Gerritsen, D. Neil Granger and Guy Zimmerman
January 8-14, 1996; Santa Fe, New Mexico

The Cell Cycle
Organizers: Steven I. Reed and Joan Ruderman
January 11-17, 1996; Taos, New Mexico

Blood Stem Cell and Bone Marrow Transplants
Organizers: Robert Peter Gale, Richard E. Champlin and James O. Armitage
January 15-21, 1996; Keystone, Colorado

Application/Abstract Deadline: Sept. 27, 1995

Molecular Biology of HIV
Organizers: B. Matija Peterlin and Bryan Cullen
January 17-23, 1996; Taos, New Mexico

Hepatitis C and Beyond
Organizers: Richard H. Decker and Robert H. Purcell
January 23-29, 1996; Burlington, Vermont

Tissue Engineering
Organizers: Jeffrey A. Hubbell and A. Hari Reddi
Wound Repair in Context
Organizers: Richard A. Clark and John McPherson
January 23-29, 1996; Taos, New Mexico

The Molecular Biology of the Cardiovascular System
Organizers: Jeffrey M. Leiden, Victor J. Dzau, Richard Lawn, Christine Seidman, Robert Rosenberg and R. SandersWilliams
January 29-February 4, 1996; Keystone, Colorado

Breast and Prostate Cancer: Basic Mechanisms
Organizers: Mina Bissell and Timothy Thompson
January 29-February 4, 1996; Taos, New Mexico

Cell Polarity
Organizers: Keith E. Mostov and Pietro De Camilli
February 1-7, 1996; Lake Tahoe, California

Application/Abstract Deadline: Oct. 4, 1995

Ion Channels as Therapeutic Targets
Organizers: Michael Cahalan, K. George Chandy, Doug Hanson and Alan North
February 4-10, 1996; Tamarron, Colorado

Gene Therapy for Hematopoietic Stem Cells in Genetic Disease and Cancer
Organizers: Stefan Karlsson, Keith Humphries and Paul Tolstoshev
February 4-10, 1996; Taos, New Mexico

Cell Migration
Organizers: Michael P. Sheetz, Rick Horwitz and Doug Lauffenburger
February 4-10, 1996; Santa Fe, New Mexico

Neural Peptides
Organizers: Richard E. Mains, James F. Battey and RobertA.Steiner
February 8-14, 1996; Lake Tahoe, California

Inductive Interactions during Vertebrate Embryogenesis
Organizers: Steven C. Pruitt, Marianne Bronner-Fraser, Robert M. Grainger, Mary Mullins, Christopher V.E. Wright and Richard R. Behringer
February 8-14, 1996; Hilton Head Island, South Carolina

Molecular Mechanisms in DNA Replication and Recombination
Organizers: Michael O'Donnell and Stephen C. West
February 10-16, 1996; Taos, New Mexico

Cell Biology of Virus Entry, Replication and Pathogenesis
Organizers: Richard W. Compans, Ari Helenius and Michael B.A. Oldstone
February 10-16, 1996; Santa Fe, New Mexico

Application/Abstract Deadline: Oct. 18, 1995

Molecular Regulation of Platelet Production
Organizers: Martin J. Murphy, Alan M. Gewirtz and Si Lok
The Hematopoietic Microenvironment
Organizers: Armand Keating and Joel Greenberger
Febru ary 16-22, 1996; Taos, New Mexico

Exploring and Exploiting Antibody and Ig Superfamily Combining Sites
Organizers: Edgar Haber and James S. Huston
February 22-28, 1996; Taos, New Mexico

Molecular Helminthology: An Integrated Approach
Organizers: James L. Bennett, Timothy G. Geary, Donald L. Riddle, Richard M. Maizels, Philip LoVerde and Valarie Williamson
February 22-28, 1996; Santa Fe, New Mexico

Molecular Approaches to the Function of Intercellular Junctions
Organizers: Bruce J. Nicholson, Daniel Goodenough, Pamela Cowin and Peter Bryant
March 1-7, 1996; Lake Tahoe, California

Viral Genome Replication
Organizers: Paul Ahlquist, Jeremy Bruenn and Eckard Wimmer
March 1-7, 1996; Tamarron, Colorado

Posttranscriptional RNA Processing
Organizers: Kenneth Stuart, Michael Green and Jack Szostak
March 11-17, 1996; Hilton Head Island, South Carolina

Molecular Basis for Drug Resistance in Bacteria, Parasites and Fungi
Organizers: Stuart B. Levy and Thomas E. Wellems
March 11-17, 1996; Park City, Utah

The Extracellular Matrix of Plants: Molecular, Cellular and Developmental Biology
Organizers: Andrew Staehelin, Michael Hahn, Norman Lewis, Andrew Mort and Keith Roberts
March 15-21, 1996; Tamarron, Colorado

Application/Abstract Deadline: Nov. 15, 1995

Steroid/Thyroid/Retinoic Acid Gene Family
Organizers: Kathryn B. Horwitz, John A. Cidlowski and Ronald Evans
March 17-23, 1996; Lake Tahoe, California

Transcriptional Mechanisms
Organizers: Thomas Shenk and Danny Reinberg
March 17-23, 1996; Taos, New Mexico

Lymphocyte Activation
Organizers: Susan L. Swain, B.J. Fowlkes and John Cambier
March 20-26, 1996; Hilton Head Island, South Carolina

Proteolytic Enzymes and Inhibitors in Biology and Medicine
Organizers: James Travis, Clarence A. Ryan, Hidsaki Nagase and Christine Debouck
March 25-31, 1996; Keystone, Colorado

Immunopathogenesis of HIV Infection
Organizers: Robert Schooley, Bruce D. Walker and Denis Henrard
March 26-April 1, 1996; Hilton Head Island, S. Carolina

Signal Transduction through Tyrosine Kinases
Organizers: Ralph A. Bradshaw, Lena Classen-Welsh, David Kaplan, Linda Pike and Klaus Seedorf
Signaling in Neuronal Development, Differentiation and Degeneration
Organizers: Mariano Barbacid, Michael Greenberg, Ronald McKay and George Yancopoulos
March 27-April 2, 1996; Taos, New Mexico

The Conduct of Science: Keeping the Faith
Organizers: John Bailer, Jeff Williams
May 2-5, 1996; Keystone, Colorado

* Registration in any meeting in a shaded group qualifies for attendance in any meeting in that group.

For more information, please contact: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498
Phone: (970) 262-1230 Fax: (970) 262-1525 E-mail: symposia@keystone.org

What More of US *Means*

When the first Europeans visited Easter Island in 1722, they found a barren land and exhausted people; 3,000 inhabitants were fighting for what remained of a lush and fertile Polynesian island. With their growing population quickly consuming its natural resources, the island's dense forests, rich soil, and abundant wildlife were nearly gone.

Today Easter Island has become a symbol of what could happen to the entire world if the human population continues to expand at its present rate. Just 200 years ago, the total world population hovered around one billion. By 1930, that number had doubled. Between 1950 and 1994, it more than doubled again, jumping from 2.6 billion to 5.7 billion, and 1 billion people are now added every 11 years. Since the mid-1800s, the world's population has quadrupled.

This astounding population growth comes on the heels of the industrial revolution and major innovations in technology and medicine. According to geographer and independent scholar Robert Kates, the present surge is one of three in human history: the first two coincided with the emergence of toolmaking and the spread of agriculture. "We are now in the last phase of the third major population surge, the completion of a demographic transition from a world with high rates of births and deaths to one with low rates," he wrote in the October 1994 issue of *Scientific American.*

Most of this century's population growth has taken place in the developing countries, not because of a sharp rise in birth rates, but rather a dramatic drop in the number of deaths, thanks in part to victories over such common diseases as malaria, smallpox, and cholera— "nature's first line of defense against the expansion of *homo sapiens,*" said Samuel Preston of the Population Studies Center at the University of Pennsylvania in a 1993 speech. Better living conditions, such as housing, nutrition, and sanitation, have also helped many more children survive.

Population vs. Environment

A major question now is the effect of this growing population on the environment that helped it develop in the first place. The search for answers to this question has become increasingly complex and controversial. The field of population and environment dynamics now includes demographers, ecologists, economists, physicists, biologists, anthropologists, geographers, nutritionists, and political scientists, many of whom have entered the debate in the last several years, introducing new sets of variables to ongoing research. In 1992, the National Academy of Sciences and Britain's Royal Society issued an unprecedented joint statement on the dangerous trends in population and environmental degradation. The Union of Concerned Scientists in Boston followed up that same year with a similar document.

How many people Earth can support depends on how many wear cotton and how many polyester; on how many eat meat and how many bean sprouts; on how many want parks and how many parking lots.

"Demographers have also just entered the discussion in the last two or three years because decent data on both sides were not available before," says Ronald R. Rindfuss, director of the Carolina Population Center in Chapel Hill, North Carolina. "And some of the sensationalism in the macrodebate had turned young scholars off. But now it seems the questions are starting to be better framed, so they're researchable in ways you can get a decent answer."

Jinsenh Tart

In general, ecologists and biologists tend to be pessimistic about the damage growing populations cause to the environment, based on their assumption that ecosystems have a limited "carrying capacity," or ability to support life. Eventually, they say, the environment will no longer be able to renew itself and will collapse. Economists, on the other hand, are often optimistic; they trust the free market and human ingenuity to develop new technology for coping with ever-larger numbers and scarce resources.

"But no science so far has been able to actually quantify the role of population on the environment," says Robert Engelman, director of the Population and Environment Program at Population Action International in Washington, DC. "At this point, there's no direct way to prove that, say, 80 million more people in a region had a particular impact on the water or air quality. However, it is clear that population is often the critical variable that can cause the degradation and even collapse of natural systems."

The pessimists in the debate often cite Reverend Thomas Robert Malthus, who predicted in 1798 that "the population growth rate would always promptly win a race against the rate of the growth of food, eventually leading to worldwide starvation," as well as "misery, vice, and premature death." His current followers, called Malthusians, have issued similar warnings,

such as Stanford University ecologist Paul Ehrlich's publication in 1968 of *The Population Bomb* and in 1990 of *The Population Explosion.* Ehrlich passionately argues that human numbers must diminish or they will trigger mass environmental destruction.

Even in the 18th century, Malthus had his opponents, notably the Marquis de Condorcet, who claimed the human mind would be able to remove all obstacles to human progress. Some experts today insist that the same ingenuity that created antibiotics and pesticides will help the world sustain its growing numbers and save it from an Easter Island–like fate. For example, University of Maryland economist Julian Simon has argued that ongoing advances in technology and science, such as genetic engineering and superefficient farming, will continue to guarantee the needs of future generations. Others believe that better resource management practices will enable heavily populated communities to thrive.

Any successful approach to solving environmental problems related to population growth must respect and incorporate these different points of view, according to Michael Brower, former director of research at the Union of Concerned Scientists. And over the past 10 years, the arguments have become increasingly subtle, says Sanjay Baliga, research analyst with the Program in Economics and Population at the World Resources Institute. "Because new subtleties are popping up all the time, we need the interaction between disciplines in order to more fully understand the issue," Baliga says.

Bad News and Good News

It is widely assumed that as populations grow, so will the pressure new generations exert on natural resources. The list of environmental problems aggravated by growing populations includes deforestation and desertification, loss of topsoil, poisoning of drinking water and pollution of oceans, shrinking wetlands, shortage of fuels such as firewood, exhaustion of oil reserves and of various mineral resources, siltation in rivers and estuaries, dropping water tables, erosion of the ozone layer, loss of species and wilderness areas, global warming, rising sea levels, nuclear waste, air pollution, and acid rain.

"In the past 10,000 years, since the dawn of agriculture, humans have deforested a net area the size of the continental U.S., mostly using it for cropland," writes Kates. "Water, in an amount greater than the contents of Lake Huron, is diverted every year from the hydrosphere for human use. Half the ecosystems of the ice-free lands of the earth have been modified, managed, or utilized by people. The flows of materials and energy that are removed from their natural settings or synthesized now rival the flows of such materials within nature itself. And half of those changes happened within our lifetimes."

Any population increase often means putting more land under cultivation and raising the production per acre, steps that can require more capital, fertilizers, pesticides, and water irrigation. In the United States, almost all arable land is now under production, leading to soil erosion that averages 8 tons per acre per year, according to Cornell University ecologist David Pimentel. Eighty-five percent of water used in the United States is for agriculture, with the remainder for industrial and public use. Water consumption naturally rises with population growth, and the country's huge groundwater aquifers are disappearing. Parts of Texas and Arizona have already been pumped dry and can no longer be farmed, says Pimentel.

In addition, rapidly growing populations in Mexico, the Philippines, Indonesia, Brazil, and El Salvador have caused environmentally fragile lands to be overfarmed and depleted. The loss of natural habitats to more and more cropland, pastures, roads, and urban spread, Ehrlich warns, "reduces the biological diversity of plants and animals. Some of these natural biota are vital for recycling organic wastes, degrading chemical pollutants, and purifying water and soil." However, sophisticated farming techniques that increase the yield per acre and engineer plants to fend off predators show promise, argue the optimists.

"But water tables are quickly dropping in the developing world, especially in the Middle East and Africa," says Engelman. "The numbers of people there have risen beyond the water's ability to renew itself. And in Zambia, for example, population size has generally exceeded the forest's ability to regenerate. So the forest has stopped, and is now moving back."

In addition, while the world population was doubling between the 1950s and 1980, its commercial energy consumption increased threefold. As a result of the higher demand, energy suppliers are mining more coal and building more dams and hydroelectric and nuclear power plants. The increased activity has led to higher levels of polluting emissions and waste.

No science so far has been able to quantify the role of population on the environment.

Levels of carbon dioxide and methane in the atmosphere are also tied to a region's population size. Some scientists propose the simple equation that twice as many people add twice as much carbon dioxide. With more than 20 million people, Mexico City has the largest population of any city in the world, and the worst air pollution. Demographers predict its population will grow to more than 30 million people, while Calcutta, Greater Bombay, Greater Cairo, Jakarta, and Seoul will reach 15–20 million each. In the United States alone, ever-increasing car driving and industrial activities now pump an estimated 23 million tons of sulfur dioxide into the atmosphere. The acid rain this chemical helps create then damages aquatic and forest life, often thousands of miles away from the source.

However, some experts argue that environmental problems are not solely a result of population size; rather, consumers in industrialized societies tend to have a much greater impact than those in less-developed communities. For example, the per capita emission of carbon dioxide in the United States is about 30 times larger than it is in India, according to Nathan

Population in the world's major regions, 1750-1995

Year	Total	Population in millions (%) Europe and USSR	North America	Latin America[a]	Africa	Asia and Oceania
1750	694	144 (20.7)	1 (0.1)	10 (1.4)	100 (14.4)	439 63.3
1900	1,571	423 (26.9)	81 (5.2)	63 (4.0)	141 (9.0)	863 (54.9)
1950	2,520	549[b] (21.8)	166 (6.6)	166 (6.6)	224 (8.9)	1,416[c] (56.2)
1975	4,077	676 (16.6)	239 (5.9)	320 (7.8)	414 (10.2)	2,427 (59.5)
1995	5,716	727 (12.7)	293 (5.1)	482 (8.4)	728 (12.7)	3,487 (61.0)

[a]Includes Mexico, Central America, and South America.
[b]1950 and later: excludes Asiatic republics of the former USSR.
[c]1950 and later: includes Asiatic republics of the former USSR.

Adapted from *Consequences*, vol. 1, no. 2, 1995.

Keyfitz of the International Institute for Applied Systems Analysis in Austria. And as wealth increases, so can consumption, manufacturing, waste, and pressure on the environment. "Scenarios of likely future emissions of greenhouse gases are, of course, dependent on the expected increase of the world population," Keyfitz said in a 1993 speech. "There is, however, no simple and straightforward relationship. The amount of energy being used by people very much depends on their economic status."

In the past 10,000 years, humans have deforested a net area the size of the continental U.S.

"How many people Earth can support depends in part on how many will wear cotton and how many polyester; on how many will eat meat and how many bean sprouts; on how many will want parks and how many will want parking lots," writes Joel E. Cohen, researcher in the Laboratory of Populations at Rockefeller University, in the July 21 issue of *Science*. "These choices will change in time and so will the number of people Earth can support," he said.

But a burgeoning population and greater wealth are not a guaranteed recipe for environmental disaster, according to Michael Mortimore, a research associate of the Overseas Development Institute in London. In an October 1994 article in *Environment* magazine, Mortimore and associate Mary Tiffen discussed a 60-year study of the resource management practices of Akamba farmers in the Machakos District in Kenya. Between 1932 and 1989, the district's population grew from 240,000 to 1,393,000. In the 1930s, analysts had predicted that rapid population growth, unreliable rainfall, frequent moisture stress, low soil fertility, and high erosion would most probably result in population-induced degradation on a large scale.

But over the decades, the farmers were introduced to a wide variety of farming techniques, including terracing, that were designed to conserve water, prevent soil erosion, and ensure an efficient system of nutrient cycling through plants, animals, and soil. They began to use manure on their crops, rather than inorganic fertilizers, as well as to feed their livestock in stalls instead of pastures, systematically cultivate their trees for firewood, and sell their produce.

"Increasing population density has had positive effects in Machakos," Mortimore and Tiffen reported. "The increasing scarcity (and value) of land promoted investment, both in conservation and in yield-enhancing improvements. The Machakos study has shown that a high-density population in an area that is steep and dry can be sustained through—and perhaps be driven by—a combination of exogenous and endogenous practices and much local initiative."

In many regions, however, higher population densities do result in degraded living conditions, including entrenched poverty and limited access to food. Poverty in the developing world "will increase until nature itself curbs the human population with mass starvation," predicted Henry Kendall, MIT professor of physics and the 1990 Nobel Prize winner in physics, in an August 1993 issue of *Los Angeles Times Magazine*. Public health experts also view high population densities as ripe conditions for the breeding and spread of epidemics, bacterial and viral. For example, plagues often thrive in the most crowded conditions, where they are able to migrate quickly. Experts on global warming attribute rising temperatures to increased populations and human activity. Plus, higher air pollution levels can cause or aggravate asthma and other respiratory illnesses, while untreated and undertreated water spawns intestinal disorders and fatal diseases. And when food production must be increased, there is a greater risk of contamination from the increased amount of fertilizers and pesticides often required.

In recent years, high population densities and limited natural resources have been blamed for the outbreaks of war in some regions. With the highest fertility rate in the world, more than eight children for every woman, Rwanda's civil war has been partially blamed on rampant population growth. Tim Wirth, undersecretary of state for global affairs, said in a 1994 speech that environmental destruction is now an additional variable that can lead to war in troubled countries. "The nation's once rich agricultural land is so severely depleted and degraded that between 1980 and 1990, during a time of unprecedented population growth, food production fell by 20 percent," Wirth said of Rwanda. He also warned that Haiti's already high population of 7 million is expected to double in the next 18 years.

When severe enough, environmental health problems begin to force a decline in population growth. For example, Russia's environment is one of the most severely damaged in the world today. Unchecked industrialization and a cynical disregard for environmental laws under communism have left a heavily polluted nation whose people struggle daily with poor air, water, and soil pollution problems. The price has been a 15% rise in infant mortality (only 40% of newborns are born healthy) and an average longevity of 57 years for males. Although there are many reasons for the population downturn, including alcoholism and the stresses of major political and economic upheaval, the main cause is ecological, said Alexei Yablokov, ecology and population adviser to President Boris Yeltsin. Russian scientists view the country's declining numbers as a national catastrophe.

No Easy Solutions

With around 1.2 billion people, China now boasts the world's largest population; India is second with nearly 1 billion. The United States, with more than 262 million inhabitants, is the fastest-growing industrialized nation. As an indicator of future growth, researchers often study a nation's total fertility rate (TFR), the average number of children a woman will bear in a lifetime. Italy has the lowest TFR, with 1.3 children, while West Africa's Mali has one of the highest, with 7.3. Family planning programs have brought India's TFR down to 3.4. Women in the United States today bear an average of two children each, a TFR of 2.0.

Twelve percent of the world's population resides in Africa, where the annual growth rate is 3%, according to H.W.O. Okoth-Ogendo, professor at the Centre for African Family Studies in Kenya. In a 1993 speech, Okoth-Ogendo noted that fertility management practices in Nigeria led to a drop in the TFR from an average of 8.1 in the period from 1969– 1979 to 6.7 in 1985 and 5.4 in 1993.

Decisions concerning family size are a very private matter and cannot be ruled without treading upon human rights.

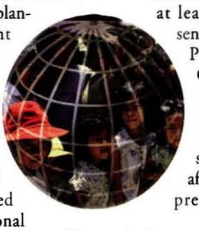
Urbanized and industrialized nations tend toward smaller family sizes, while agrarian communities still prefer large fam-

ilies, in part because they depend on the labor. Social traditions can encourage high fertility rates in countries dominated by subsistence economies. Rural parents often view children as "productive assets" and as a source of security in their old age, wrote University of Cambridge economics professor Partha S. Dasgupta in the February 1995 issue of *Scientific American*. Parents need their children to help provide the family's current and future income. But "the need for many hands can lead to a destructive situation," such as "greater crowding and susceptibility to disease as well as to more pressure on environmental resources," he adds. The key is to provide conditions that encourage couples to limit how many children they produce. By improving social and economic conditions, such as "providing cheap fuel and potable water," the usefulness of extra hands will drop. "When a child becomes perceived as expensive, we may finally have a hope of dislodging the rapacious hold of high fertility rates," said Dasgupta.

The process of finding solutions to the population problem is often politically and emotionally charged. Over the years, different countries have tried various methods to hold down their population growth. Some are controversial, including forced sterilization practices in some regions of India, and the one child per couple policy in China. In 1994, global delegates met in Cairo for the International Conference on Population and the Environment, where they considered alternative ways to lower national fertility rates. They discussed voluntary methods such as promoting modern contraceptives, encouraging economic development, improving infant and child mortality, raising the status of women through education and employment opportunities, and changing the attitudes of men toward women.

"Decisions concerning family size are a very private matter and cannot be ruled by laws or general regulations without treading upon the human rights ratified again and again in UN declarations," said Keyfitz. "Wherever people have full reproductive choice without coercion, they have chosen to have smaller families if opportunities to regulate fertility are available."

"It's better to stabilize the population, and not count on technology," says Engelman. "But people don't respond to coercion, so we have to give up the idea of population control. Instead, there is a pent-up demand for family planning and access to the right contraceptives. So if you combine relatively feasible things, such as access to education, with better access to medical care, you can dramatically change attitudes."



Like other government and private agencies, the United States Agency for International Development (USAID) has been working to fulfill the promises made at the 1993 population conference in Cairo. One strategy involves encouraging environmental organizations abroad "to think more about the connection between environment and population," says Richard Cincotta, a fellow for the Western Consortium for Public Health at USAID. "It's important to look at how environmental programs can be combined with family planning programs. For us, population/environment is a tool to raise awareness of the need for family planning.

"The problem is that it's a long-term effort," says Cincotta. "You have to collect enormous amounts of data and understand how the various population/environment models work in order to create effective policies. But in the meantime, policy makers tend to respond to presentations that are simple and even sensational, although in reality the question is often much more complex than you can ever convey in a policy presentation."

Nevertheless, scientists continue to research, develop models, and try to quantify population effects on the environment. For example, the East-West Center in Hawaii began a project this year to help develop large-scale population and environment models, using a model developed by the International Institute for Applied Systems Analysis which illustrates the relationships among population growth, economic growth, and environmental change. The East-West Center hopes to apply such modeling principles to the Cebu region in the Philippines.

"Such models have been around since at least 1975," says Eric Jensen, senior fellow in the Program on Population at the East-West Center. "They always consist of a set of equations that describe a system of production and the way in which the environment serves as input and in turn is affected by output. But they can predict the effects only to the extent that the builders put them into the models. While some models may build in an extrapolation of current trends and predict catastrophe, others build in more realistic ameliorating impacts of society and economy and make less drastic predictions."

Recent changes are a result of there being more of us, and of a much more complex world.

In recent years, nongovernmental environmental organizations have also created or stepped up their population programs, including the Sierra Club, National Wildlife Federation, and the Natural Resources Defense Council. "We're just starting to understand many things that weren't looked at before, such as equity, how resources are distributed, and how that affects the way humans interact with their environment," says Karen Kalla, director of the International Population Program at the Sierra Club. "There is a recognition now that the issue is not as simple as just looking at numbers. A lot of the recent changes are a result of there just being more of us, and of a much more complex world."

Rebecca Clay

LIVESTOCK LEGACY

Behind those pristine packages of pork chops at the market is a dirty little secret. Actually, it's not so little: the 60 million hogs in the United States produce an estimated 100 million tons of feces and urine each year, according to statistics from the U.S. Department of Agriculture and the American Society of Agricultural Engineers. And it's not such a secret anymore: last summer a series of hog waste spills fouled streams and rivers in Iowa and North Carolina, the two top hog-producing states in the country.

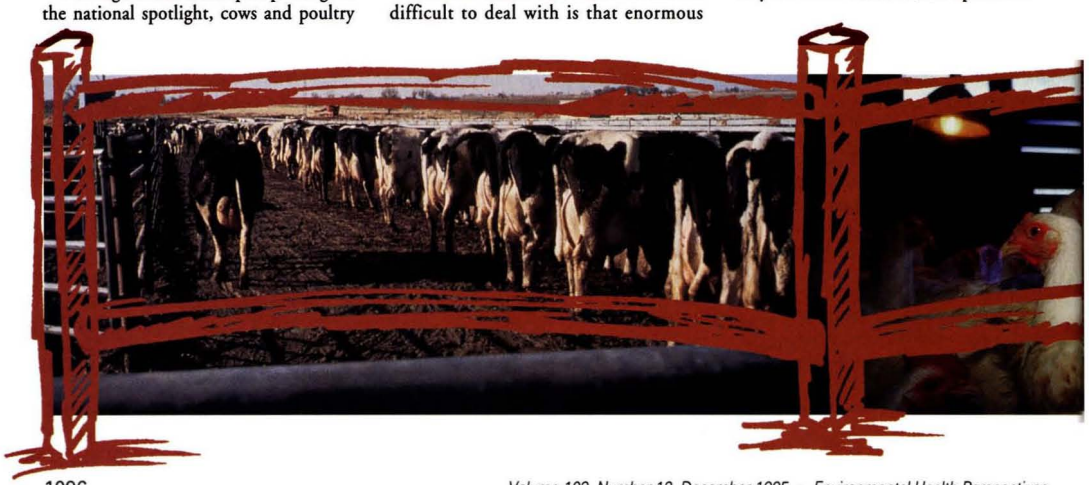
Livestock waste spills can introduce enteric pathogens and excess nutrients into surface waters. The waste can also contaminate groundwater with nitrates and contaminate air with ammonia and odors so offensive that they make people angry and depressed. While livestock waste regulations vary from state to state, most are based largely on voluntary compliance. Opinions on the best way to keep livestock waste out of the air and water vary widely. At issue are the level and degree of both regulation and waste management technology.

Although recent waste spills put hogs in the national spotlight, cows and poultry also produce their share of waste. The United States's 46.5 million milk and beef cows produce 500 million tons of waste per year, and the 7.5 billion chickens and turkeys produce 300 million tons of waste per year. These figures are conservative: altogether, livestock produce a staggering billion tons of waste annually, according to a paper by B.L. Harris, an extension specialist at the Crop and Soil Science Department at Texas A&M University, and his colleagues in the proceedings of the 1994 Great Plains Animal Waste Conference on Confined Animal Production and Water Quality.

Managing that much waste would not be easy under the best of circumstances. But the problem is exacerbated by the fact that there is little demand for the waste. Before the advent of chemical fertilizers, farmers typically raised both livestock and crops and used the animal waste as fertilizer. Today most farmers specialize in either crops or livestock, which means that the waste would have to be hauled to the crop farms. And using chemical fertilizers is cheaper.

Another reason that livestock waste is difficult to deal with is that enormous amounts are produced in relatively small areas. Most livestock operations pack huge numbers of animals into confinement buildings or feedlots, a practice that makes sense economically because in general the more animals raised in one place, the cheaper the cost per animal. In North Carolina, for instance, 10,000-head hog operations are not uncommon. (Hog confinement operations usually allot an average of about eight square feet per animal.) Beef cattle feedlots can hold up to 50,000–100,000 head in Texas, the state that produces the most feedlot cattle. And operations that produce broiler chickens raise as many as 400,000 at a time in Arkansas, the top broiler-producing state.

To make matters worse, livestock operations are often clustered near processing plants because the closer the livestock operations are to a plant, the cheaper the costs of transporting the animals there. Most of North Carolina's 7.6 million hogs are raised near a Smithfield Foods-owned hog-processing plant in the eastern part of the state. About 80% of the hogs are raised on only 10% of the state's 7,000 operations.



Lagoon Spills

The North Carolina hog industry has tripled in size since 1990, making it the fastest-growing as well as the largest in the country. This growth has come at a cost, however. Most waste from hogs and cows raised in confinement is collected in lagoons, which are large, shallow pits dug into the ground. The waste solids sink to the bottom of the lagoon and are broken down by anaerobic bacteria over a period of months. In theory, operators keep the lagoons from overflowing by spraying the liquid that rises to the surface on nearby fields.

In practice, however, these lagoons do not necessarily contain the waste. The most dramatic evidence for this came on June 21 of this year, when North Carolina suffered the largest agricultural waste spill in its history: a 7.5-acre, 12-foot-deep lagoon leaked 25 million gallons of hog waste into the headwaters of the New River near Richlands. The waste from the 10,000-head operation, owned by Oceanview Farms, contaminated the water for several miles downstream, increasing the levels of nitrogen, phosphorus, and other nutrients. When nutrient levels dramtically increase in rivers and other bodies of water, algae grow furiously, consuming most of the dissolved oxygen and asphyxiating the other aquatic organisms living there. An estimated 5,000 fish died as a result of the Oceanview Farms spill. Nine subsequent waste lagoon spills—six in North Carolina and three in Iowa—showed that this was not an isolated occurrence.

Waste from livestock operations is particularly copious and nutrient-rich because animals raised in confinement are fed plentiful amounts of high-quality food. "We want animals as fat as we can get them; we want cows to give as much milk as possible. If you put a lot in the front, you get a lot out the back," says David Holsinger, state non-point source coordinator at the North Carolina Division of Environmental Management (DEM).

Downstream of the spill, the New River also had high fecal coliform bacteria counts. Fecal pathogens that can be transmitted from livestock to people include enteric bateria such as *Salmonella* and *Shigella* and protozoa such as *Cryptosporidium* and *Giardia*. People could potentially be exposed to these pathogens by fishing or swimming in contaminated waters or by eating shellfish, which are filter-feeders and can concentrate pathogens.

EPA

Susan Schiffman—The amount of anger people have over odor pollution is tremendous.

Livestock waste has been implicated in outbreaks of human disease, notably the spring 1993 cryptosporidium infection that afflicted 4,000 people in Milwaukee through the public water supply. But the link is difficult to trace conclusively. "The human health effects [of livestock waste] are unknown," says James Oliver of the biology department at the University of North Carolina at Charlotte. "There are lots of enteric pathogens in fecal coliform [bacteria], and you would expect there to be an increased health risk, but there are no studies showing that. The spill in the New River provides an interesting opportunity to study any health effects," he says, adding that he and his colleagues are planning such a study.

When investigating the Oceanview Farms hog waste spill into the New River, North Carolina DEM officials found that the lagoon had not been operated properly. An irrigation pipe had been bored through the lagoon's earthen wall, which weakened it. The truck-sized hole through which the waste spilled was near the pipe. In addition, the lagoon had been overfilled: the wastewater nearly reached the top of the lagoon rather than stopping 20 inches short of the top as stipulated in the operator's waste management plan. Moreover, the operator had failed to clear enough acreage for spraying the wastewater from the lagoon: only 44 of the 102 acres stipulated in the waste management plan had been cleared. Ironically, this was the first lagoon to receive a permit under stricter state standards for animal waste adopted in 1993. The DEM fined Oceanview Farms $110,000, but the company will appeal, said Bill Johnson, vice president of Coastal Ag-Development, Inc., the registered agent for the company, in a 23 August 1995 article by the Associated Press. "We have submitted a formal written report [to the state] that outlines how we complied with the waste management plan for Oceanview Farms," he said.

In response to the series of livestock waste spills, North Carolina Governor James Hunt ordered the DEM to investigate the lagoons on the state's largest hog operations. The investigators found 109 operations that were discharging hog waste directly into streams and rivers, 124 lagoons that were so full that they were likely to overflow or burst, and 526 that were nearing that critical point of fullness. Although record June rains were cited as a reason for the lagoon problems, lagoons are supposed to be able to handle heavy rainfall, says DEM spokesman Don Reuter.

The waste spills in North Carolina are a warning to all livestock-producing states. "Problems become public here first primarily because the animal confinement industry has taken off due to the laxity of

Reagan M. Waskom

Paying for spraying. Overspraying fields with liquid animal waste can lead to excessive odor and to runoff in nearby streams.

the political climate [in North Carolina], but the problems are basically the same nationwide," says the DEM's Holsinger.

Chronic Seepage and Runoff

Although spills focus attention on the hazards of livestock waste, the greatest threats are chronic seepage from lagoons and runoff from the fields where the lagoon liquids are sprayed, according to Kenneth Pollig, an environmental engineer in the groundwater section of the North Carolina DEM. Some waste lagoons are lined with compacted clay or plastic, but most are not. "The main route of contamination is through the soil. Waste migrates into nearby streams and aquifers," says Pollig. Livestock waste contamination can increase the level of nitrates in groundwater, which can cause methemoglobinemia or "blue baby syndrome." In this rare but potentially fatal disease, intestinal bacteria metabolize the nitrates to nitrites, which oxidize the iron in hemoglobin, rendering it incapable of binding oxygen. Babies less than six months old are particularly susceptible to this syndrome, in part because their digestive tracts are less acidic than those of adults, which favors the growth of the nitrate-converting bacteria.

Groundwater contamination is of particular concern in the coastal plains of eastern North and South Carolina, where the water table is only 15–20 feet below the surface of the soil. "Twenty-five percent of the lagoons in the Carolinas are in the coastal plains, and the bottom of the lagoon can dip below the water table," says Pollig, citing a 1994 doctoral thesis by Maolin Zheng of Clemson University's Department of Agricultural Engineering. Zheng studied 36 hog and poultry waste lagoons and found that 65% of them leaked into the groundwater. "Some hog farmer can basically dig [a waste lagoon] wherever he wants and build it however he wants," says Pollig. "But that's changing now."

Groundwater contamination is also a problem is in Weld County, Colorado, where 500,000 beef cattle are raised in feedlots. "The fields are loaded with manure. It's too expensive to haul away," says Reagan Waskom, an extension water quality specialist at Colorado State University. The towns around the feedlots have high nitrate levels in their groundwater—about 20 ppm, which is double the EPA standard, says Waskom.

Another source of water contamination is runoff from the fields where livestock waste is applied. Although some poultry waste is collected in lagoons, most poultry operators use a "dry system" that involves putting, for example, sawdust on the floor to catch the waste and then plowing the sawdust into fields. Waste from lagoons is supposed to be sprayed on fields at agronomic rates; in other words, the plants growing in the fields are supposed to be able to take up all the nutrients in the waste. But many operators spray too much liquid on too little land, says William Holman, a lobbyist for the North Carolina chapter of the Sierra Club, resulting in runoff.

Airborne Health Effects

"Too much spray also leads to drift [through the air], which carries odor," says Holsinger, who was on North Carolina's Swine Odor Task Force. While odor transmission and control are poorly understood, one thing is clear: living downwind of hog operations adversely affects people's moods, according to a 1995 study published in the *Brain Research Bulletin* by Susan Schiffman and her colleagues in the Department of Psychiatry at the Duke University Medical Center.

The study compared the moods of 44 people living near hog operations with those of a control group (matched for age, gender, race, and years of education). The subjects rated their moods by filling out Profile of Mood States questionnaires (POMS), which reveal transient mood shifts. Subjects living near hog operations filled out POMS on days when they could smell hog odors, and the results showed that they are more tense, angry, and depressed, as well as more tired and confused than average.

"The amount of anger people have over odor pollution is tremendous. The smell gets into bedding, carpets, and drapes. People can't sell their houses because no one wants to live near a hog farm," says Schiffman, who was also on the state Swine Odor Task Force. "Dairies and poultry farms also smell. Farmers are following all the laws and don't know what to do. There is no legislation for odor standards—there has not been enough research to set odor standards or to know how to intervene and fix the problem."

Besides being affected by living near livestock operations, people may be affected by working in them. Workers in livestock confinement buildings breathe in dust from waste and feed, which may cause or exacerbate respiratory diseases such as asthma, bronchitis, and even chronic obstructive pulmonary disease. However, there is only anecdotal evidence that breathing in livestock waste dust causes chronic pulmonary disease, says John Pickrell of the environmental toxicology department at Kansas State University in Manhattan.

Other little-understood but potentially adverse effects of airborne livestock waste include global warming and atmospheric nitrogen deposition. Waste lagoons may contribute to global warming because they produce the greenhouse gases carbon dioxide and methane. Lagoons may also contribute to atmospheric nitrogen deposition because they produce ammonia, which evaporates both directly from the lagoons and from the wastewater sprayed on fields. "When you drive by a lagoon you can smell the ammonia," says James Pinckney, visiting assistant research professor at the Institute of Marine Sciences (IMS) of the

University of North Carolina at Chapel Hill. Atmospheric nitrogen rains down into streams, rivers, lakes, and coastal waters. The increased nitrogen then contributes to the algae blooms that deprive fish and other aquatic organisms of oxygen.

Although there is no direct evidence that livestock waste lagoons are contributing to acid rain, they are likely to be a major source, says Pinckney, who works with IMS researcher Hans Paerl. Their studies have shown that the average amount of ammonia in eastern North Carolina's rainfall increased by about 25% from 1990 to 1995, which coincides with the increase in hog farming. Paerl plans to determine how much of this ammonia comes from waste lagoons by measuring the compound's nitrogen isotope ratio, which is characteristic of the source.

Waste Regulation

The EPA requires no-discharge systems for confined livestock operations and considers waste lagoons to be non-discharging. The basic requirement is that lagoons must be able to accommodate all runoff except in the event of rainfall greater than a 24-hour, 25-year storm—that is, the amount of rain that falls in 24 hours during the biggest storm in a 25-year period.

Seepage from lagoons and spray and runoff from waste-treated fields are, like other non-point sources of pollution, poorly regulated. "Non-point pollution is a kind of big, amorphous beast that no one can really characterize," says Holsinger. While the EPA estimates that livestock production contributes between one-third and one-half of the non-point surface water pollution in the United States, this estimate is controversial.

The EPA has the authority to regulate livestock waste under the Clean Water Act, but instead largely leaves this to the individual states. But state standards are not all that strict. Texas has approached the task of controlling water contamination by livestock waste "more seriously than most states," wrote John Sweeten, associate department head and extension program leader for agricultural engineering at Texas A&M University, in the proceedings of the 1994 Great Plains Animal Waste Conference on Confined Animal Production and Water Quality. But even so, he says, only 50–70% of dairy farms are in compliance with no-discharge requirements in Erath County, Texas, where about 70,000 dairy cows are kept within a 50-mile radius.

North Carolina may represent the other end of the regulatory spectrum. "North Carolina standards and enforcement are weak. There was virtually no enforcement until this summer. It was complaint-driven," says the Sierra Club's Holman. When corporate hog farming began booming in North Carolina, "we predicted how bad the odor would be and that the lagoons would leak, but no one in the government would listen to us. And now it's happening," says Don Webb, a former hog farmer who is now the president of the Alliance for a Responsible Swine Industry. "The government turned its back on us for rich, powerful people," Webb says. Schiffman of the Duke University Medical Center concurs, saying, "State regulators looked the other direction."

North Carolina regulators are paying more attention to livestock waste now. Under bills enacted this summer, livestock operators must be trained to apply waste on land and must pass a certification test, and the state must inspect all new waste lagoons. In addition, new hog operations must be located at least 1,500 feet from houses and 2,500 feet from schools, hospitals, and churches. These distances, according to Holsinger, were the suggested standards of the National Pork Producer's Association.

Solutions to Management Problems

Despite the new regulations in North Carolina, Holsinger and others think that the regulations do not go far enough. Commenting on the new location requirements for hog operations, Holsinger says, "Fifteen hundred feet is about a quarter of a mile, which is nothing compared to a six-acre lagoon." Furthermore, operators of old lagoons do not have to comply with most of these new regulations. Holsinger calls for inspecting and, if necessary, retrofitting older waste lagoons with liners. The North Carolina DEM is now studying leakage in unlined lagoons. Holsinger and others also call for keeping lagoons away from surface waters. "Some old lagoons are right next to streams. It would be best if they were relocated," says Roger Thorpe, water quality supervisor at the North Carolina Department of Environmental Health and Natural Resources.

Some North Carolina legislators also want tougher regulations. State representative Howard Hunter wants counties and towns to be able to pass zoning laws to keep hog operators from building on unsuitable sites. "Rural areas are exempt from zoning," says Holsinger. "A little agricultural community can't do anything about a mega-hog farm moving in and bringing with it odor and groundwater problems." U.S. Congressman Charlie Rose wants the EPA to regulate livestock waste more stringently. But that would

Texas Agricultural Extension Service

Sniffing out answers. John Sweeten (left) and a technician use scentometers to test odor strength at a cattle operation.

merely provide the state officials with an excuse to avoid dealing with livestock waste because they would be able to blame the EPA for any problems, countered the *Fayetteville Observer-Times* in a 30 August 1995 editorial.

Others call for tightening existing state regulations, which are not necessarily consistent with each other. "There's no coordination of regulatory agencies," says Deanne Morse, livestock waste management specialist at the University of California, Davis, citing California regulations that are designed to control mosquitoes but that have unforeseen adverse effects on lagoon integrity. Operators are told to lower lagoon levels to kill the floating weeds where mosquitoes like to lay their eggs, but when the water level in a clay-lined lagoon goes up and down, the cycle of wetness and dryness makes the lagoon more likely to crack, she says.

Besides disagreeing about the level and extent of regulatory reform, people disagree about the level of technology that is necessary to treat livestock waste safely. Some say that when used properly, current waste treatment methods are adequate. "There are data on both sides of the fence, but my personal experience is that it's a question of [the operator's] management," says Morse. "[Waste lagoons] are not an exciting place to go. It's the last thing [operators] want to do, but it's an effort they need to make."

Reagan M. Waskom

Blue lagoon? Catastrophe can result when waste lagoons are allowed to overfill or when leaks occur due to improper lining.

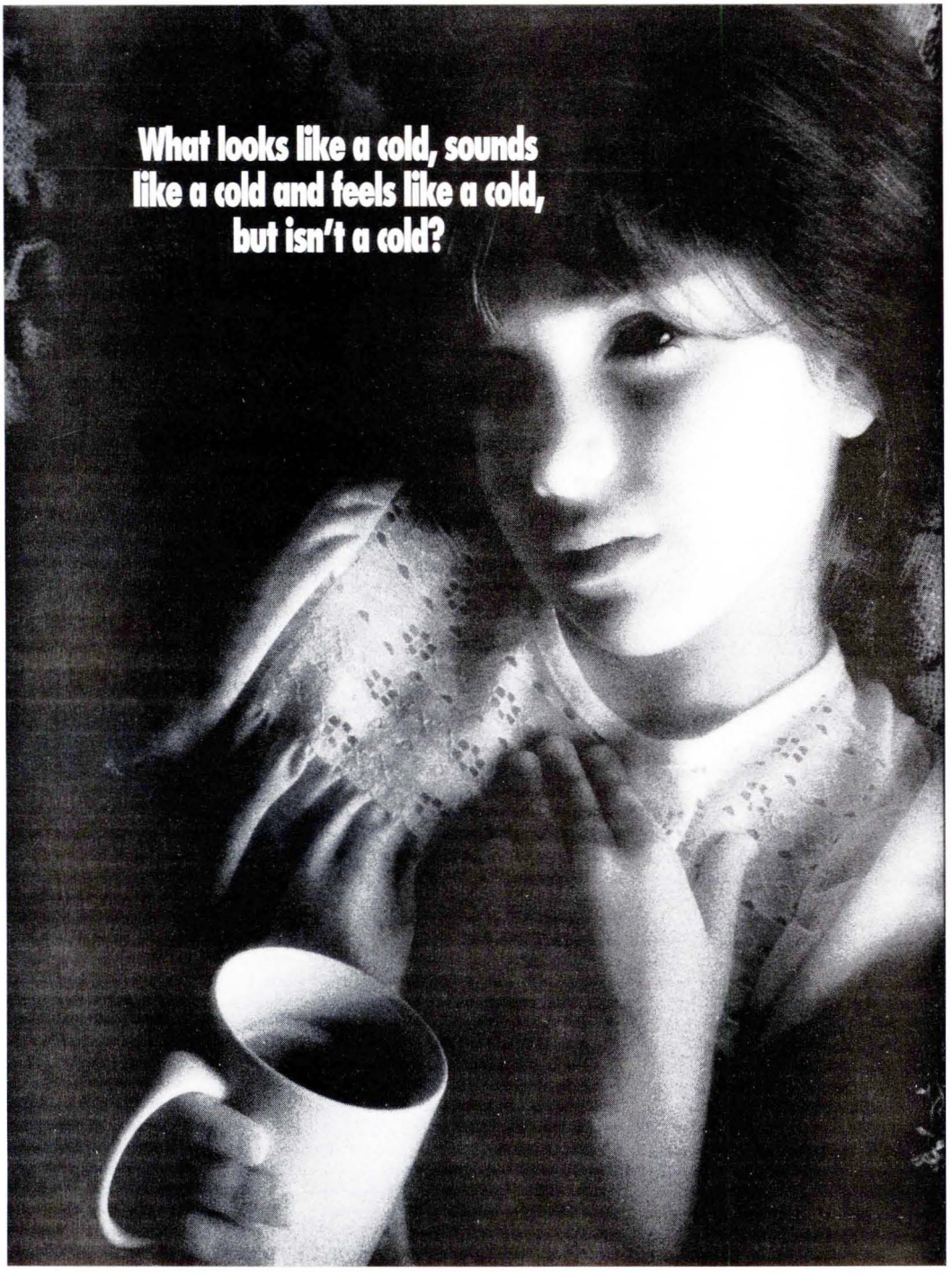
NCPFA

Walter Cherry—We need to find a balance between producing livestock and protecting the environment.

Others say that livestock operators need to adopt new waste treatment technologies. The Sierra Club's Holman would like livestock operations to use more advanced treatments such as those used for human waste but, he says, the animal industry says they are too expensive. Walter Cherry, president of the North Carolina Pork Producers Association, agrees that livestock operators need to protect water supplies and control odor but emphasizes that "we need systems that adequately address all factors and are still economically feasible. We need to find a balance between producing livestock and protecting the environment." The North Carolina Pork Producers Association is funding a study by the Duke University Medical Center's Schiffman to identify the odor-causing compounds and determine ways of managing lagoons to decrease the odor.

While there is no consensus on how to deal with livestock waste, almost everyone agrees that there is a need to educate livestock operators better and fund more research to determine the best ways to manage livestock waste. As devastating as the hog lagoon spills last summer were, they may ultimately benefit the health of the environment and public health by forcing people on all sides of the issue to face the considerable problems that livestock waste can cause.

Robin Meadows



Asthma. But asthma can be much more serious. So if your child has a cough that won't go away, is often short of breath, or wheezes a lot, especially at night or after running, don't treat it yourself. Go to your doctor or clinic.

Breathe easier. Ask your doctor if it's asthma.

National Asthma Education and Prevention Program

National Heart, Lung, and Blood Institute; National Institutes of Health; Public Health Service; U.S. Department of Health and Human Services.

CONTRABAND in the Stratosphere

With the 1 January 1996 deadline for ceasing production of chlorofluorocarbons (CFCs) in the industrialized world rapidly approaching, those who would make money nefariously have found a new way to breach the law. They are smuggling what some estimate to be huge quantities of these compounds, used primarily as refrigerants in air conditioners and refrigerators, into the United States.

Production of CFCs is being phased out because they damage the earth's stratospheric ozone layer, which prevents much of the sun's ultraviolet light from reaching the earth. Degradation of this layer can cause an increase in skin cancer and a reduction of crop yields. Some scientists believe a severe enough decrease could lead to the elimination of many species.

The Largest Hole

The phaseout of CFCs, and its consequences, had its genesis in the 1970s when scientific evidence began to mount that these compounds were damaging the earth's ozone layer. As CFCs escape from junked or faulty compressors and hoses, or when used as solvents, they rise to the stratosphere. There they are transformed by ultraviolet radiation into chlorine atoms, which play a role in catalytic ozone depletion.

Scientists estimate that since the late 1950s, the ozone layer over Europe and North America has decreased by 10%. And just last September the United Nations World Meteorological Organization announced that the largest hole ever measured in the earth's ozone layer—a 3.86 million square mile gap—existed over Antarctica. Figures also showed that this year, the typical springtime decline in ozone levels over the Southern Hemisphere was 10% greater than the previous year.

In an attempt to halt such depletion, an international accord known as the Montreal Protocol was adopted in 1987. Calling for a 50% phaseout of CFCs by 1998, the protocol has been ratified by more than 140 countries. As part of the original protocol, an agreement was made to continue to meet every few years to review the rapidly evolving science relative to ozone depletion.

When a second meeting was held in London during 1990, clouds were darkening on the ozone horizon. In response, the phaseout was accelerated to 100% by the year 2000. As negative ozone figures kept coming in, that figure too was amended in Copenhagen in 1992, when developed countries agreed to eliminate the production of CFCs as of 1 January 1996, except for a few essential uses. Developing countries received a 10-year extension. The "essential" exceptions allow for the production of CFCs for use as propellants in aerosol sprays for use by asthmatics, and for the use of small quantities in laboratories.

Taxed into Oblivion?

To accelerate the switch to new equipment that can use alternative refrigerants, the United States levied a hefty, annually graduated excise tax on all new and imported CFCs, beginning in 1990. The tax does not apply to CFCs that are recovered and recycled in the United States, since it is presumed they have already been taxed.

During its first year of implementation, the tax was set at $1.37 per pound of CFC. The tax has risen rapidly to $5.35 per pound this year and will continue to rise an additional $0.45 per year. And while the jury is out as to the effectiveness of the tax in speeding the transition, there is little doubt that it has created a large profit margin for those selling contraband product.

With a 30-pound tank of CFC on the legitimate market currently selling for $250, the tax accounts for over half of the price. Smugglers selling CFCs at or near the market price can pocket what would otherwise go into the government's coffers, in addition to the normal profit. Because equipment built to operate with CFCs cannot use the new refrigerants being introduced, a substantial market for CFCs will remain for some time to come. And, as the supply of CFCs decreases and the tax increases, the situation will look all the more attractive to those interested in skirting the law.

Operation Cool Breeze

Three agencies have authority over CFC distribution: the Customs Service, the EPA, and the Internal Revenue Service. Each has introduced measures to counter the increasingly lucrative black market. A Customs Service task force by the name of "Operation Cool Breeze" has resulted in the prosecution of four criminal CFC smuggling cases in the United States, each of which was brought to court this year in Miami, Florida.

Miami has become the focus of this operation because "it is a convenient location for this sort of trade," says Thomas Watts-FitzGerald, chief of the Environmental Enforcement Section of the U.S. Attorney's Office in Miami. "We are a major port and a major trading hub for the Central, Latin, and South American markets."

Yet not all of the contraband involved in the cases prosecuted in Miami have come in through that port. Some of the CFCs flowed into the United States through New York and Newark, New Jersey, as well. These cases ended up in southern Florida's venue because the CFCs had subsequently been shipped there under pretense of being routed to another country, when it fact they were diverted into the U.S. market.

The largest case prosecuted to date was that of Irma Henneberg of Fort Lauderdale, Florida, who was found guilty in August of 34 charges relating to falsely manifested cargo. The intent was to conceal the smuggling of almost 4,000 tons of CFCs, with a retail value of $52 million, and an associated tax loss of some $32 million. But Keith Prager, assistant special agent in charge with the Customs Service in Miami, says, "We know there are people bigger than her out there." Each count car-

ries a maximum penalty of five years in prison and a fine of $250,000.

The Henneberg case is typical of the kinds of subterfuge being used by smugglers of CFCs. Because developing countries are still permitted to produce and use CFCs, it is not illegal to ship them through the United States on a tax-free, bonded basis. This status provides that the goods are, for legal purposes, not considered to be in the United States, even though they may be on a pier or in a warehouse on U.S. soil, provided they are passing through from one country to another. "Henneberg was convicted of filing 34 false documents with customs to make it appear as though all of the CFC had been reshipped out of the U.S., when in fact it had not been," explained Watts-FitzGerald.

To date, CFC smuggling has been a white-collar crime. "The key to black market smuggling is to get it introduced into legitimate commerce. The people we have arrested so far have all been associated with the legitimate CFC market," said Prager.

Opening a Loophole

In addition to the excise tax, another governmental regulation on CFCs was implemented in 1990, which some experts feel eventually gave further impetus to smugglers. This regulation involved controls on the amounts of CFCs produced and imported into the United States. Its purpose was to ensure compliance with international control agreements.

The controls used 1986 as a base year and required that CFC production and importation, which had been on a growth curve between 1986 and 1990, be cut back to 1986 levels. To meet this requirement, the EPA gave producers and importers quotas based on their 1986 levels of activity. The total U.S. 1995 production quota is 150 million pounds, 70 million of which is allotted to DuPont, the leading producer of CFCs in 1986. Quota compliance is carefully tracked by the EPA, which requires quarterly activity reports from involved companies.

A move in 1994 to lift the import quota on used CFCs further opened the floodgates to contraband. Until that time, the import quotas applied to both virgin and recycled CFCs equally. Then, as of 1 January 1994, the import quota on used CFCs was lifted. As with the original limitations, this too was done so that the United States would be in compliance with international agreements. As production controls grew ever more stringent, the international community reduced them on used product, both to ease the pain of transition and to prevent the release of used CFCs into the atmosphere by promoting recycling. As a practical matter, with the EPA no longer watching, smugglers were emboldened to mismark virgin CFCs as recycled material.

"A lot of people have imported virgin refrigerant and called it used, or contaminated it slightly to appear as though it has been used . . . to get around the allowances," said Dave Stirpe, executive director of the Alliance for Responsible Atmospheric Policy (ARAP), an industry group in Arlington, Virginia, composed of producers and users of CFCs and their alternatives.

To plug this loophole, the EPA has recently reentered the picture, this time not with quotas, but with a petition system, requiring importers to gain permission to bring used refrigerant into the United States. "You have to document where it came from and where you are going to recycle it," said Stirpe.

This loophole is not the only threat to the efficacy of the new regulations on CFCs, however. Members of the House Energy and Environment Subcommittee are questioning the impending ban and some have introduced legislation to thwart it. "I am convinced . . . there has not been a sufficient showing of scientific evidence to justify the current and rapidly approaching ban date," said Representative John Doolittle (R-California) in an article in *The Environmental Health Letter.* Doolittle has introduced a bill (HR 2367) to postpone the ban. Representative Tom DeLay (R-Texas) has introduced legislation (HR 475) that would repeal the ban altogether.

An Inundated Market

Those involved in law enforcement are reluctant to speculate as to the sources of the contraband CFCs. The chemicals involved in the cases prosecuted thus far came from England and India, according to Watts-FitzGerald. It is suspected that some people in Russia and China are growing rich from such illegal trade.

Projections as to the quantity of CFCs being smuggled into the United States are also hard to come by. One joint industry–government estimate placed the quantity at 40 million pounds per year, or about 25% of the current production and import allotment under EPA regulations. An industry representative at an EPA-sponsored seminar estimated that 90% of the CFC market in southern Florida was being satisfied with smuggled product, according to Watts-Fitzgerald. Whatever the specific figures, smuggling is having a substantial impact on the marketplace.

Sharon Gidumal, a senior environmental specialist at DuPont, said her company started noticing something amiss over a year ago, when CFC prices began to drop to an unrealistically low level. "We believed, knowing our costs and those of our competitors, that they could not have made the material for what it was being sold at," she said. "There was a signal being sent to the marketplace that there really was no shortage of CFCs, there's no reason to get out of CFCs. They are plentiful. We felt it was the wrong thing to do for the environment and that businesses that had legitimately invested in CFC alternatives were being harmed by this illegal activity."

The availability of contraband "put the market into a price tailspin," Gidumal added. "It doesn't take much in a commodity business to cause a price war, and that is what this has done . . . the bottom dropped out of the market." As a result, DuPont decided not to produce its full 1995 EPA allowance of CFCs.

The black market has also been causing headaches for those who service air conditioners and their customers because a significant portion of the contraband product is contaminated. The Air Conditioning & Refrigeration Institute in Arlington, Virginia, specifies that recycled CFCs should have no more than 0.5% contaminants, but, "we have found contraband that has come in from off-shore with five, six, and seven percent contaminants," said Simon Oulouhojian, president of the Mobile Air Conditioning Society-Worldwide, an Upper Darby, Pennsylvania-based industry group. Contaminated CFCs can leak through hoses and damage systems, resulting in air conditioning having to be redone, according to Oulouhojian.

Replacements

CFCs are a melange of chemical compounds. Their designations were developed by DuPont in the 1930s as an esoteric code intended to keep competitors from knowing the products' chemical makeup. That code has long since been revealed and is used by the entire industry.

The most widely used compound is CFC-12, also known as R-12, which carries the DuPont trade name Freon. Its primary use is as a refrigerant in residential refrigerators and mobile air conditioners. The automotive industry used about half the worldwide production of CFC-12, or some 125 million pounds per year.

Other CFCs, such as 11, 113, 114, and 115, are used in the production of foam rubber and rigid insulating foam for appliances and construction, and as solvents, especially in the electronics industry. All of these CFCs are being replaced with an

equally confusing number of hydrofluorocarbons or hydrochlorofluorocarbons, known as HFCs and HCFCs, respectively. In the past two years, for example, the automotive industry has made a complete switch to HFC-134a, the replacement of choice for new cars. HCFC-22, which has always been used in residential central air conditioning units, is now also being used in large commercial chillers and in commercial refrigeration.

Yet these replacements for CFCs are no panacea. It is known, for example, that HCFCs can also deplete the earth's ozone layer, albeit at a considerably slower rate than do CFCs. To address this problem, the 1992 meeting in Copenhagen also adopted a complex phaseout schedule for HCFCs. The agreement calls for the reduction of HCFCs in five stages beginning in 2004, with a total phaseout by 2030. And, although HFCs have not been associated with ozone depletion, both they and HCFCs have recently been cited as possible acid rain culprits.

In a paper published in the July 27 issue of *Nature*, T.K. Tromp and colleagues at Atmospheric and Environmental Research, Inc. in Cambridge, Massachusetts, note that atmospheric degradation of several HFCs and HCFCs "is expected to produce trifluoroacetate (TFA), which is removed from the atmosphere mainly by rain." And while the authors find that the concentrations of TFA for the year 2010 will be "well below the concentrations thought to inhibit plant growth," they say that "TFA could attain appreciable concentrations ($>10^2$ μg) in the local surface waters of seasonal wetlands within a few decades."

The paper goes on to state, "The ecological importance of many of these ubiquitous wetlands lies not only in their acting as habitat for many rare and endangered plant and animal species, but also in their use by migratory and wintering waterfowl for foraging and resting during winter and early spring."

A number of alternative refrigeration technologies that can use inert gas as the heat transfer medium are at various stages of development, but when they will be available and economically practical is up in the air.

Work is being done on several fronts to perfect Stirling cycle refrigeration units which can make use of helium. Although the technology is almost ready for commercialization, it is most efficient in small refrigerators and for low-temperature applications. Additionally, the Stirling cycle cannot be used as a drop-in replacement for compressors in existing refrigerators because other refrigeration hardware must be changed as well. For these reasons the U.S. appliance industry has given Stirling a cold shoulder, although it seems to be receiving a warmer reception in Europe.

Another promising refrigeration technology involves a thermoacoustic refrigeration system, which uses sound (see **Innovations**, *EHP*, vol. 102, no. 9). It was invented at Los Alamos National Laboratory in New Mexico with the initial intent of providing cold storage for biological samples aboard space shuttles. Steve Garrett, a primary developer of thermoacoustic refrigeration at the Naval Postgraduate School in Monterey, California, who is now a professor of acoustics at Pennsylvania State, says he has put much time into the thermoacoustic system because he believes it is an environmental and humanitarian necessity. "Refrigeration is not a glamorous field," he says, "but if you look at it realistically, refrigeration has done more to increase the life span of humans than pharmacology. More people used to die of food poisoning than used to die of the other diseases we can now cure." As a result, he continues, "When developing countries start to make money, the first thing they are going to want is refrigeration. This will create the double pressure of an unmet need and restrictions on chemicals. This leads me to conclude that unless you have an inert gas-based refrigeration system, or a solid-state system, you are at risk with regard to satisfying refrigeration requirements."

But for the time being, the need for CFCs and their questionable replacements will continue, as will the war against those who would make money on illegally trafficking in these compounds. Watts-FitzGerald, for one, doesn't mind. "This is the side of God and the angels," he says. "People who are environmentally concerned look at this and say, 'Hey, this is a good thing to be doing.' And the domestic industry says the same thing for reasons that are persuasive to them. Basically, except for the evildoers, you really don't have anybody saying, 'Why are you doing that?' and in the environmental area that is not always the case."

Victor Chase

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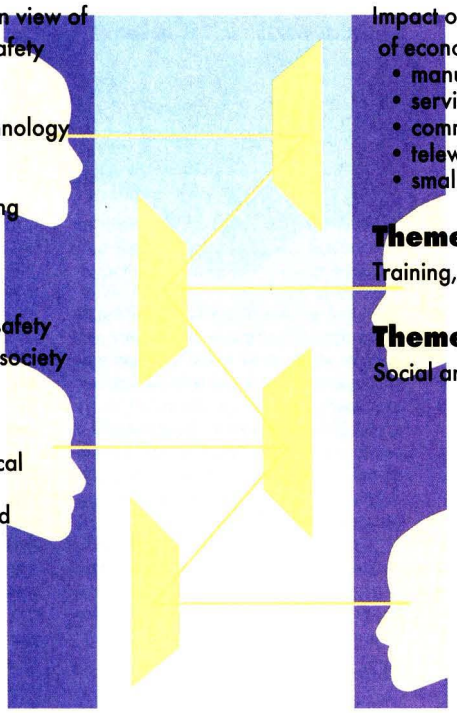

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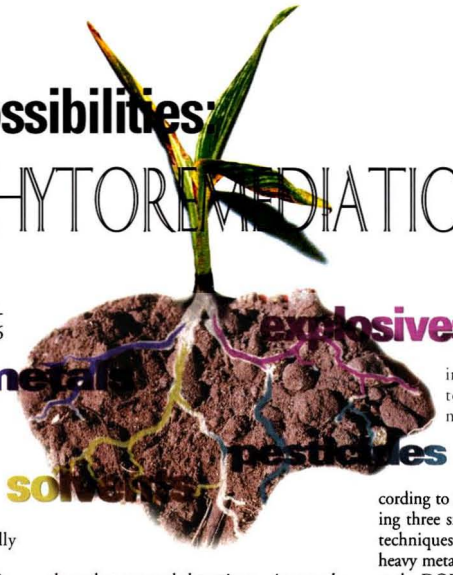
Absorbing Possibilities: PHYTOREMEDIATION



Joseph Tart

Confronted with the task of decontaminating soil made radioactive by the 1986 Chernobyl nuclear accident, American and Ukrainian scientists are field testing the ability of Indian mustard plants to clean the soil in the region by absorbing radioactive metals such as cesium and strontium. In Iowa, where soil and groundwater contamination by the pesticide atrazine is a concern, researchers are testing how well poplar trees can remove the potentially cancer-causing chemical from the soil.

These are examples of phytoremediation—an approach to cleaning up contamination that is attracting increasing attention from scientists and regulators because it appears to be cheaper than chemical and engineering-oriented methods and may also offer immediate and long-term environmental benefits.

New Name, Old Idea

Ilya Raskin, a professor of plant biology at Rutgers University in New Jersey, defines phytoremediation, as "the use of plants for environmental remediation. That involves removing organics and metals from soils and water." Raskin, a biochemist and plant physiologist who coined the term, notes that using plants to alter the environment "has been around forever, since the time plants were used to drain swamps."

What is new, he asserts, is the systematic, scientific investigation of how plants can be used to decontaminate soil and water. Interest in phytoremediation has been growing as the United States continues to face the daunting task of cleaning up a wide range of sites contaminated with toxic heavy metals such as selenium and cadmium, as well as organic compounds including pesticides, explosives, and solvents.

Scientists have found that many plants naturally absorb metals from the ground and store them in their tissues. Plants, like animals, need metals such as zinc and copper for growth. In many instances, according to Raskin, plants can't distinguish between heavy metals such as cadmium and those that are needed nutrients. Among the metal-absorbing plants is *Streptanthus polygaloides*, which grows in California on nickel-contaminated soil and accumulates large amounts of the metal. Members of the genus *Thlaspi* also take up large amounts of heavy metals. These plants are called hyperaccumulators—their tissues an contain from 1,000 to 10,000 parts per million (ppm) of certain heavy metals.

Organic compounds can be degraded by enzymes expressed in the plant membranes of poplar trees. These plants may also stimulate the growth of chemical-degrading bacteria around their roots.

Pluses

The potential benefits of phytoremediation seem to be as numerous as the problems it might address. One reason phytoremediation is gaining attention is because it is potentially cheaper than conventional treatment approaches such as incineration and soil washing, a chemically based, energy intensive approach.

Burt Ensley, president of Phytotech, a firm in Monmouth Junction, New Jersey, seeking to turn phytoremediation into a money maker, says washing metal-contaminated soil can cost about $250 per cubic yard. One EPA project that cleaned up 19,000 tons of contaminated soil cost over $7 million, approximately $400 per ton (one ton is roughly equivalent to one cubic meter). Incineration costs range from $400 to $1200 per ton (for explosives). And an incineration project to clean up explosives-contaminated soil at the Department of Energy's Idaho National Engineering Laboratory cost $4,000 per ton to clean hot spots at the naval proving ground.

That compares with estimates of $80 per cubic yard for phytoremediation, according to Rashalee Levine, who is managing three small field studies exploring new techniques that may be used to clean up heavy metal and radioactive contamination at the DOE weapons facilities and labs.

Phytoremediation is also being explored because it may increase the slow pace of hazardous waste cleanup. "In 1995, few hazardous was sites have been cleaned-up . . . because of the impracticality or cost of engineering solutions," Rufus Chaney, a scientist at the USDA's Environmental Chemistry Laboratory told phytoremediation researchers at a conference at the University of Missouri this spring. Although some types of phytoremediation may take longer than conventional methods at a site, more sites may be cleaned up simultaneously because the cost for each one is less.

Still another attraction of this technology is that it may leave topsoil in usable condition and reduce the amount of contaminated material to be landfilled or incinerated, distinct advantages over the chemical and engineering technologies used now to treat contaminated soil, according to Leon Kochian, a USDA scientist and professor of plant biology at Cornell University. For example, phytoremediation of toxic heavy metals actually reduces the volume of contaminated material. According to Ensley, removing heavy metal-contaminated soil from two and a half acres to a depth of about 18 inches creates about 5,000 tons of soil that must be disposed of in a hazardous landfill. In contrast, plants that take up the metal and are burned leave a residue of between 25 and 30 tons of ash to be disposed of. "It's an immense reduction of mass and volume," Ensley says.

Phytoremediation may also have direct health benefits. "This technique seems particularly attractive for the cleanup of lead in soils," says Robert Tucker, director of the Eco Policy Center at Rutgers University. "Actually having [ground] cover on property is a way to decrease exposure risk. Kids may not play in an area where there is plant growth. And the presence of plants also limits the direct hand-to-mouth exchange of dirt in children." Tucker, who is the former head of the division of research in the New Jersey Department of Environmental Protection, cites a number of small phytoremediation demonstration projects in the state, including one where plants are being used to remove lead from lagoon sediments near a facility where lead tetraethyl was made for leaded gasoline.

Minuses

Phytoremediation also has its drawbacks, which even its ardent champions are quick to acknowledge. For one thing, it is a time-consuming process that can take several growing seasons to clean a site. "Suppose there's a site that's contaminated with heavy metals that's just been bought by a real estate developer and he wants to build a K-Mart on it," says Ensley. "This technology is probably going to be a problem for him, because he doesn't want to sit on that property for two or three years while it gets cleaned up. He's going to want it cleaned up now."

Vegetation that absorbs toxic heavy metals may also pose a risk to wildlife that eat the plants, Ensley acknowledges. The possible scenario is that these harmful metals can work their way up the food chain. For instance, moles or voles that eat metal-contaminated plants are eaten by predators, which then become victims of metal intoxication. To address this problem, Ensley says, "All we can do is when we're doing the field trials of phytoremediation, make sure that's one of the things we measure . . . One of the things we'll have to do is trap insects off the plants and analyze them, and trap moles and voles and analyze them."

While Ensley suggests that insecticides might be used to prevent insects from eating heavy metal-containing plants, he says that such plants aren't appetizing to insects in the first place. "We've discovered that some insects that you would normally expect to eat these plants, when they have metals in them, won't eat them. That hasn't been published, but we have seen that," he says.

But Robert Boyd, associate professor of botany and microbiology at Auburn University, worries that insects might adapt to eating such plants. "I would predict these phytoremediation crops would be subject to pests as they become adapted to these plants," he says. That might lead to heavy metals working their way through the food chain. Boyd says that this whole issue remains to be explored. "There is a knowledge void."

Ensley emphasizes that there's more to phytoremediation than merely putting plants in the ground and letting them do the work. He says that cleanup areas have to be engineered to prevent flooding and erosion. And phytoremediation isn't necessarily chemical free. Researchers talk of needing chelating agents that will free metals and other contaminants from soil particles to allow them to be taken up by the plants. Researchers also need to find the depth to which plants can sink their roots to clean up contamination.

Finding the Right Plants

In spite of these concerns, phytoremediation research is widespread. Scientists at numerous laboratories are exploring the power of plants to cope with contamination, both of metals and of toxic organics.

Thlaspi, like other hyperacculumulators, isn't very good at phytoremediation, says Ensley. These plants are too small and grow too slowly. As a result of screening various plants, Ensley has found that *Brassica*, the genus to which broccoli and Indian mustard belong, do a much better job. Because they grow faster and have more tissue, they can take more metal out of the soil. In the Ukraine, Ensley, Raskin, and their Ukrainian colleagues are studying dozens of varieties of Indian mustard and related plants to see how good they are at removing radioactivity.

In the United States, Norman Terry, professor of plant biology at the University of California at Berkeley is exploring the possibility of using Indian mustard to remove naturally occurring selenium from soil. A necessary nutrient, selenium can leach into water. In high amounts, this metal can poison wildlife and livestock. In laboratory research, Terry has found that Indian mustard not only takes up selenium but converts it into dimethyl selenide, a gas which he describes as relatively nontoxic. "We're trying to genetically alter plants so that we step up the volatilization, so that much of the selenium removed by the plant from the soil goes straight into the atmosphere," he says. According to Terry, there are huge amounts of this gas in the atmosphere from volcanoes, soil, and plants, and it is continually recycled. The amount that would be added via phytoremediation would be negligible, he insists. Terry has not yet tested the genetically engineered plants in the field.

Raskin is also creating transgenic plants to improve their ability to take up metals from soil. He has added the gene for the protein metallothionein, which binds metals, to several plants that have yet to be field tested. Raskin has found that even though Indian mustard is a terrestrial plant, it can remove heavy metals from water.

The DOE began field tests this year on plots that are several hundred square feet to see how well plants take up cadmium, zinc, and radioactive cesium and strontium. Plants including Indian mustard, rape, and turnip—all varieties of *Brassica*—and grasses are being tested at a contaminated site in Butte, Montana, said Kochian, who helped screen plants in the lab for their ability to take up metals prior to field testing. Alfalfa and beans are being

Steven C. McCutcheon/EPA

Nature's kidneys. Researchers are showing that some plants, such as parrot feather, can absorb metals, solvents, and pesticides from soil and water.

Steven C. McCutcheon

Beaver pond. Eurasian water milfoil growing in a beaver pond absorbs TNT from runoff of contaminated soil.

tested at the Idaho National Engineering Laboratory for their ability to accumulate radioactive cesium and strontium. The uranium-absorbing ability of sunflower plants is being tested at a DOE facility in Ashtabula, Ohio.

While some plants may be able to decontaminate soil by simply absorbing metals, others break down organic compounds, and can also enlist soil bacteria to detoxify these compounds.

In his field research, Jerald Schnoor, professor of civil and environmental engineering at the University of Iowa, has found that poplar trees can break down between 10% and 20% of atrazine in soil. Schnoor found that poplars detoxify atrazine in two ways. They absorb atrazine through their roots and break it down, possibly by the enzymes dehalogenase and laccase, to several harmless compounds, including short-chain metabolites. "That was a surprise to us," says Schnoor. He expected only that the trees would stimulate bacteria living around the trees' roots to attack and degrade atrazine, which they do. Exudates—sugars, alcohols, and volatile acids—are secreted by the trees and "seem to enhance the rate of microbial transformation of atrazine," says Schnoor. In sandy soil, virtually 100% of the atrazine was metabolized.

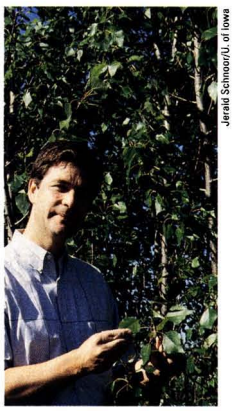
At the University of Washington, Stuart Strand, a professor in the College of Forest Resources, and Milton Gordon, a professor of biochemistry, have found in lab experiments using small poplar trees that monooxygenase enzymes break down trichloroethylene, a suspected carcinogen and groundwater contaminant, to carbon dioxide. Although the majority of TCE is transpired to the atmosphere, the breakdown is still significant as a potential cleanup technique.

Strand says that the plant enzymes appear to be solely responsible for the breakdown, and he finds the same results in tissue culture experiments in the laboratory. The researchers are currently testing poplar trees in the field to see if the lab results are borne out.

EPA researchers are studying plants such as parrot feather weed and Eurasian water milfoil for their ability to break down the explosive TNT, a toxic compound which contaminates both groundwater and soil at U.S. Army ammunition facilities.

These plants, and the variety of poplars studied by Schnoor, contain the enzyme nitroreductase, which can rapidly break down TNT, explains Steven McCutcheon, an environmental engineer with the EPA's Athens, Georgia, laboratory. McCutcheon and his colleague David Young at Auburn University have tested Eurasian milfoil on 2–4 inches of soil from the Alabama Army ammunition plant contaminated with 5,000 ppm of TNT. The soil, which is essentially sterile, was put in small plastic pools, covered with water, and the plants were added. "Within a week the dissolved TNT is near detection; a few days later it's below detection," McCutcheon said. The TNT is broken down and becomes part of the lignin or plant structure. Toxicity analysis is needed, McCutcheon says, to determine if the TNT breakdown products represent a residual risk. However, he said, in experiments, tadpoles and snails have thrived in the pools with the plants, but are unable to live in the control pools that do not contain the plants. In the spring, the EPA and the Army Environmental Center will be field testing these plants on a one-eighth of an acre wetland at an Army ammunition plant in Milan, Tennessee.

McCutcheon's team has compiled some information on the breakdown pathways of chlorinated solvents, as well as identified four plant proteins that degrade organics. The team has also developed an antibody assay that allows them to screen plants for the presence of nitroreductase, thus allowing them to find native plants that can be used in phytoremediation. About 20% of the aquatic plants tested contain the nitroreductase, McCutcheon said.



Jerald Schnoor/U. of Iowa

Poplar conception. Jerald Schnoor has found that poplar trees can break down atrazine in soil.

Future of Phytoremediation

Phytoremediation is still in its early stages. While many scientists, engineers, and regulators are optimistic that it will eventually be used to clean up organic and metallic contaminants, at least two or three years more of field tests and analyses are necessary to validate the initial, small-scale field tests. Issues like soil characteristics and length of the growing season will also have to be taken into account. Scientists must also determine what sites are most amenable to phytoremediation. Other issues such as the potential impact on wildlife remain to be fully explored. Simultaneously, researchers working in the lab are trying to better detail the processes behind phytoremediation to possibly improve it.

While it may offer a number of advantages, it is not, Schnoor cautions, a panacea. Still, the information gathered thus far, says McCutcheon, "establishes that phytoremediation and ecological engineering are powerful approaches that should be fully explored."

Harvey Black

Suggested Reading

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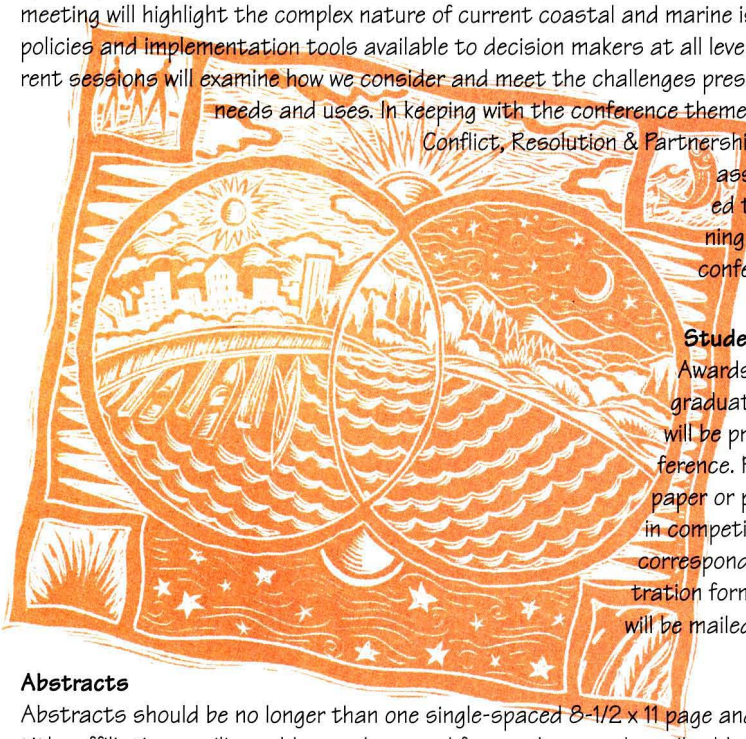

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Use of Outpatient Clinics as a Health Indicator for Communities around a Coal-Fired Power Plant

Ayana I. Goren,[1] Sarah Hellmann,[1] and Eduard D. Glaser[2]

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The permit to operate the first coal-fired power plant in Israel was issued with the condition that a comprehensive network to monitor its effects on the environment, health, and agriculture must be installed and operated around the plant. The health monitoring system consists of four studies, which started 1 year prior to the operation of the plant and were carried out for 10 years. In the framework of the health monitoring system, a study of requests for health services was carried out. In this survey, 8 clinics of the Sick Fund, served by 16 physicians, were followed up. The clinics were located as near as possible to air pollution monitoring stations and represent expected different levels of pollution. A health recorder summarized each day's visits to each physician and tabulated the total visits for each day and the visits due to respiratory tract complaints. Multivariate stepwise regressions on total as well as on respiratory complaints were carried out. The independent variables in the regressions were sulfur dioxide, meteorological parameters (such as temperature and humidity), and flu epidemics. Temperature was almost always significantly correlated with respiratory complaints, but less correlated with total visits among adults and children. Sulfur dioxide, most meteorological parameters, and flu epidemics were not meaningful explanatory factors in the regressions. Ambient air pollution levels did not exceed the Israeli air quality standards or the more stringent local air quality standards; the monthly and annual average sulfur dioxide and nitrogen oxides values were very low. *Key words:* air pollution, community study, outpatient clinics, pediatrics, pulmonary diseases. *Environ Health Perspect* 103:1110–1115(1995)

The permit to build and operate the first (1400 megawatt) coal-fired power plant in Israel stipulated that a comprehensive network be installed to monitor its effects on the environment, health, and agriculture. An epidemiological monitoring program was designed in response to the health monitoring requirement (*1*).

Four types of studies were included in the epidemiological monitoring program: mortality analyses, monitoring of requests for health services, studies of pulmonary symptoms and lung function in schoolchildren, and panel studies of adults, both with and without chronic obstructive pulmonary diseases. Baseline data were gathered for at least 1 year prior to the operation of the first unit of the power plant. Subsequent data were collected until the end of 1990.

This epidemiological monitoring program was aimed at detecting any spatial or temporal changes in the health status of the study population, taking into account possible confounding factors (such as meteorological parameters and flu epidemics) that could act similarly to air pollution. Here we evaluate requests for health services in outpatient clinics. This survey was designed to point out short-term health effects with minimal time delay. Ongoing analysis of the data could demonstrate a difference in demand for medical services between areas with different levels of pollution.

Baseline data gathered before the operation of the power plant enabled a comparison with the seasonal distribution of visits before possible changes in air quality.

Methods

About 70,000 people live within a 10-km radius of the power plant. The population in this area was relatively stable throughout the time of the follow-up. About 80% of this population was insured under "The General Sick Fund." The use of health services in 8 clinics of the Sick Fund, served by 16 physicians (each clinic has one pediatrician for children younger than 14 years and one general physician for adults) was recorded. The size of the physicians' practices did not change substantially over the years of the follow-up. The number of patients in each of the clinics followed up varied from 600 to 2100. In the area expected to be most polluted, about 7000 patients were followed up. The total number of patients followed up in this study was about 30,000 persons.

The distribution of the eight clinics represent a gradient of expected low, medium, and high pollution, according to the environmental impact statement presented by the Israel Electric Company to the Ministry of Health. The clinics were located as near as possible to continuous air pollution monitoring stations (Fig. 1). The people using these clinics lived in the vicinity; therefore, the location of the clinics served as a good estimate of exposure to air pollutants in the community. The physicians kept records, registering all visits of patients and each patient's diagnosis.

Once a week, a health recorder summarized each day's visits to each physician and separately tabulated the total visits for each day as well as the number of visits for respiratory tract complaints. Air pollution and meteorological data were gathered and analyzed by the Association of Towns for Environmental Protection.

The environmental monitoring network consists of 12 monitoring stations, of which 10 are stationary and 2 are mobile. The monitoring stations are fully automatic and measure the following data: SO_2, NO_x, O_3, CO, total hydrocarbons, and meteorological parameters. Not all monitoring stations perform all measurements. The instruments are automatically calibrated and continuously measure the levels of ambient air pollution. The data are sent by radio to the Association of Towns and fed into a computer that stores and analyzes them. Daily averages of air pollution measurements were given to the health recorder for inclusion in the statistical analysis of visits to outpatient clinics.

Data concerning flu epidemics during November to April of each year were gathered nationwide by the epidemiological department of the Ministry of Health. Most flu cases were diagnosed on symptomatology. A combined file was created including daily visits to outpatient clinics, daily air pollution concentrations, daily meteorological data, and daily flu data.

Statistical analysis of use of outpatient clinics in the vicinity of the power plant was carried out using SPSS (McGraw-

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Hill, New York) and BMDP (Berkeley, California) programs. The ratio between the daily number of visits and the size of the physician's practice (size = number of patients assigned by the Sick Fund to the physician) (visit ratio) was calculated both for visits with respiratory tract complaints and for the total number of visits, for every day and physician. The daily averages of these ratios for each day of the week and for each month were calculated. The calculations were carried out separately for visits to pediatricians and for visits to general physicians.

Monthly averages for all the survey years were calculated from the daily averages, both for respiratory diseases and for the total number of visits among children and adults. In the next step, data of environmental measurements from the nearest monitoring stations were added to the database of the outpatient clinics.

From flu monitoring data, daily averages of flu visits were calculated for children and for adults. These flu data were also added to the database of the outpatient clinics. Multivariate stepwise regressions on the daily visit ratios were performed separately for each year of the study for both pediatricians and general physicians. Daily visits due to respiratory tract complaints as well as visits due to all reasons were the dependent variables in the multivariate analysis. Daily ambient SO_2 concentrations, meteorological parameters, and flu-data were the independent variables in these regressions. To follow up possible changes over time in the use of outpatient clinics, a one way analysis of variance was carried out, in which the studied effect was the year of study. The meteorological parameters as well as SO_2 were entered as covariates in the analysis of variance.

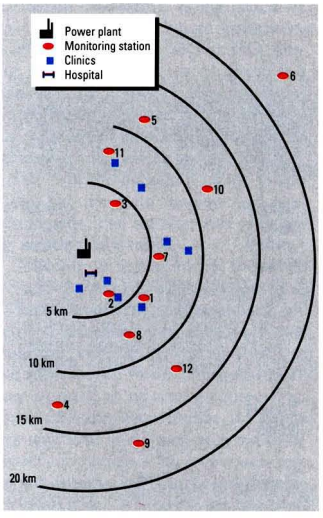

Figure 1. Site of the power plant, outpatient clinics, and environmental monitoring stations. Station 11, expected low pollution; station 2, expected medium pollution; station 7, expected high pollution.

Results

Analysis of the daily averages of visit ratios showed an increased use of clinics on Sundays, the first workday of the week in Israel. This trend characterized both visits to pediatricians and visits to general physicians.

In Figures 2 and 3 the visit ratios for monthly averages of total visits and of visits due to respiratory tract complaints are presented. Analysis of monthly averages of outpatient clinic visits showed seasonal trends.

Among adults, a seasonal trend of more frequent use of outpatient clinics due to respiratory tract complaints was observed during the months December through March throughout the follow-up. A seasonal trend of more frequent use of pediatric outpatient clinics during winter months due to respiratory tract complaints was also observed (Fig. 3). A seasonal trend was not observed for the total visits to practices served by general physicians (Fig. 2). However, the total number of visits to pediatricians peaked during winter months, possibly because respiratory complaints account for a major part of the total visits to pediatricians.

No consistent trend of change in the use of adult clinics due to respiratory tract complaints was observed between 1982 and 1990. A substantial decline in

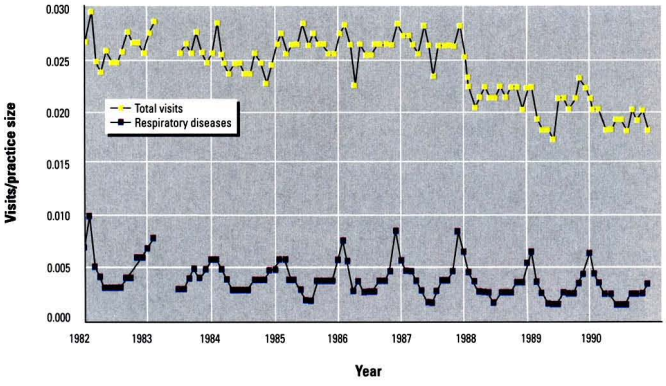

Figure 2. Monthly averages of clinic visits by adults, expressed as a ratio to the size of physicians' practices.

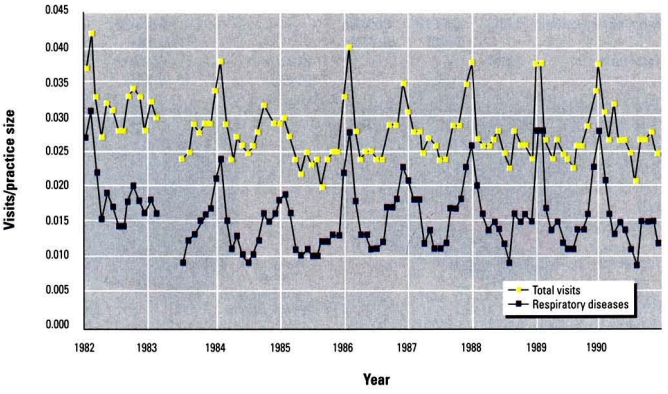

Figure 3. Monthly averages of clinic visits by children, expressed as a ratio to the size of physicians' practices.

the total number of visits occurred between 1988 and 1990 (Fig. 2). For children, a trend of moderate decline in the total number of visits, and especially in the number of visits due to respiratory tract complaints, was observed between 1982 and 1984. There is no obvious trend for the following years (Fig. 3).

Multivariate statistical analyses were carried out to determine the dominant environmental factors explaining the use of medical services in the power plant area. By means of multiple stepwise regressions, the effects of meteorological parameters (such as temperature, barometric pressure, humidity, and precipitation), flu epidemics, and air pollutants on the use of outpatient clinics were analyzed.

For each year of study, a multiple regression analysis which included all the above-mentioned parameters was carried out. Regression equations were calculated separately for respiratory complaints and for total visits, both for children and adults. The coefficients for the variables which appeared in the regression equations are presented in Table 1 for each year of the study. The major explanatory factor for use of outpatient clinics among both children and adults was ambient temperature. Most coefficients for temperature were negative and highly significant; i.e., at lower temperatures, higher numbers of clinic visits were observed. In some regressions, further explanatory factors for use of medical services occurred; a flu epidemic was the most frequent additional explanatory factor. The part of the variance explained by the regressions was between 6% and 32%.

In the analysis of changes in use of outpatient clinics over time, the year of follow-up was entered as the main effect, and all other variables such as tempera-

Table 1. Coefficients in the regression equations for respiratory diseases and for total number of visits among children and adults[a]

Dependent variable	Adults						Children					
	R^2	Temperature	BP	Precipitation	SO_2	Flu epidemics	R^2	Temperature	BP	Precipitation	SO_2	Flu epidemics
1982												
Respiratory diseases	0.2279	0.000296 (0.000)	—	—	—	0.000043 (0.0035)	0.0595	-0.000688 (0.000)	—	—	—	—
Total visits	—	—	—	—	—	—	0.0154	—	—	—	—	0.000262 (0.0010)
1983												
Respiratory diseases	0.2522	-0.000182 (0.000)	—	-0.000112 (0.0053)	0.084721 (0.0003)	0.000303 (0.000)	0.1005	-0.000399 (0.000)	-0.000115 (0.0270)	—	—	—
Total visits	0.0167	—	—	—	—	-0.000444 (0.0001)	0.0280	-0.000374 (0.0001)	—	—	—	—
1984												
Respiratory diseases	0.1588	-0.000195 (0.000)	0.000047 (0.0294)	—	—	—	0.3246	-0.000483 (0.000)	0.000179 (0.0197)	—	—	0.000352 (0.000)
Total visits	0.0048	—	0.000152 (0.0143)	—	—	—	0.1490	—	0.000416 (0.000)	—	—	0.000423 (0.000)
1985												
Respiratory diseases	0.1665	-0.000214 (0.000)	—	-0.000025 (0.0273)	0.061329 (0.0134)	—	0.1924	-0.000352 (0.000)	—	—	—	0.000170 (0.000)
Total visits	—	—	—	—	—	—	0.0580	—	—	—	—	0.000293 (0.000)
1986												
Respiratory diseases	0.2256	-0.000301 (0.000)	—	—	—	0.000054 (0.0218)	0.2907	—	0.000521 (0.000)	0.000204 (0.0035)	0.26535 (0.0172)	0.000306 (0.000)
Total visits	0.230	—	—	—	—	-0.000239 (0.000)	0.2038	0.00068 (0.0006)	0.000849 (0.000)	0.00032 (0.0020)	—	0.000473 (0.000)
1987												
Respiratory diseases	0.1819	-0.000156 (0.000)	0.000075 (0.0069)	—	—	0.000192 (0.000)	0.1503	—	0.000425 (0.000)	—	—	-0.000189 (0.000)
Total visits	0.0105	—	—	-0.000213 (0.0450)	—	0.000232 (0.0105)	0.0671	0.000319 (0.0029)	0.000459 (0.0001)	—	—	0.000228 (0.000)
1988												
Respiratory diseases	0.1695	-0.000114 (0.000)	0.00001 (0.0068)	—	—	0.000166 (0.000)	0.1903	-0.000348 (0.000)	—	-0.000251 (0.0148)	-0.236965 (0.0001)	0.000287 (0.000)
Total visits	0.0090	—	—	—	—	0.000246 (0.0010)	0.1341	—	—	-0.000262 (0.0270)	-0.148155 (0.0474)	0.000395 (0.000)
1989												
Respiratory diseases	0.2066	-0.000234 (0.000)	-0.000119 (0.2111)	—	—	—	0.2575	-0.00105 (0.000)	-0.000615 (0.0146)	—	—	—
Total visits	—	—	—	—	—	—	0.1383	-0.000838 (0.0002)	—	—	—	—
1990												
Respiratory diseases	0.1948	-0.000291 (0.000)	—	—	—	—	0.2405	-0.000671 (0.0001)	—	—	—	0.000334 (0.0050)
Total visits	—	—	—	—	—	—	0.1451	-0.00052 (0.0219)	—	—	—	0.000421 (0.0075)

BP, barometric pressure.
[a] p-value for the variable in regression equation in parentheses.

ture, precipitation, barometric pressure, and SO_2 were entered as covariates. Significant deviations from the average use of pediatric and adult outpatient clinics occurred over time, but the effect of the year of study was not consistent. In other words, a significant increase in use of outpatient clinics was observed for some years, while in other years a decrease was observed.

During the time period of the study, the ambient air pollution levels did not exceed the Israeli air quality standards or the more stringent local standards (half of the Israeli standard), based on measurements of SO_2 and NO_x at the 12 monitoring stations (Fig. 1). In fact, the annual average SO_2 and NO_x values were very low. For instance, the 1989 annual SO_2 averages did not exceed 20 $\mu g/m^3$, less than a quarter of the U.S. standard, at any monitoring station (Table 2).

Because of the difficulty of following multiyear trends of such low levels of annual average concentrations, we decided to focus on half-hourly concentrations, for which there is also an Israeli ambient standard. We counted the number of air pollution "events" in which the half-hourly averages for SO_2 or NO_x were above an arbitrary threshold. The threshold values chosen were 183 $\mu g/m^3$ (70 ppb) for SO_2 and 235 $\mu g/m^3$ (125 ppb) for NO_x, which are about one-quarter of the local air quality standards and about one-eighth of the Israeli air quality standards. In Figure 4 the summary of events for SO_2 and for NO_x during 1981–1992 is presented. From this summary, an increase in the total number of events measured by the monitoring stations can be observed. Part of the increase in the number of events originated from the operation of the power plant, but the main increase was connected with other sources, such as industry and especially traffic.

The sources responsible for the events were identified according to the wind direction measured at the time of the event. The highest number of SO_2 events from the power plant was measured at the monitoring station located in the area expected to be most polluted (Fig. 5); a markedly lower number of events was registered in the area expected to be moderately polluted. No events were registered during 1987–1992 in the monitoring station located in the expected low-pollution area. The highest number of NO_x events from all sources was measured in the monitoring station located in the area expected to be most polluted (Fig. 6); a markedly lower number of

Table 2. Monthly and yearly average sulfur dioxide concentrations ($\mu g/m^3$) for 10 monitoring stations in 1989

Station	1	2	4	5	6	7	8	10	11	12
January	7	19	8	12	20	7	11	8	0	5
February	5	17	9	15	26	13	8	11	11	6
March	5	12	5	5	15	13	7	7	5	5
April	7	23	8	13	32	12	10	14	23	9
May	9	21	11	10	16	13	5	9	8	8
June	10	21	8	14	13	5	11	7	7	9
July	11	8	4	5	5	18	5	11	0	10
August	13	7	4	5	8	23	8	10	1	10
September	11	11	4	6	4	10	6	7	1	11
October	12	12	12	10	9	7	6	8	7	5
November	5	11	7	10	11	12	4	7	6	5
December	6	19	14	14	18	15	5	11	22	20
Yearly average	8	15	8	10	15	12	7	9	8	9

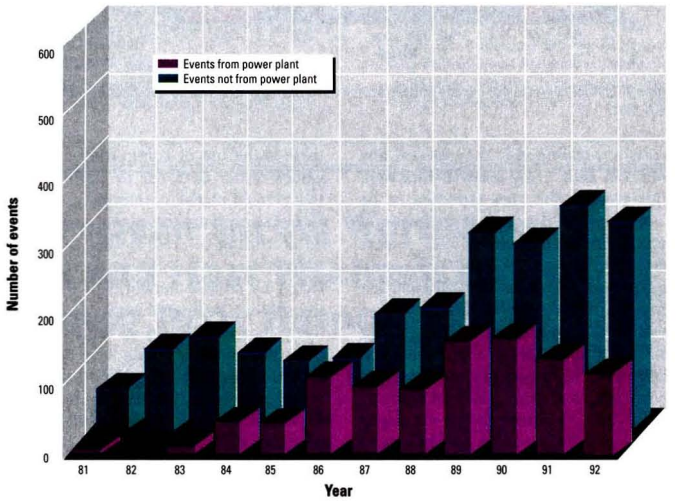

Figure 4. Summary of sulfur dioxide and nitrogen oxides "events" measured at 10 permanently sited monitoring stations during the period 1981–1992.

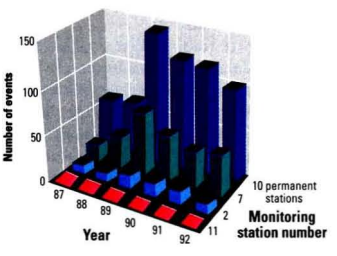

Figure 5. Sulfur dioxide "events" from the power plant in expected low-pollution (station 11), medium-pollution (station 2), and high-pollution (station 7) areas and for the 10 permanently sited monitoring stations during 1987–1992.

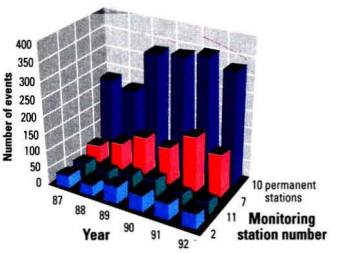

Figure 6. Nitrogen oxides "events" measured around the power plant in the expected low-pollution (station 11), medium-pollution (station 2), and high-pollution (station 7) areas and for the 10 permanently sited monitoring stations during 1987–1992.

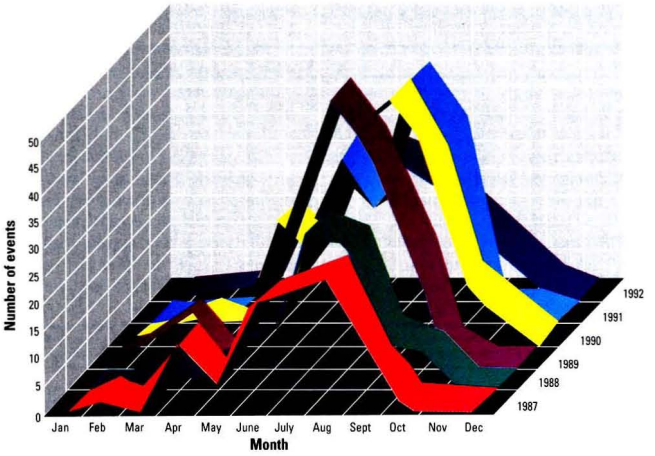

Figure 7. Distribution of sulfur dioxide and nitrogen oxides "events" from the power plant by month during 1987–1992.

events was registered in the expected low pollution area. Only a few events were registered in the area expected to be moderately polluted. A substantial increase in the number of NO_x events measured between 1987 and 1992 could be observed. In the monthly distribution of events from the power plant (Fig. 7), a peak in the number of events during summer months (June–September) was registered. The reason that the pollution events occurred at this coastal site primarily during summer days is the strong radiative heating of the ground, combined with an on-shore sea breeze (3–5 m/sec), which together gave rise to a strong convective layer extending to a height of several hundred meters. When the plumes from the high stacks entered the convective layer, fumigation occurred and pollution was brought down to ground level. In contrast, no significant convective layer developed during typical winter weather.

Discussion

Epidemiological investigations of health effects of ambient air pollution have often focused on community or regional morbidity and mortality (*2–4*). Short-term effects on morbidity have been sought by examining correlations of pollution levels based on networks of monitoring stations with rates of hospital admissions (*3-6*), emergency room visits (*2,7–10*), or outpatient clinic visits (*11–13*). Such community-based epidemiological studies are subject to a number of limitations; primary among these is the use of community exposure values rather than use of exposures of individuals in the community. Nevertheless, when morbidity data are collected according to well-defined and uniform criteria and pollution data are collected by reliable networks of monitoring stations, such studies provide a relatively inexpensive and valuable tool. In our study morbidity data were collected according to well-defined and uniform criteria. However, we have not been able to distinguish between acute and planned visits nor could we identify multiple visits of the same patients. Air pollution data were collected by a reliable network of monitoring stations, located in the vicinity of the Sick Fund clinics. All available data collected over the entire follow-up period have been used in the analysis. Regarding air pollutants, past studies have measured primarily airborne SO_2 and particulate matter arising from industries.

In highly industrialized areas, SO_2 and particulate concentrations are well correlated with one another and with some detectable health effects (*3*). In the vicinity of the Hadera power plant, particulate matter has only been measured by weekly high-volume sampling. No measurements of small-diameter particulate matter have been carried out by the monitoring network in the power plant area. Microscopic analyses of high-volume samples have shown that particulates in this region mainly originate from natural sources. This is due to the soils and vegetation and to the use of electrostatic precipitators which reduce fly ash emissions from the power plant to low levels. Sulfur dioxide concentration alone has therefore been taken in this study as a rough estimate of health-related environmental pollution resulting from the power plant. Meteorological data have been almost complete, and all available data were used in the multivariate analysis. Similar to our findings, Kardaun et al. (*11*), in their study in Amsterdam, showed seasonal variations in the use of family practices. Marty (*12*), in a Swiss study, showed an increase in acute respiratory illnesses among children during fall and winter months (low temperatures, high humidity) similar to our results. Bates et al. (*7*) have found variations in emergency visits by day of the week, similar to our findings of excess visits on Sundays. The excess emergency room visits on Sundays observed by Bates may have resulted from lack of health services in the community on Sundays, while excess in outpatient clinic visits on Sundays in our study are apparently due to use of the first opportunity to consult a community physician after the weekend. The trend of moderate decline in the utilization of "The General Sick Fund" medical services during the years of this study may have resulted from consulting more often private pediatricians instead of using the Sick Fund medical services.

Kucerova et al. (*13*) also used morbidity data as recorded by pediatricians in the community. They registered a higher incidence of respiratory diseases ($p<0.01$) with higher mean duration for contaminated versus clean areas. In our survey we could not find an effect of SO_2 exposure on use of outpatient clinics, apparently due to extremely low SO_2 levels even in the regions expected to be more polluted. Moreover, the number of events of SO_2 peaked during summer months, whereas visits due to respiratory complaints peaked during winter months.

Similar to our findings, Rebmann et al. (*14*), in their health study carried out among preschool children in southwestern Germany, did not find a correlation between cases of croup and the degree of air pollution, apparently because of relatively low pollution levels. The epidemiological monitoring program carried out in the vicinity of the Hadera power plant, included a health study among schoolchildren in which pulmonary symptoms and lung functions had been assessed. In the framework of this study we also could not show any deleterious health effect among the children residing

in the areas expected to be affected by the operation of the power plant as compared to the expected low-pollution areas. Even among the most sensitive part of the population, adults suffering from chronic obstructive pulmonary diseases, the follow-up failed to show any deterioration in their lung function or differences in the severity of their respiratory complaints as compared to a panel of a similar population residing in a rural, clean area with no major environmental polluting source. Air pollution levels measured around the coal-fired power plant in Hadera were low and did not seem to cause adverse health effects.

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Genetic Control of Cadmium Tolerance in *Drosophila melanogaster*

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Flies from a transgenic line of *Drosophila melanogaster* with two copies of the metallothionein allele Mtn^3 were more tolerant to cadmium than strains with only one copy of the gene. However, flies with the Mtn^3 allele were as tolerant as flies with the Mtn^1 allele, despite the level of expression of Mtn^1 being three times higher than that of Mtn^3. We propose that the substitution of Lys-40 (in Mtn^3) for Glu-40 (in Mtn^1) accounts for a reduction in binding affinity of Mtn^1, which offsets the increased expression levels. *Key words*: cadmium tolerance, *Drosophila melanogaster*, gene duplication, life span, metallothionein, *Mtn* gene, viability. *Environ Health Perspect* 103:1116–1118 (1995)

One of the two metallothionein genes in *Drosophila melanogaster*, *Mtn*, occurs in two predominant alleles: Mtn^1, which was found at frequencies of 85% and 95% in American and European samples, respectively (*1*–3), and Mtn^3, which was the minority allele in the American and European samples, but was fixed in a sample from Congo (*2*). Structurally, Mtn^1 differs from Mtn^3 in three respects: 1) it has two base substitutions in the promoter region, 2) it lacks a 49-base pair segment in the 3' untranslated region, and 3) it has a base substitution in the C-terminal codon. Studies in sibling species suggest that Mtn^3 is the ancestral allele. RNA measurements indicate that Mtn^1 strains accumulate mRNA at a level approximately three times greater than do Mtn^3 strains, probably the consequence of one of the first two differences listed above (*2*).

Metallothionein seems to be a very monomorphic protein in most species. To our knowledge, *Drosophila* presents the only case in which genetic polymorphism of metallothionein has been studied; we have observed polymorphism both in the coding sequence and the presence of duplications (*2,4*). The occurrence of Lys-40 in Mtn^3 in place of the Glu-40 found in Mtn^1 may have a profound effect. This C-terminal residue is next to one of the Cys-*X*-Cys groups responsible for metal binding. Coordination of Cd^{2+} ions by the thiolate groups of four cysteine residues creates an excess negative charge that is thought to be neutralized by basic amino acids in the vicinity (*5*). Thus, the presence of Glu in that position may affect the binding capacity of the metallothionein produced by Mtn^1. Despite numerous attempts, it has not been possible to purify *Drosophila* metallothionein to test this hypothesis, so we resorted to the indirect approach of measuring tolerance to metal toxicity in flies of various genotypes.

Increased transcriptional activity in any of several duplications of the Mtn^1 allele is always accompanied by increased tolerance to cadmium and copper ions (*4*). The lower level of expression of Mtn^3, therefore, should lead to reduced metal tolerance, unless its potentially more efficient protein counters this effect. This argument assumes that the two alleles have comparable translational efficiency.

To discover whether altered levels of Mtn^3 product lead to correspondingly modified tolerance to toxic metals, we generated a transgenic line of *Drosophila melanogaster* with two copies of the Mtn^3 allele. As in the case of Mtn^1 natural duplications, flies with this synthetic duplication were more tolerant to cadmium than strains with only one copy of the gene. Comparison of the cadmium tolerance of Mtn^1 and Mtn^3 strains, however, showed that the two strains are quite similar; this suggests that the increased transcript level in Mtn^1 exactly compensates for a reduction in either its metal-binding efficiency or its stability, caused by the amino acid substitution.

Materials and Methods

Flies of a Canton S strain with allele Mtn^1 were crossed to a Samarkand strain (S500) with allele Mtn^3, and females of the progeny were back-crossed to Samarkand males. Females of this second generation were then individually mated to Samarkand males. After allowing these females to lay eggs for a few days, DNA was extracted from them and tested for presence of the Mtn^1 allele by Southern blotting. Vials with progeny from females that were heterozygous were kept, and vials in which the female proved to be homozygous for Mtn^3 were discarded. This procedure was repeated for 11 generations, at which time Mtn^1 homozygous stocks were established. Thus, stocks were created in which the majority of the genetic background was the same as that in the Samarkand Mtn^3 stock but which were homozygous for the Mtn^1 allele. Two of these lines, SCS1 and SCS2, were used for tests of metal tolerance.

Stocks with a duplication for the Mtn^3 allele were produced by P-element-mediated transformation with a vector carrying a copy of the Mtn^3 allele including 370 base pairs (bp) of the promoter region. We have shown that this segment of the promoter is sufficient for normal expression of the *Mtn* gene (*6*). Transformed flies were crossed to a strain carrying Mtn^3, and the third-chromosome marker, "red" (C301). The strain derived from this cross, C313, was homozygous for the Mtn^3 allele at two different sites. Level of expression was determined by northern analysis as previously done (*4*); we determined that the amount of RNA loaded in all lanes was comparable by visual inspection of ethidium-bromide-stained rRNA after electrophoresis.

Males, eclosed over a 24-hr period, were sorted in groups of 20 and kept on normal food for a 6-hr period. At this point they were transferred to treatment vials containing Instant Drosophila Medium (Carolina Biologicals, Burlington, North Carolina) supplemented with 0, 0.1, 0.5, or 1.0 mM $CdCl_2$. Surviving flies were counted every 24 hr and transferred to fresh vials with the corresponding medium every 3 days. We determined the half-life of different genotypes in the various treatments from survival curves. All genotypes and concentrations tested were tested simultaneously, with 4 vials of 20 flies for each genotype and each treatment. This unit experiment was repeated three times for each comparison.

Results and Discussion

Using P-element-mediated transformation, we produced flies carrying an extra copy of the Mtn^3 allele. Estimates of metallothionein RNA, by northern analysis, confirmed that these transformed flies (C313)

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accumulate higher levels of *Mtn* RNA than flies with a single copy of the gene (C301) (Fig. 1).

Flies with two copies of the Mtn^3 allele are more tolerant to cadmium than flies with a single copy, as is shown in Figure 2 and Table 1. Tolerance here is measured as the half-life of adult males in various media; Figure 2A shows a set of survival curves of the type used to obtain the half-life values reported in Figure 2B. These results indicate that the allele Mtn^3 is capable of participating in metal detoxification, and we propose that the failure to find duplications for this allele in natural populations is probably due to its infrequent occurrence in Europe. It is in Europe where duplications of Mtn^1 seem to have originated, in response to the agricultural use of copper salts for antimicrobial purposes (*2*).

In addition to studying flies with one and two copies of Mtn^3, we also determined the tolerance of a new allele, Mtn^{K45} (CK45), which has the same sequence and RNA level as Mtn^3 (data not shown) but which has an insertion of 14 bp (GTTACAATCGTTAC) in the 3' untranslated region (111 bp downstream of the termination codon). With respect to cadmium tolerance, Mtn^{K45} is indistinguishable from Mtn^3.

Table 2 and Figure 3 show that there is no significant difference between Mtn^1 (SCS1 and SCS2) and Mtn^3 (S500) flies with respect to their tolerance to various concentrations of cadmium. At the lowest cadmium concentration (0.1 mM), Mtn^1 appears to have a slight advantage, but analysis of variance indicates that overall the two alleles are not significantly different. Table 2 also shows that both Mtn^1 and Mtn^3 are more sensitive to cadmium than a duplication for Mtn^1 (statistically significant difference), as was expected based on our previous work (*4*). In Table 2, the half-life of flies was standardized as a percent of their half-life in control medium.

Despite a threefold difference in expression level of Mtn^1 and Mtn^3, the half-life in cadmium of flies carrying these alleles is not significantly different. Since the only known difference between the two alleles, other than in the level of expression, is in the substitution of Lys-40 (in Mtn^3) with Glu-40 (in Mtn^1), this result supports the hypothesis that the protein of Mtn^1 has reduced binding affinity or stability, which is compensated by its increased expression levels. It should be noted that metallothionein is probably quickly removed from the cytosol, either by aggregation or degra-

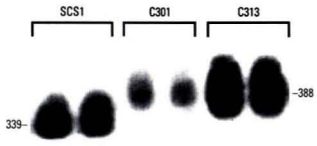
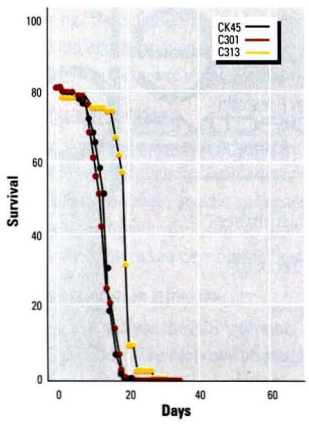


Figure 1. Autoradiograph of a northern blot using an *Mtn* cDNA probe and RNA extracted from flies induced with 1.0 mM $CdCl_2$ for 48 hr. Total RNA, 10 µg per lane, was used (*2*).



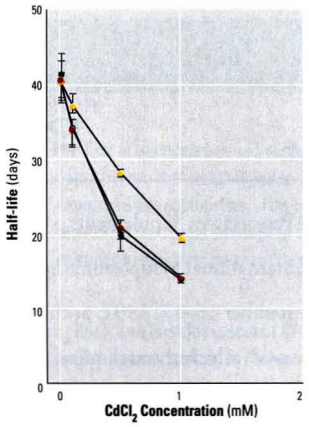


Figure 2. (A) Survival of adult males in medium supplemented with 1.0 mM $CdCl_2$. The data from four vials for each strain are combined here. Half-life estimates were obtained from plots such as this. For each strain and each $CdCl_2$ the experiment was repeated three times. (B) Half-life of adult males in medium supplemented with 0, 0.1, 0.5, or 1.0 mM $CdCl_2$. Vertical bars indicate standard errors.

Table 1. Half-life of adult male flies, in days, in medium with varying concentration of cadmium chloride[a]

	Mean (SE)			
Strain	Control	0.1 mM $CdCl_2$	0.5 mM $CdCl_2$	1 mM $CdCl_2$
C313 *(Dup(Mtn^3)]*	40 (3)	37 (2)	28 (0.5)	19 (1)
C301 *(Mtn^3)*	41 (3)	34 (2)	21 (1)	14 (0.5)
CK45 *(Mtn^{K45})*	41 (3)	34 (2)	20 (2)	14 (0.5)

[a] $N = 3$. Analysis of variance indicates that, with respect to cadmium tolerance, C313 *Dup(Mtn^3)* is significantly different from the other two strains ($p<0.01$), which are not significantly different from one another.

Table 2. Half-life of adult male flies, in days, in medium with varying concentrations of cadmium chloride, as percent of the half-life in control medium without cadmium[a]

	Mean (SE)			
Strain	Control	0.1 mM $CdCl_2$	0.5 mM $CdCl_2$	1 mM $CdCl_2$
DupH22 (Mtn^1)	100 (6)	112 (15)	92 (4)	59 (4)
SCS1 *(Mtn^1)*	100 (7)	82 (2)	55 (4)	34 (5)
SCS2 *(Mtn^1)*	100 (6)	78 (6)	48 (6)	33 (4)
S500 *(Mtn^3)*	100 (5)	98 (8)	52 (4)	37 (5)

[a] $N = 3$. Analysis of variance indicates that, with respect to cadmium tolerance, *DupH22 (Mtn^1)* is significantly different from the other three strains ($p<0.01$), which are not significantly different from one another.

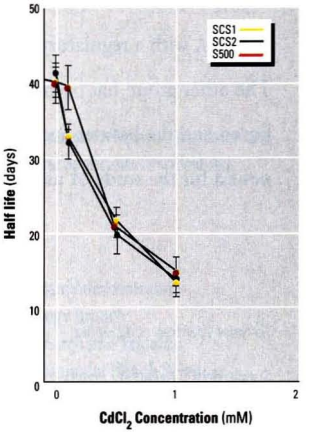


Figure 3. Half-life of adult males in medium supplemented with 0, 0.1, 0.5, or 1.0 mM $CdCl_2$. Vertical bars indicate standard errors.

dation, when not fully complexed with metal ions; we expect Mtn^1 and Mtn^3 flies to have comparable levels of soluble metallothionein, but larger amounts of Mtn^1 protein need to be synthesized to maintain that level.

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THE NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

Announces Request for Applications

ENDOCRINE-DISRUPTING CHEMICALS AND WOMEN'S HEALTH OUTCOMES

PURPOSE

Research on the health effects of chemicals and other exposures which are suspected to disrupt the normal activity of the endocrine system is a high priority of the National Institute of Environmental Health Sciences (NIEHS). Exposure to these chemicals may have broad-based systemic effects and may increase a human risk to hormonal cancers. Accordingly, the goal of this Request for Applications (RFA) is to encourage toxicologic, basic science and epidemiologic research on the human health effects of exposure to chemicals which mimic, antagonize or indirectly alter the activity of hormones. Of particular interest is the health effects associated with exposure to women, since such exposures may affect both the woman herself and future offspring. Research is encouraged to determine the endocrine potential of a variety of chemicals, understand their biological activity, and understand the biologic consequences during early development, the reproductive period and later life. Research on the offspring of exposed women is also needed to understand the transgenerational effects of these exposures.

RESEARCH GOALS

The goals and scope of this initiative are twofold. The first is to encourage and support mechanistically based research on the health effects of endocrine disruptors at concentrations that are commonly found in the environment. Experimental work on the cellular, molecular, genetic and systemic effects of exposures are appropriate. The second area of emphasis is to examine emerging hypothesis in human populations that complement the recent findings in the laboratory and in wildlife. Emphasis should be placed on development and validation of methods to precisely measure these exposures in human populations.

MECHANISMS OF SUPPORT

This RFA will use the NIH individual research grant (R01), and First Independent Research Support and Transition Awards (FIRST/R29). The costs and project period for R01 applications submitted in response to the RFA may not exceed $100,000 (direct costs)/per year.

FUNDS AVAILABLE

This RFA is supported jointly by NIEHS and the NIH Office of Research on Women's Health. The expected number of awards is 8 to 10.

DEADLINES:

Application: January 18, 1996

INQUIRIES

A complete copy of this RFA can be found in the NIH Guide. TO GRANTS AND CONTRACTS Written and telephone inquiries concerning this RFA are encouraged. The opportunity to clarify any issues or questions from potential applicants is welcomed.

Direct inquiries to:

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Reduced Birthweight and Length in the Offspring of Females Exposed to PCDFs, PCP, and Lindane

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The objective of this study was to investigate a broad range of adverse health outcomes and their potential association to wood preservative used in daycare centers. This article focuses on reproductive effects. A sample of 221 exposed teachers was provided by the employer's liability insurers. A comparison group (n = 189) insured by the same two organizations was recruited from nonexposed daycare centers. In a face-to-face interview, job history and reproductive history of 398 female teachers were ascertained. Data on exposure were provided, including measurements on concentration of pentachlorophenol (PCP) and lindane in wood panels, and of PCP, lindane, polychlorinated dibenzo-p-dioxins and dibenzofurans in indoor air. An exposure matrix based on individual job history, independent exposure information from each center, and reproductive history was set up with regard to the vulnerable time windows for each pregnancy. Using this approach, 49 exposed and 507 nonexposed pregnancies were identified, including 32 exposed and 386 nonexposed live births. For subgroup analyses the observations were restricted to independent pregnancies, excluding multiple and consecutive births. The data were analyzed with linear regression techniques, taking confounders into account. The crude median difference between exposed and nonexposed was 175 g in birthweight and 2 cm in length. Controlling for confounders, the results show a significantly reduced birthweight (p = 0.04) and length (p = 0.02) in exposed pregnancies, even after restricting the data to independent pregnancies and pregnancies for which data could be validated from the mother's health cards. These differences were not explained by differences in gestational age, indicating that a toxic effect, which could cause small-for-date newborns, might have affected the fetus. *Key words*: birth length, birthweight, fetotoxic effects, lindane, PCDDs, PCDFs, pentachlorophenol, wood preservatives. *Environ Health Perspect* 103:1120–1125 (1995)

Wood preservatives were extensively used in former western Germany. Not only was exterior wood treated for preservation, but paneling and other interior wood structures were treated, an especially popular practice in the 1960s. Wood preservative preparations contained pentachlorophenol (PCP) and γ–hexachlorocyclohexane (HCH) as biocidal substances. Wood preservatives were also contaminated with trace amounts of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs, PCDFs), which were formed during production processes. These compounds have the potential to volatilize and become entrained in ambient air or dust particles, thus becoming available for human contact. PCDDs and PCDFs have been found in indoor air of exposed daycare centers at picogram per meter levels, with higher chlorinated congeners such as hexa-, hepta-, and octachlorodibenzo-p-dioxins and furans (HxCDD, HxCDF, HpCDD, HpCDF, OCDD, OCDF) as major contaminants (*1*).

No epidemiological study regarding reproductive effects of mixed exposure to PCP, HCH, and PCDDs and PCDFs is available so far. In a case study, 22 of 90 women with histories of multiple spontaneous abortions, unexplained infertility, menstrual disorders, or early onset of menopause were found to have elevated blood levels of PCP (>25 µg/l) and/or HCH (>100 µg/l) (*2*). In women with fertility problems, chlorinated hydrocarbons such as HCH were found in higher concentrations in follicular fluid and cervical mucus from women who remained infertile compared to women who ultimately conceived (*3*).

Regarding human exposure to PCDDs, PCDFs, and related compounds during pregnancy, only a few studies are available so far. A study on reproductive effects due to the contamination of soil with PCDDs and PCDFs in eastern Missouri (n = 386 exposed, n = 772 nonexposed) identified a nonsignificantly increased risk ratio of 1.5 for low birthweight (<2500 g) and an average reduction of 20 g for the offspring of mothers living in the vicinity of the exposed areas (*4*). Decreased birthweights were reported for pregnancies in Taiwanese women who consumed rice oil contaminated with large amounts of polychlorinated biphenyls and PCDFs (*5,6*). The difference in birthweights between eight exposed and eight nonexposed pregnancies was about 510 g. In an Austrian study on health effects in the vicinity of a copper recovery plant releasing heavy metals and PCDD/PCDF pollution, a reduction of birthweight, length, and head circumference was detected (*7*). However, only the latter achieved statistical significance in the crude analysis presented.

A reduction in birthweight has been demonstrated for HCH in mice (*8*). Embryolethality (*9–12*) and decreased fetal or gestational weight gain (*9,13*) were reported in rats and/or hamsters exposed to PCP.

Thus, whether chronic, low-level exposure causes adverse effects on human reproduction is controversial. Our hypothesis was that indoor exposure to a mixture of PCP, HCH, and PCDDs/PCDFs reduces birthweight and birth length in the offspring of mothers exposed during pregnancy.

Methods

In a cross-sectional investigation in 1987–1988, exposed employees insured under the employer's liability scheme, working in daycare centers treated with wood preservatives in the State of Hamburg and its vicinity, were invited to participate in the study. The control group, also insured under the same scheme, was directly recruited from untreated daycare centers, each of which was located close to one of the exposed daycare centers. Of those identified as exposed under the employer's liability scheme, 68% participated in the study; 62% of the workforce in daycare centers not treated with wood preservatives participated.

The study population consisted of 410 daycare workers (men and women), 210 with known exposure and 200 without. In the course of the investigation, 12 employees from the nonexposed group were found to have been exposed at a former daycare center. One of the persons with employer's liability insurance had not experienced occupational exposure to wood preservatives. Thus, the exposed group consisted of 221 persons and the nonexposed group of 189.

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Two hundred fourteen of the exposed group and 184 of the control group were women. They contributed 556 pregnancies with an individual maximum of 5 pregnancies.

A broad range of adverse health outcomes was investigated. Medical checkups with blood sampling and 24-hr urine samples and face-to-face interviews inquiring about occupational and reproductive history were undertaken. We asked the women to bring their official medical record documents (mother's health card) to validate data on gestational age, pregnancy outcome, birthweight, and length. Informed consent was obtained for all data collection procedures. Female medical students who had undergone interview training conducted the interviews.

In addition, individual occupational history and lifestyle exposure were ascertained: occupational conditions (six time periods), smoking (three periods of active smoking, four periods of nonsmoking), alcohol consumption (three periods of higher consumption), exposure to wood preservatives in private or weekend homes (three or five periods, respectively, subject's information supported by product and brand names). For each pregnancy, information on the outcome and its date, gestational age, parity, complications, desire for a child (woman/partner), malformations, and birthweight and length was requested, providing a full reproductive history of up to five pregnancies for all women. Age of the mother at conception and parity were computed using these data.

Measurements from the wood paneling and of the indoor air were conducted independently of this study. In 1986 the Federal State of Hamburg initiated a screening program to detect wood preservative exposure in all daycare centers in Hamburg. In facilities with a PCP concentration in the wood exceeding 100 ppm, indoor air measurements were conducted, or, if only few wood panels existed (area in m^2/indoor volume in m^3 <0.2), the paneling was removed (*14*). No such program existed outside Hamburg. However, communities and church-owned daycare centers on the border of Hamburg also followed this approach. In our sample, PCP concentrations in the wood >100 ppm had been measured in 24 facilities. The control group was working in 35 nonexposed facilities. Not all indoor air measurements were complete for all three components (PCP, HCH, and PCDDs, and PCDFs). In the exposed group the median concentration of PCDDs/PCDFs in toxic equivalency factors for 2,3,7,8-TCDD (TEFs), used by the Federal Health Office in Germany, was 0.5 pg/m^3. The PCP concentration was about 0.25 µg/m^3, and HCH was about 0.2 µg/m3 (median). The PCDD/PCDF indoor concentration and the PCP concentration in wood were correlated (Spearman $r = 0.48$, $n = 19$, $p = 0.039$), but not indoor PCDD/PCDF and HCH in the wood paneling (Spearman $r = -0.31$, $n = 13$, $p = 0.28$). However, to avoid assumptions about indoor air pollution, a facility was defined in this study as exposed when the wood paneling showed PCP concentrations higher than 100 ppm.

For single pregnancies, exposure to wood preservatives was checked for each time window (uncertainty ±1 month). A pregnancy was defined as being exposed if the employee worked in any of the 24 exposed facilities at any time during her pregnancy.

In a similar way, the values of potential confounders were addressed. Active smoking, alcohol consumption, and exposure to wood preservatives in private homes was checked according to personal history data for each time window of the pregnancy (±1 month). All pregnancies before or after such risk periods were defined as nonexposed with regard to wood preservatives or the potential confounders. Although some indoor air measurements were available for most occupational facilities, exposure to wood preservatives in daycare centers and in private homes was dichotomized (exposure versus nonexposure). Extrapolation from the measurements in 1986 and 1987, consideration of the duration of exposure in the respective time window (either in months or in weekly working hours), or their combination was assumed to provide less valid information.

For women who smoked during pregnancy, the months of smoking, not the number of cigarettes, were taken into account because the reported number was thought to be less reliable due to social pressures against smoking. For active smokers, the period of smoking in relation to gestational age was computed. The "usual" amount of alcohol consumed was determined by using the frequency of consumption (daily, three to four times a week, one to two times a week, about once a month, never) and the amount of wine/beer or liquor consumed (0.02–0.5 l) and its respective concentration of alcohol. This same amount was used for past alcohol consumption. For periods in which a woman said she drank more, the amount was doubled.

Age of the mother, parity, and gestational age (according to the mother's health card or comparable documents) were controlled for in the analyses. The increase in weight during pregnancy is not linear. The squared values of gestational age show a more appropriate relation and were taken into consideration when evaluating birthweight.

Exposure effects were adjusted for height and weight of the mothers. For both variables measurements taken during 1987–1988 were used, since no information was available on these measurements before pregnancy.

The analyses were conducted using SAS software (SAS Institute, Cary, North Carolina) and included descriptive information and results of multiple regression analyses on birthweight and birth length (*15,16*). The multivariate normal distribution of the two outcome variables in models, including all predictors, their homoscedasticity, and collinearity of the predictors, was checked.

The 556 observations (pregnancies) contributed by 398 are not independent. Thus, a subgroup including the first exposed pregnancy for women who had at least one exposed pregnancy and the first pregnancy for women who only had unexposed pregnancies, excluding twins, was also analyzed. To take occupational status also into account, regression analyses were conducted in six groups: 1) the total group of observations excluding twins: two outliers in the distribution of birthweights (>6200 g) had to be eliminated to achieve a multivariate normal distribution and one outlier in birth length smaller than 34 cm had to be eliminated; 2) a subgroup in which the weight and length could be validated according to official medical documents (mother's health card); 3) a subgroup consisting of all pregnancies during which the mother was employed for at least 1 month of the pregnancy; 4) a subgroup based on the total group with restriction to first exposed and first nonexposed pregnancy; 5) a subgroup formed by including only those pregnancies of group 2 and group 4; 6) a subgroup formed by including only those pregnancies of group 3 and group 4.

The final model, with all confounders that do not disturb the exposure–weight or exposure–length relation eliminated (*17*), is presented for the total group (group 1). Regarding the results of the other subgroups, only the exposure effects controlling for all other confounders are presented.

The two outliers in the analysis of birthweights and the one outlier in birth lengths were excluded for statistical reasons only (no normal distribution of the residuals). Other than the fact that these birthweights (>6200 g) could not be validated from the mother's health card, there is no medical justification to exclude these two outliers (see Fig. 2). Thus, the analysis was repeated including these outliers with a

correction by transforming weights according to Blom (*18*). The newborn with the birth length of 27 cm (see Fig. 2), however, came from a 22-year-old mother with a severe atopic skin disease, who was under treatment during pregnancy. Thus we did not include this case in the analysis.

Results

Overall, only 49 of 556 pregnancies occurred in the time window of occupational exposure to wood preservatives (Table 1). Thirty-two exposed and 386 nonexposed pregnancies were carried to full term and resulted in live birth. Restrictions on single first exposed and first unexposed pregnancies reduced the number of exposed pregnancies to 32. Of these, only 27 were first-exposed live births (Table 1). Exposed pregnancies ended more often as induced abortions, spontaneous abortions, or as cesarean sections (Table 1).

Two births with twins (2 of 32, 6.3%) led to a higher prevalence of twins in the exposed pregnancies than in the nonexposed. Complications during pregnancies were more frequent in the nonexposed group. Validation of pregnancy data according to medical documents (mother's health card) was possible in more than 80% of exposed and only in about 50% of nonexposed pregnancies (Table 1). This fact is explained by the introduction of the mother's health card in 1969–1970 and its coincidence with popular use of wood preservatives after 1970.

Table 2 shows the rank and outcome of exposed pregnancies which resulted in live births. The 32 live births originated from 29 women. Three women each had two exposed live births. Nine women had both exposed and nonexposed children. Of these, the single first exposed or first nonexposed was taken into consideration for the subgroup of independent observations.

Birthweights were reduced by about 150 g in exposed pregnancies taking all observations into account, as well as those restricted to the first exposed pregnancies and the pregnancies for which data could be validated from the mother's health card (Table 3). In exposed pregnancies, the babies were also shorter (50 cm in comparison to 52 cm in nonexposed; Table 3). Figure 1 shows that the reduction in birthweights cannot be attributed to single outliers, but is due to a general shift in the distribution of the weights. From Figure 2, it is obvious that weight and length co-scatter. Only one observation (length of 27 cm) seems to be outlying.

Potential confounders were not equally distributed among exposed and nonex-

Table 1. Outcome of pregnancies of women exposed to wood preservatives and of women not exposed: all and first pregnancies

Outcome	All exposed ($n = 49$)	All nonexposed ($n = 507$)	First exposed ($n = 32$)	First nonexposed ($n = 256$)
Induced abortion (%)	20.4	14.4	25.0	14.8
Miscarriage (%)	14.3	8.7	18.8	7.0
Stillbirth (%)	0	0.8	0	0.8
Cesarean section (%)	14.3	5.7	12.5	6.3
Regular birth (%)	51.0	70.4	43.8	71.1
	Last two outcomes, exposed ($n = 32$)	Last two outcomes, nonexposed ($n = 386$)[a]	First exposed with live births ($n = 27$)	First nonexposed with live birth ($n = 231$)
Complications during pregnancy (%)	15.6	20.8	14.8	21.7
Twins (%)	6.3	1.0	0	0
Gender: male[b]	46.9	50.0	48.2	49.4
Verified with mother's health card (%)	87.5	50.3	85.2	53.7

[a]Includes four pregnancies for which data on birthweight and length are missing.
[b]Gender data are missing for 0.8% of all nonexposed pregancies and for 0.4% of first nonexposed pregancies.

Table 2. Exposed pregnancies with outcome and rank

No. of exposed live births	Frequency	Pregnancy rank					Exposure during pregnancy to wood preservatives				
		1	2	3	4	5	1	2	3	4	5
1	1	IA	IA	LB			No	Yes	Yes		
1	1	IA	IA	Twins			No	Yes	Yes		
1	1	IA	IA	LB	SA		Yes	Yes	Yes	No	
1	1	IA	CS				No	Yes			
1	1	IA	LB				No	Yes			
1	1	SB	SA	SA	CS	IA	No	Yes	No	Yes	No
1	1	SA	LB	LB	LB	IA	No	Yes	No	No	Yes
1	2	CS					Yes				
1	1	CS (twins)					Yes				
1	1	CS	SA	SA	LB		No	No	Yes	Yes	
1	1	CS	SA	CS	CS		No	Yes	Yes	No	
1	8	LB					Yes				
1	1	LB	IA				Yes	No			
1	1	LB	SA	SA	LB		No	Yes	Yes	Yes	
1	1	LB	SA	LB			No	Yes	Yes		
1	1	LB	LB				No	Yes			
1	1	LB	LB				Yes	No			
1	1	LB	LB	LB			No	No	Yes		
2	1	IA	LB	IA	LB		Yes	Yes	Yes	Yes	
2	1	IA	LB	CS	LB		No	No	Yes	Yes	
2	1	LB	LB				Yes	Yes			

Abbreviations: IA, induced abortion; SA, spontaneous abortion; LB, live birth; CS, cesarean section.

Table 3. Median and 5th and 95th percentiles of birthweight and birth length for offspring of women exposed to wood preservatives and of women not exposed, with and without verification by the mother's health card

Outcome		All exposed	All nonexposed	First exposed	First nonexposed
Without verification					
Birthweight (g)	*n*	32	382	27	230
	Median	3175	3350	3200	3345
	5th/95th	2430/3910	2400/4500	2780/3910	2450/4500
Birth length (cm)	*n*	31	379	26	229
	Median	50	52	50.5	52
	5th/95th	47/53	47/57	48/53	47/57
With verification					
Birthweight (g)	*n*	28	192	23	123
	Median	3115	3350	3060	3370
	5th/95th	2430/3870	2570/4200	2780/3740	2570/4150
Birth length (cm)	*n*	50	52	50.0	52
	5th/95th	47/53	47/56	48/53	48/56

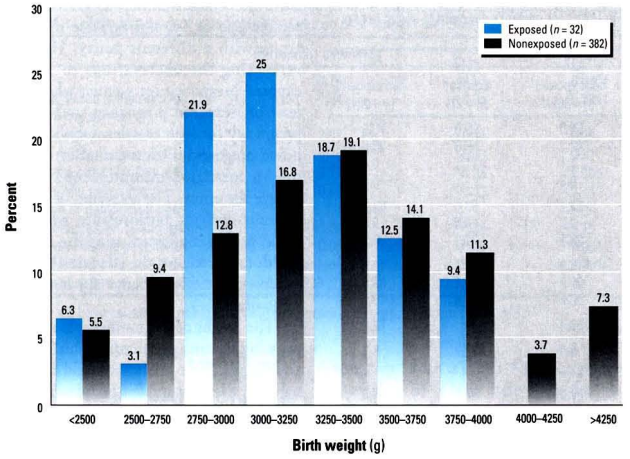

Figure 1. Birthweights for exposed and nonexposed pregnancies. Numbers above bars are actual percentages.

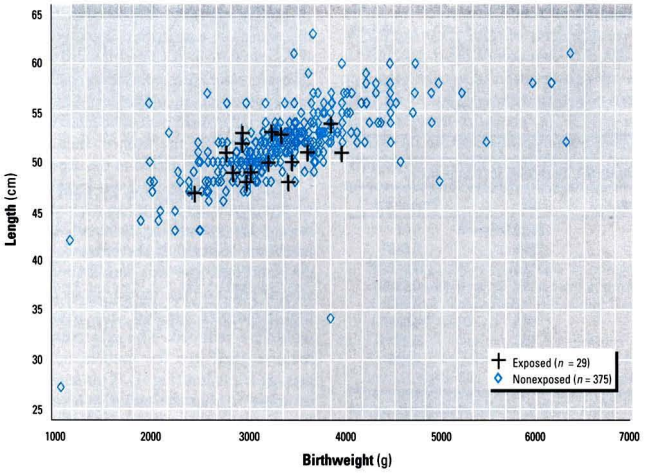

Figure 2. Scatterplot of birthweight and size, exposed and nonexposed pregnancies. Spearman rank correlation of weight and length: nonexposed, $r = 0.70$, $p = 0.001$; exposed, $r = 0.52$, $p = 0.004$.

posed pregnancies (Table 4). Women who were exposed during pregnancy tended to be older and to have a higher parity. There were only minor differences for smoking during pregnancy and for alcohol consumption. All exposed pregnancies occurred in employed women. Exposure to wood preservatives in private homes occurred more frequently for exposed pregnancies. Also, the desire for a child was more prevalent in exposed pregnancies.

For the group including all observations with live births, we estimate that a given exposed birth could have been on average 217 g heavier if it had not been exposed, controlling for necessary confounders (Table 5). The effect was even stronger when the analysis was restricted to observations which were validated by the mother's health card (-259 g; Table 6) or to observations which presented the first exposed or nonexposed pregnancies (-303 g; Table 6). The association is weaker and does not gain statistical significance in the subgroup of women who were employed during pregnancy when two outliers are included ($p = 0.078$).

The birth length of exposed children was statistically significantly reduced in exposed pregnancies (-1.34 cm, Table 7). This reduction is of nearly equal length in all subgroups (Table 7). However, the effect does not gain statistical significance in three of the five subgroups.

Discussion

Recruitment of the exposed group was based on information provided by the employer's liability insurers. The control group, also insured under the same scheme, had to be approached in a different way. To reduce potential differences, we asked nonexposed daycare centers in the vicinity of the exposed facilities to participate. The proportion of participation was lower in the reference group (62%) compared to the exposed group (68%). However, there are no hints of selection biases with regard to educational level, smoking, and total number of children (data not displayed). Also a restriction to pregnancies of female teachers of the exposed daycare centers did not change the results (birthweight: -272.5 g, $p = 0.02$, $n = 224$; birth length: -1.37 cm, $p = 0.039$, $n = 221$).

The sampling of the daycare centers and the ascertainment of indoor exposure was performed without knowledge of birthweights and lengths. Birthweights and lengths, however, were ascertained with some knowledge of the exposure and could therefore be biased. Birth data such as weight, length, gestational age, complication during pregnancy, and sex of child was, for the majority of observations (85% of the exposed, 54% of the nonexposed; Table 1), validated from medical notes documented in the mother's health card. In the subgroup with validated information, the results are not dependent on the recall of the mother. This subgroup includes only pregnancies which occurred between 1969 and 1987. The findings did not change when the analysis was restricted to this subgroup (Table 7).

Recall, however, might affect the information on smoking and alcohol consumption. Nevertheless, there is no reason to suspect that a misclassification of these confounders is related to exposure (Table 4).

The reduction in birth length compared to birthweight is less impressive in the five subgroups (Table 7). However, it should be borne in mind that the measurement of the length of a newborn is more dependent on individual techniques than weight is. Consequently, measurement errors are more likely with birth length, and thus the chance of detecting an effect for this outcome is smaller.

In the total group, the effect of exposure could be due to few exposed mothers

Table 4. Distribution of potential confounders (percentages) in births for mothers exposed to wood preservatives and nonexposed (excluding twins)

Confounder		All exposed pregnancies (n = 32)	All nonexposed (n = 382)	First exposed (n = 27)	First nonexposed (n = 231)
Age at conception (years)	<25	25.0	56.5	29.6	70.6
	25–35	59.4	42.2	59.3	28.6
	>35	15.6	1.1	11.1	0.4
	Unknown		0.3		0.4
Smoking during pregnancy	No	65.6	69.1	63.0	67.1
	≤3 months	12.5	2.6	14.8	2.6
	>3 months	21.9	28.3	22.2	30.3
Consumption of alcoholic beverages	No	12.5	14.9	11.1	12.6
	≤12 g/day	71.9	64.9	74.1	68.0
	>12 g/day	15.6	20.2	14.8	19.5
Employed		100	63.4	100	72.7
Wood preservatives in private homes		21.9	5.0	22.2	6.1
Desire for a child	Woman	93.8	87.6 (1 unknown)	92.6	85.3 (1 unknown)
	Partner	87.5	87.6 (9 unknown)	88.9	86.2 (4 unknown)
Parity: nullipara		65.6	59.2	70.4	97.6
Gestational age	Median	9.2 months	9.2 months	9.2 months	9.2 months
	5th/95th percentile	8.3/9.4	8.3/9.7	8.7/9.4	8.3/9.7
Height of mother	Median	166.4 cm	164.1 cm	166.4 cm	164.4 cm
	5th/95th percentile	157/184	151/177	157/184	151/176
Weight of the mother at examination	Median	66.2 kg	61.4 kg	61.9 kg	65.9 kg
	5th/95th percentile	51.5/85.7	50.0/84.4	50.0/84.4	50.5/85.0

Table 5. Effect of the exposure of wood preservatives on birthweight, controlling for confounders[a]

Predictors	Parameter estimates	SE	Probability
Intercept	127.7	340.3	0.71
Exposure to wood preservatives	-217.1	105.8	0.0409
in daycare centers (with two outliers included)	(-230.3)	(119.4)	(0.0423)[b]
Gestational age * gestational age (months^2)	33.1	3.6	0.0001
Age at conception (years)	10.6	6.3	0.092
Gender of the child (f = 1, m = 2)	180.1	52.5	0.0007
Complications during pregnancy	-61.4	66.3	0.36
Explained variance: R^2 = 21.5	F-value: 21.66	df = 5	0.0001
Probability of a normal distribution of the residual: 0.25			

[a] n = 402, n = 10 missing variables in some predictors, two outliers >6200 g are excluded, except where indicated.
[b] Based on Blom-transformed values to achieve a multivariate normal distribution: $y_i = \Psi(r_i - 3/8)/(n + 1/4)$, with Ψ = inverse cumulative normal (Probit) function, r_i = rank, and n = number of nonmissing observations (*18*).

with more than one baby. Due to the small sample size, it was not possible to analyze strata with a different parity. However, restricting the observations to the first exposed pregnancy for women who had at least one exposed pregnancy, and the first pregnancy for women who only had unexposed pregnancies (thus excluding an effect due to correlated measurements) did not reduce the effect. The inclusion of two statistically outlying birthweights, which also could not be confirmed using the mother's health card, reduced the effect in three subgroups, but did not change the results substantially.

The height of the mother was a statistically significant predictor of birthweight and length. This predictor could be eliminated in the final models (Tables 5 and 6). Height of the mother is, however, included in the comparisons in Table 7. An effect could also be seen for the woman's weight, which was ascertained at the examination, not before the pregnancy. Thus, this variable is only a substitute. Height and weight of the fathers could not be controlled for due to lack of information. However, there is no reason to assume that the distribution of these potential confounders is different in exposed and nonexposed pregnancies. Additionally, there is no evidence that diseases suffered by the women, such as diabetes mellitus, are related to the exposure.

The effect of exposure was independent of gestational age. Thus, an adverse effect on the fetus resulting in small-for-date newborns is likely. If the effect depended on gestational age, this would support an adverse effect on the mother in such a way that gestational age and thus birthweight would be reduced. The effect does not manifest itself in a few outliers, but in an average reduction (Fig. 1). This stresses the assumption that the majority of exposed pregnancies were affected, not just a few sensitive ones.

One limitation of this study is the absence of an indicator of the body burden,

Table 6. Comparison of the effects of wood preservatives on birthweight and length in the five subgroups controlling for all potential confounders

Subgroup	Birthweight n	Birthweight Parameter estimate	Birthweight SE	Birthweight Probability	Birth length n	Birth length Parameter Estimate	Birth length SE	Birth length Probability
Data validated from mother's health card[a]	216	-259.2	104.8	0.0142	215	-1.22	0.54	0.0242
Women employed during pregnancy	265	-220.2	110.2	0.0468	263	-1.12	0.65	0.0865
(with two outliers included)	(267)	-191.6	123.3	(0.078)[b]				
First exposed or nonexposed pregnancy	253	-303.2	119.2	0.0142	252	-1.34	0.67	0.0477
(with two outliers included)	(255)	(-286.7)	(134.2)	(0.018)[b]				
Data validated from mother's health card[a]	145	-317.0	121.2	0.0099	144	-1.09	0.61	0.0764
Woman employed during pregnancy	187	-310.9	126.5	0.015	187	-1.44	0.75	0.0542
(with two outliers included)	(189)	(-277.2)	(148.0)	(0.031)[b]				

[a] The weights of the two outliers were not confirmed in the mother's health card.
[b] Based on Blom-transformed values in order to achieve a multivariate normal distribution: $y_i = \Psi(r_i - 3/8)/(n + 1/4)$, with Ψ = inverse cumulative normal (Probit) function, r_i = rank, and n = number of nonmissing observations (*18*).

Table 7. Effect of the exposure of wood preservatives on birth length controlling for confounders[a]

Predictors	Parameter estimates	SE	Probability
Intercept	32.14	3.37	0.0001
Exposure to wood preservatives in daycare centers	-1.34	0.35	0.0201
Gestational age (months)	2.02	0.35	0.001
Age of the mother (years)	0.03	0.03	0.31
Gender of the child ($f = 1$, $m = 2$)	0.79	0.28	0.0052
Smoking during pregnancy (proportion of gestational age)	-0.49	0.33	0.14
Alcohol consumption during pregnancy	-0.34	0.24	0.15
Complications during pregnancy	-0.66	0.36	0.0643
Explained variance: $R^2 = 12.2\%$	F-value: 7.86	df = 7	0.0001
Probability of a normal distribution of the residual: 0.19			

[a] $n = 401$, $n = 8$ have missing variables in some predictors, one outlier <34 cm is ignored.

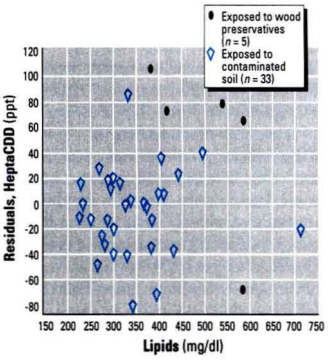

Figure 3. Residuals of the blood concentration of heptaCDD per lipid basis (ppt) not explained by age and the Abdel-Malek Index (*22*). Residuals = actual values – predicted values; predicted heptaCDD = age * 2.15 - Abel-Malek Index * 0.10; Abel-Malek Index = $f_s * 10^6 * (\text{weight})^{1.2} * (\text{height})^{3.3}$; $f_s = 3$ for women, 4 for man (*14*); lipids = sum of cholesterol and triglycerides. Explained variance: $R^2 = 33.1\%$.

since no individual measurements of PCDDs/PCDFs, PCP, or HCH during or after pregnancy are available. One reason for this is that the pregnancies took place before exposure and potential health effect had been determined. However, measurements of PCP in children from daycare centers exposed to wood preservative before and after removing the wood paneling show a clear reduction of PCP in urine samples from about 17 µg/l to 4 µg/l (*19*). Additionally, few measurements of PCDD/PCDF in fat samples exist from persons exposed to wood preservatives. The comparison of five findings, which were ascertained by ERGO Forschungsgesellschaft in Hamburg with 33 measurements in an adult group exposed to contaminated soil (*20*) ascertained by the same laboratory, does not reveal a significant increase in TEFs (*21*) but significantly higher values for heptaCDD, a congener which is typical for wood preservatives (Fig. 3). Thus, these additional findings support the assumption that indoor exposure could have increased the body burden of PCP and PCDDs/ PCDFs.

The detrimental effect, however, cannot be attributed to one single group of substances but to three groups: PCP, HCH, or PCDDs/PCDFs. Our findings of an adverse effect seem to confirm previous findings on these compounds (*12–13*). In summary, the results of this study stress the need for future investigation of the effects of wood preservatives and of PCDDs and PCDFs on the development of the fetus, as reduced birthweight is a childhood risk factor for a range of adverse health effects.

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Pesticides in Household Dust and Soil: Exposure Pathways for Children of Agricultural Families

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Children of agricultural families are likely to be exposed to agricultural chemicals, even if they are not involved in farm activities. This study was designed to determine whether such children are exposed to higher levels of pesticides than children whose parents are not involved in agriculture and whose homes are not close to farms. Household dust and soil samples were collected in children's play areas from 59 residences in eastern Washington State (26 farming, 22 farmworker, and 11 nonfarming families). The majority of the farm families lived within 200 feet of an operating apple or pear orchard, whereas all reference homes were located at least a quarter of a mile from an orchard. Four organophosphorous (OP) insecticides commonly used on tree fruit were targeted for analysis: azinphosmethyl, chlorpyrifos, parathion, and phosmet. Samples were extracted and analyzed by gas chromatography/mass selective detection. Pesticide concentrations in household dust were significantly higher than in soil for all groups. OP levels for farmer/farmworker families ranged from nondetectable to 930 ng/g in soil (0.93 ppm) and from nondetectable to 17,000 ng/g in dust (17 ppm); all four OP compounds were found in 62% of household dust samples, and two-thirds of the farm homes contained at least one OP above 1000 ng/g. Residues were found less frequently in reference homes, and all levels were below 1000 ng/g. Household dust concentrations for all four target compounds were significantly lower in reference homes when compared to farmer/farmworker homes (Mann-Whitney U test; $p < 0.05$). These results demonstrate that children of agricultural families have a higher potential for exposure to OP pesticides than children of nonfarm families in this region. Measureable residues of a toxicity I compound registered exclusively for agricultural use (azinphosmethyl) were found in household dust samples from all study homes, suggesting that low-level exposure to such chemicals occurs throughout the region. Children's total and cumulative exposure to this pesticide class from household dust, soil, and other sources warrants further investigation. *Key words*: agriculture, azinphosmethyl, children, chlorpyrifos, household dust, insecticides, organophosphates, parathion, pesticides, phosmet, soil. *Environ Health Perspect* 103:1126–1134 (1995)

Concern about residential pesticide exposures among children has increased recently with the reported associations between residential pesticide use and childhood leukemia (*1–3*). Substantial research has focused on pesticide exposure after indoor and lawn applications (*4–8*), and a recent study demonstrated that individuals who contact treated indoor surfaces can absorb measurable amounts of the compound through the skin (*9*). In cases of residential misapplication, exposures have resulted in pesticide-related illnesses (*10,11*). Studies designed to characterize children's exposure to pesticides in the general population indicate that the largest number of pesticides and the highest concentrations are found in household dust compared to air, soil, and food (*12,13*). However, few of these studies have been conducted in or near agricultural regions, where one might expect relatively higher exposures for residents due to both residential and agricultural pesticide use.

Children of farmers and agricultural field workers are likely to have a high potential for pesticide exposure, even if they are not involved in farm activities related to exposure. Pesticide exposure could occur from a number of sources such as contaminated soil, dust, work clothing, water, and food, or through drift, the deposition of a pesticide off target. In many agricultural communities, residential home sites are close to or surrounded by fields or orchards. Pesticides can be tracked into the home on shoes or by pets and become part of a household dust "reservoir." Pesticide residues in indoor environments are not subject to degradative environmental processes such as sun, rain, and soil microbial activity, and may thus persist longer in the house than in outdoor soil.

Household dust and yard soil are considered significant sources of exposure to pesticide residues and other toxicants for small children and toddlers (*13*). Young children spend a large portion of their time on the floor or ground and can easily come in direct contact with yard soil or dust by putting hands and objects in their mouths frequently and thereby ingesting soil or dust. Studies using tracer elements to quantify soil ingestion have estimated that children in the United States can ingest from 10 to 1300 mg of soil/day; in children with a pica history the level can reach 5000 mg/day (*14–17*). EPA investigators estimated the potential health risks to children for the soil and dust pathway to be 12 times that of adults (*18*).

Government reporting of pesticide poisoning cases is one indicator of the hazards or risks associated with pesticide use on the farm or in the home. In 1991, 39% of pesticide incidents reported to all agencies in Washington State were agriculturally related (*19*). One case that demonstrates the potentially serious nature of post-application exposures involved a 20-month-old child who developed acute poisoning from ingesting ethyl parathion-contaminated soil. However, present reporting data do not allow assessment of the overall prevalence or severity of chronic exposures to pesticides for children in agricultural settings. Reliance on such statistics is limited by at least three factors: 1) reported cases generally involve only acute intoxications (subacute or chronic effects are likely to remain unreported), 2) even acute cases may not be recognized or reported consistently by physicians as pesticide related, and 3) cases tend to provide little information for exposure mitigation. Thus, properly focused environmental sampling represents a more reliable and preventive approach for investigating public health concerns related to children's exposure to pesticides in agricultural and residential settings.

Organochlorine and arsenical compounds were the first pesticide classes studied in the home environment, due primarily to their widespread use, persistence, and chronic health effects (*20–23*). However, during the past 20 years there has been a dramatic increase in the use of less persis-

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tent but more acutely toxic organophosphorus (OP) pesticides. Acute effects of OP exposure are well known, but chronic effects are not well characterized, and available information pertains primarily to adults (*24–28*). Thus, major gaps exist in our knowledge of the health effects of chronic pesticide exposure in children (*29*). No published studies have examined the neurotoxic effects of low-level pesticide exposure to children.

The primary objective of this study was to evaluate the potential for chronic exposures of children to pesticides in and around the homes of farmers and agricultural workers. The study had two specific aims: to determine to what extent household dust and surface soil from children's play areas contain agricultural pesticides, and to determine if children of agricultural families live in homes that contain higher levels of pesticides than homes of nonfarm children. An attempt was also made to identify risk factors for elevated residential pesticide levels in the study population.

Methods

Study design. This study employed a cross-sectional environmental sampling strategy during the 1992 pesticide spray season. Targeted residences were those of agricultural families, including both farmers and nonseasonal farmworkers, and nonagricultural reference families. Sampling goals were to collect household dust using a vacuum sampler from carpeted entryways and indoor play areas and to collect surface soil from outdoor play areas at each residence. The greater Wenatchee area in eastern Washington State was chosen for study because its residents are engaged predominantly in agricultural production of tree fruits, including apples, pears, and cherries.

Four OP pesticides commonly used during the spray season were targeted for analysis: azinphosmethyl [*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl)-phosphorodithioate (CAS no. 86-50-0)], phosmet [*N*-(mercaptomethyl)-phthalimide *S*-(*O,O*-dimethylphosphorodithioate (CAS no. 732-11-6)], chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (CAS no. 2921-88-2)], and ethyl parathion [*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate (CAS no. 56-38-2)]. These pesticides were identified as the most commonly used OPs for apple production. Parathion registration was canceled for use in orchards in 1991 by the U.S. Environmental Protection Agency due to its high acute toxicity and the frequency of reported poisonings nationwide, but continued use of existing stock was allowed through the 1992 spray season.

Recruitment. Participating families were recruited from Chelan and Douglas counties with the assistance of several commercial and social service organizations. Service organizations recruited farmworkers by mailing letters to their members that described the study and asked interested families to contact the organization or the university directly. Reference families were also recruited using these procedures; several of the reference families included an employee of the service organizations. Farmers were sent a similar letter through the Washington Growers Clearinghouse Association. When a positive response was received, the family was contacted by phone and screened for eligibility. All procedures involving human subjects were reviewed and were approved by the University of Washington Human Subjects Review Committee before the study began.

Farmer and farmworker family selection was based on the following eligibility criteria: at least one child between the ages of 1 and 6 years and at least one family member living in the home employed as an orchardist, fieldworker, and/or pesticide applicator. Reference family eligibility factors were: no family member working in the farm industry, no family member having direct contact with agricultural pesticides, and the residence situated more than one-quarter mile from a commercial orchard or crop. Although most farmer and reference families were of Caucasian background, the majority of farmworkers were Hispanic.

Soil sampling and analysis. Participating families were asked to identify their children's outdoor play areas, including sandboxes, front and back lawns, and driveways. Five locations within these designated play areas were chosen for sampling. A 26 cm × 26 cm template was placed on the ground, and the top 0.5–1 cm soil layer was scraped with the edge of a 5-inch stainless-steel spatula. The five samples were composited for each home, transported on dry ice, and stored at -20°C. Samples were analyzed within 12 months of collection.

Samples were thawed to room temperature and sieved through a 425-µm stainless mesh to remove large nonsoil debris. Wet samples were dried in a desiccator for 5–16 hr. A portion of each sieved sample was submitted to the University of Washington Forest Research Laboratory for determination of moisture content. All samples contained <10% moisture at the time of extraction.

A sonication method was adapted from Nigg (*30*) and is described in detail elsewhere (*31*). Five-gram soil samples were pre-wet with 400 µl distilled water and refrigerated at 4°C for 15–18 hr. We added 50 ml acetone and sonicated the soil at 20 kHz for 1 min in an ultrasonic processor with a 0.5-inch tapped horn (Heat Systems-Ultrasonics, Inc., Farmingdale, New York). The clear supernatants were separated from soil solids and evaporated to near dryness under a purified nitrogen stream and then partitioned between hexane (2 ml) and water (40 ml). The hexane layer was separated and dried over anhydrous sodium sulfate.

We prepared standard OP solutions at 1 mg/ml of each analyte in acetone using neat materials (≥98% purity) purchased from Chem Service (West Chester, Pennsylvania). Further dilutions were made in hexane to prepare OP calibrant solutions. We used 1 ng/ml tributylphosphate as a GC internal standard in all samples. Quantification of the target OPs was performed by GC/mass selective detection (MSD), in selected ion monitoring mode using a Hewlett-Packard gas chromatograph 5890A series II equipped with 5971 mass selective detector and a 15-m × 0.25-mm i.d. J&W capillary column with 0.25 µm DB-1701 bonded phase. Selected ions were acquired for each analyte; two confirmation masses, and one mass (typically the most abundant in that compound's electron impact mass spectrum) for quantitation.

We determined the analytical limit of detection (LOD) by running analytical standards in solvent (no matrix effect). The method limit of quantitation (MLOQ) was determined by running analytical standards in a soil extract (matrix effect). Relative ion intensities and simultaneity were used to confirm each positive detection. Samples with quantitation ion response, but without qualifier ion response were defined as having concentrations below the MLOQ. Samples with no ion response were designated as below the limit of detection. These limits are specified in Table 1. In most cases the LOD and MLOQ were similar. Extraction of the OP compounds from soil was virtually complete, with extraction efficiencies ranging from 90% to 110%. Final OP concentration results were reported as nanograms of pesticide per gram soil, without correcting for the minimal moisture content of the soil.

Household dust sampling and analysis. Household dust was collected using the high-volume, small-surface sampler (HVS-3; Cascade Stamp Sampling Systems, Bend, Oregon) from two carpeted or rug-covered areas in each home: 1) 3 ft inside the main entryway, and 2) in an area where children commonly played. The HVS-3 is a cyclone-equipped vacuum sampler developed for U.S. EPA, which collects small

Table 1. Instrument limits of detection (LOD), method limits of quantitation (MLOQ), and extraction efficiencies for analysis of targeted organophosphorus insecticides in soil and household dust by GC/mass selective detector[a]

	LOD[b] (ng/ml)		MLOQ[c] (ng/g)		Extraction efficiency (%)[d]	
Insecticide	Soil	Dust	Soil	Dust	Soil	Dust
Azinphosmethyl	11	16	32	40	90 (10)	77 (17)
Chlorpyrifos	13	20	11	17	92 (9)	72 (14)
Phosmet	11	10	7	12	98 (11)	73 (8)
Ethyl parathion	13	16	34	11	110 (14)	106 (20)

[a]Instrument type: HP 5890A series II, with mass spectrum detector, in selected ion mode.
[b]Instrument LOD determined with analytical standards in solvent (no matrix effect); determined separately under instrument conditions used for analyzing soil and conditions for dust.
[c]MLOQ determined by spiking soil or dust extracts to account for matrix effects.
[d]Values are means with SDs in parentheses. For soil, $n = 12$: six samples fortified with organophosphorus mix at 100 ng/g soil and six samples at 500 ng/g soil. For dust, $n = 7$: four samples fortified with organophosphorus mix at 250 ng/g dust and three samples at 650 ng/g dust.

particles (>5 µm) in a teflon catch bottle (*32*). A measured area on the rug or carpet was sampled according to standard procedures described in the HVS-3 operation manual, with a target sample weight of 5 g. Samples were transported on dry ice and stored at -20°C and analyzed within 12 months of collection.

Samples were sieved through a 150-µm stainless mesh to remove large nondust debris, hair, and carpet fibers, and to yield the smaller-diameter particles shown to adhere more readily to the hands (*33*). Analyzing solvent-extracted dust proved to be much more difficult than analyzing soil, due in part to analytical interference by waxy substances and other organic components of the dust. Procedures used for dust were modifications of those described above for soil, with the addition of a filtration step and a gel permeation chromatography (GPC) clean-up procedure before GC/MSD analysis.

We pooled the two sieved dust samples from each house and sonicated 2.5 g portions in 50 ml of acetone for 1 min. Acetone extracts were concentrated under a purified nitrogen stream, solvent exchanged into cyclohexane, and filtered through 0.45-µm polytetrafluoroethylene membrane filters (Gelman Sciences, Ann Arbor, Michigan) to remove fine dust particles and precipitate. The resultant 1.5 ml cyclohexane extracts were applied to a 20-cm × 2-cm i.d. GPC column (Bio-Beads S-X3, Bio-Rad Laboratories, Richmond, California) and eluted with cyclohexane. After discarding an initial volume of 48–52 ml (depending on column), 230 ml of eluant was collected, concentrated using Kuderna-Danish flasks with Snyder columns over a hot water bath, and evaporated to 2 ml under a purified nitrogen stream. The analysis of target OPs in household dust was performed by GC/MS as described for soil, again using tributylphosphate as an internal standard and with standard OP calibrant solutions diluted in cyclohexane. The LOD and MLOQ concentrations for dust were similar and did not differ greatly from those for soil, as indicated in Table 1. Extraction of ethyl parathion from dust was complete, but for the other three OP compounds extraction efficiencies ranged from 72% to 77%. Final OP concentrations were adjusted by these values.

Quality assurance. Blank samples were prepared from solvent-rinsed laboratory-grade sand, carried into the field on each day of sampling, and processed along with the field samples. No targeted analytes were detected in the 19 field blanks (16% of field samples). Field spike samples were prepared by spiking the same sand with the target OP compounds. Samples were carried into the field on each day of sampling and processed with field samples. Results were inconsistent, ranging from 15% to 83% recovery of target analytes. Sand was used in the absence of a standard "clean" dust or soil medium at the time of the field study. It is unclear whether results from the spiked sand samples are due to pesticide instability in storage or use of this particular spiking medium. As there was doubt that sand was a representative matrix, field sample results were not adjusted by field spike recoveries. Further work on storage stability of these types of samples is needed. Reagent blanks were included during the extraction and analysis procedures; no targeted analytes were detected ($n = 3$, or 2.5% of field samples).

Participant interviews. Participants were asked about occupational pesticide use, frequency of both residential and agricultural pesticide use in and around the home during the past 6 months, and proximity of their homes to orchards. Pesticide registration numbers were collected whenever possible for verifying the active ingredient for home pesticide products. Family members who reported applying pesticides were asked about their personal protective equipment use and laundering of work clothes. Additional questions gathered information about vacuuming frequency, number of days since last vacuum cleaning, routine removal of shoes at the door, use of door mats, and presence of an indoor/outdoor pet. The survey instruments used for this study were largely adapted from EPA's Nonoccupational Pesticide Exposure Study (*12*) and the National Cancer Institute/EPA Farm Occupational Exposure Study (*34*). Interviews were conducted in Spanish when appropriate.

Statistical methods. Median values were lower than mean values in nearly all cases, suggesting skewed distribution of the residue data. Log_{10} transformation yielded approximately log-normal distributions in some but not in all groups. Therefore, nonparametric statistical tests were used to analyze the data whenever possible, including the Wilcoxon Signed-Rank, Mann-Whitney *U*, Kruskal-Wallis, and Spearman Rank Correlation tests. Analysis of variance tests were performed on some of the log_{10}-transformed data. Concentrations which fell below the method limit of quantitation (<MLOQ) were assigned one-half the MLOQ for statistical purposes.

Results

Families recruited and sampled included 26 farming families, 22 farmworker families, and 11 reference families. The average age of the farmers and farmworkers was 33 years; all had at least one young child (1–6 years). The average number of persons per household employed in the tree fruit industry was 1.0 for farming families and 1.8 for farmworker families.

Pesticide Use

Participants in the farmer study group who owned and/or managed orchards (23 of the 26 farming families) were surveyed regarding the use of pesticides during the 1992 spray season (January 1–July 1): 91% (21/23) reported using at least one of the target OP compounds, and 65% (15/23) reported the use of more than one target OP compound. Azinphosmethyl was the most commonly used OP, reported by 83% (19/23) of respondents. Chlorpyrifos was used by 57% (13/23), phosmet by 22% (5/23), and parathion use was reported by only 1 responding farmer (4%) during the 1992 spray season. Azinphosmethyl was the OP most recently sprayed, with applications ranging from 1 to 3 weeks before sampling, phosmet was used 1–4 weeks before sampling, chlor-

pyrifos 2–3 months before sampling, and parathion use was reported several months prior to sampling.

Of the 28 agriculturally employed study subjects who reported direct involvement with pesticide application, all but one (97%) reported using some personal protective equipment when applying OP pesticides, including rain suits, gloves, boots, and ventilated spray helmets with face shields. Eighty-two percent (23/28) reported leaving protective equipment outside the home, usually in a barn or shed. Eighty-nine percent (25/28) reported washing work clothes worn beneath protective equipment (jeans, shirts) after each pesticide application.

Analysis of the active ingredients reported by homeowners who used pesticide products in the home or on their lawn indicated that residues in soil and household dust samples were due primarily to agricultural use and not to home use of pesticides. One reference family reported application of chlorpyrifos to their lawn by a professional service 1 month before sampling. Soil from this reference home had a greater chlorpyrifos concentration (39 ng/g) than those found in the majority of agricultural family homes.

Soil and Household Dust

Table 2 provides the mean, median, range, and frequency of detection of each compound from soil samples by study group. A large fraction of samples had nondetectable levels (<LOD) of one or more of the targeted pesticides; many additional samples exhibited some ion response, but were below the <MLOQ. As stated previously, all such samples were assigned a value of one-half the MLOQ for statistical purposes. In soil samples from farmer/farmworker families (henceforth called Ag families), levels of the four target insecticides ranged from nondetectable to 930 ng/g, with one or more target compounds found in 58% of soils. For reference homes, residues in soil ranged from nondetectable to 39 ng/g, exceeding the MLOQ only twice (two homes had quantifiable levels of chlorpyrifos).

Household dust sampling results are presented in Table 3. In Ag family homes, levels of the four target analytes ranged from nondetectable to 17,100 ng/g. All four targeted insecticides were found in quantifiable levels in 62% of these homes (30/48). Two-thirds of the homes (32/48) had concentrations >1000 ng/g (>1 ppm) for one or more of the target compounds. Azinphosmethyl was quantified in 100% of the dust samples from agricultural residences. For reference families, OP concentrations ranged from nondetectable to 820 ng/g. Only one sample contained all four target analytes. Azinphosmethyl and phosmet were quantified in all reference household dust samples.

Median household dust levels of the target analytes were 17–100 times higher than soil levels, whether looking at the paired results from all study families or from the Ag families alone (Wilcoxon Signed-Rank test: $p<0.0001$). The box plots in Figure 1 indicate the distribution of pesticide concentrations in soil and dust samples from Ag families. Despite the high numbers of nondetectable residues in soil, paired outdoor (soil) and indoor (dust) values for the Ag families were significantly correlated for all pesticides (Spearman's rank correlation test; see Table 4). For reference families a significant correlation was observed for parathion only.

Table 2. Organophosphorus pesticide concentrations in soil (ng/gm)[a]

Pesticide	Ag families[b] (n = 48)	Reference families (n = 11)	Ag families: Farmers (n = 26)	Ag families: Farmworkers (n = 22)
Azinphosmethyl				
Mean	60	<32	84	<32
Median	<32*	<32*	<32	<32
Range	ND–814	ND–<32	ND–814	ND–172
Frequency (%)[c]	20 (42)	0 (0)	13 (50)	7 (32)
Phosmet				
Mean	26	<7	38	11
Median	<7	<7	<7	<7
Range	ND–332	ND–<7	ND–332	ND–101
Frequency (%)	8 (17)	0 (0)	5 (19)	3 (14)
Chlorpyrifos				
Mean	17	11	18	14
Median	<11	<11	<11	<11
Range	ND–234	ND–39	ND–234	ND–152
Frequency (%)	11 (23)	2 (18)	6 (23)	5 (23)
Ethyl parathion				
Mean	<34	<34	46	<34
Median	<34	<34	<34	<34
Range	ND–932	ND–<34	ND–932	ND–<34
Frequency (%)[c]	1 (2)	0 (0)	1 (4)	0 (0)

[a]Method limits of quantitation (MLOQ) in soil (ng/g): azinphosmethyl, 32; phosmet, 7; chlorpyrifos, 11; parathion, 34; ND, nondetectable; values <MLOQ assigned one-half MLOQ for statistical analysis.
[b]Ag families group combines the data from the farmers and farmworkers groups.
[c]Frequency = number of families with quantifiable sample concentrations (>MLOQ); percentages in parentheses.
*Significantly different concentrations; Wilcoxon signed-rank test, $p = 0.04$.

Agricultural and Reference Family Comparisons

A comparison of OP pesticide concentrations in household dust for Ag and reference families indicated that Ag families had significantly higher concentrations of azinphosmethyl ($p = 0.001$), chlorpyrifos ($p = 0.01$), and parathion ($p = 0.02$) (Mann-Whitney U test). Phosmet levels also appeared to be elevated ($p = 0.07$). Median values for azinphosmethyl, phosmet, and chlorpyrifos were 3–5 times higher, while parathion was 13 times greater. A significant difference in pesticides levels between soil samples from agricultural and reference homes was apparent only for azinphosmethyl (Wilcoxon Signed-Rank test: $p = 0.04$). This compound was used in many orchards 1–3 weeks before the sampling period.

Occupational Comparisons within Agricultural Family Groups

Median household dust concentrations for the Ag family groups tended to be higher in homes of farmers than in homes of farmworkers for azinphosmethyl, chlorpyrifos, and parathion, but levels were higher for phosmet in the farmworker homes (Table 3). However, differences between the two groups were statistically significant only for parathion (Mann-Whitney U test: $p = 0.0007$). Ag families were also grouped as "applicators" or "nonapplicators," based on reported direct handling of OP pesticides. Median dust concentrations were significantly higher in homes of applicators versus nonapplicators for chlorpyrifos and parathion (Mann-Whitney U test: $p = 0.02$ and $p = 0.0003$, respectively). Azinphosmethyl levels also tended to be higher for the applicators, but phosmet levels were similar across these two groupings.

A 2 × 2 contingency analysis was performed to test the null hypothesis that these two methods of occupational classification were independent: farmer/farmworker ($n = 26$ and $n = 22$); applicator/nonapplicator ($n = 28$ and $n = 20$). Results indicated a statistically significant association between the

Table 3. Organophosphorus pesticide concentrations in household dust (ng/g)[a]

Pesticide	Ag families[b] (n = 48)	Reference families (n = 11)	Farmers (n = 26)	Farmworkers (n = 22)	Applicators (n = 28)[c]	Nonapplicators (n = 20)
Azinphosmethyl						
Mean	1870	330	2090	1620	1955	1758
Median	1100*	283*	1320	951	1225	769
Range	170–11,270	134–816	171–6520	180–11,270	171–6520	179–11,270
Frequency (%)[d]	48 (100)	11 (100)	26 (100)	22 (100)	28 (100)	20 (100)
Phosmet						
Mean	2080	227	1700	2540	2108	2137
Median	519	185	415	519	523	523
Range	<12–17,100	73–658	<12–14,500	19–17,100	6–17,100	6–14,496
Frequency (%)	46 (96)	11 (100)	24 (92)	22 (100)	27 (96)	19 (95)
Chlorpyrifos						
Mean	429	168	506	338	514	318
Median	267*	53*	372	172	395‡	156‡
Range	<17–3585	<17–483	<17–3585	40–2180	8–3585	40–2182
Frequency (%)	47 (98)	9 (82)	25 (96)	22 (100)	27 (96)	20 (100)
Ethyl parathion						
Mean	365	76	591	98	516	161
Median	154*	<11*	310†	20†	273‡	<11‡
Range	<11–2786	<11–425	<11–2786	<11–440	<11–2786	<11–1847
Frequency (%)	33 (69)	3 (27)	22 (85)	11 (50)	25 (89)	9 (45)

[a]Method limits of quantitation (MLOQ) in dust (ng/g): azinphosmethyl, 40; phosmet, 12; chlorpyrifos, 17; parathion, 11; values <MLOQ assigned one-half MLOQ for statistical analysis.
[b]Ag families group combines the data from the farmers and farmworkers groups.
[c]Applicators and nonapplicators are groups within the Ag family group, based on whether orchard workers were engaged in pesticide handling (mixing, loading, application).
[d]Frequency = number of families with quantifiable sample concentrations (>MLOQ); percentages in parentheses.
*Signficant difference across groups: azinphosmethyl, $p = 0.001$; chlorpyrifos, $p = 0.01$; parathion, $p = 0.02$ (Mann-Whitney U test). †Signficant difference across groups: parathion, $p = 0.0007$ (Mann-Whitney U test). ‡Signficant difference across groups: chlorpyrifos, $p = 0.02$; parathion, $p = 0.0003$ (Mann-Whitney U test).

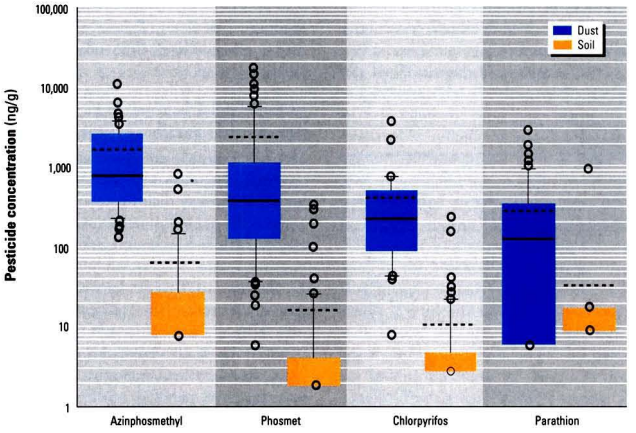

Figure 1. Box plots comparing organophosphorus pesticide concentrations in soil and household dust samples from agricultural families (farmers and farmworkers), plotted on a $\log_{10}$ scale. From the bottom to the top, the box lines in the figure represent 10th, 25th, 50th, 75th, and 90th percentiles, respectively. Circles represent outliers, and the horizontal dotted lines represent the mean concentration.

two grouping variables, with 73% of farmers categorized as pesticide applicators and 59% of farmworkers categorized as nonapplicators (chi-square test: $p = 0.02$).

Orchard Proximity

Ag family respondents categorized the proximity of their homes to any commercial orchards as <50, 50–200, or >200 ft. Thirty-three of 48 Ag families lived within 50 ft of an orchard, 7 families lived between 50 and 200 ft, and 8 families lived more than 200 ft from an orchard. By definition, all of the 11 reference homes were >1/4 mile from a commercial orchard. Nonparametric analysis of variance of Ag family data revealed a tendency for median OP concentrations in dust to decrease with increasing distance from an orchard. However, a significant difference was seen across the three proximity categories only for parathion (Kruskal-Wallis: $p = 0.005$). Due to the small numbers of subjects in the 50–200 ft and >200 ft groups, these groups were combined into a category of >50 ft from an orchard and compared again to homes <50 ft from an orchard. The box plots in Figure 2 show this distribution of OP household dust concentrations from Ag family homes with respect to proximity. Mean and median levels were higher in the proximate group for all four OP compounds, with significant differences observed for azinphosmethyl and parathion (Mann-Whitney U test: $p = 0.04$ and 0.005, respectively). Including the reference family data in this analysis strengthened the trend, with OP concentrations decreasing at increasing distance from an

Table 4. Spearman rank correlation coefficients (r) between household dust and soil organophosphate concentrations

Pesticide	Ag families (n = 48) r	Ag families (n = 48) p	Reference families (n = 11) r	Reference families (n = 11) p
Azinphosmethyl	0.49	0.001	0.05	0.87
Phosmet	0.67	<0.0001	0.23	0.48
Chlorpyrifos	0.52	0.0003	0.40	0.21
Ethyl parathion	0.35	0.02	0.81	0.01

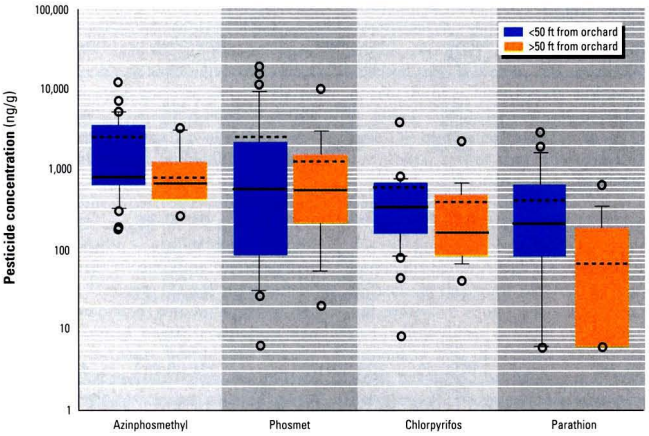

Figure 2. Box plots of organophosphorus pesticide concentration in agricultural family household dust samples comparing groups whose homes are <50 ft or >50 ft from a commercial orchard, plotted on a $\log_{10}$ scale. From the bottom to the top, the box lines in the figure represent 10th, 25th, 50th, 75th, and 90th percentiles, respectively. Circles represent outliers, and the horizontal dotted lines represent the mean concentration.

orchard for azinphosmethyl, chlorpyrifos, and parathion (Kruskal-Wallis: $p = 0.0001$, 0.02, and 0.001, respectively).

The eight Ag families who lived more than 200 ft from an orchard were distributed unevenly across the groups tested above. To determine if nonproximity to orchards confounded these analyses, tests for significant differences between farmers/farmworkers and applicators/nonapplicators were repeated excluding those eight families, but the outcome of the analyses were unchanged.

Further analysis was performed to determine if an association existed between proximity to orchards in categories of <50 ft ($n = 32$) and >50 ft ($n = 15$), and the occupational classifications of farmer ($n = 26$) and farmworker ($n = 22$). A significant association was observed between the two grouping variables (chi-square: $p = 0.04$), with 65% of those living <50 ft of an orchard categorized as farmers and 67% of those living >50 ft categorized as farmworkers. As indicated above, occupation and pesticide application activities were also interrelated grouping variables. However, an additional analysis of these variables demonstrated that pesticide application activity and homesite orchard proximity were not associated groupings (chi-square: $p>0.05$).

Analyses of variance were performed to determine which one or combination of these three interrelated variables might best explain the variability in household dust OP concentrations for Ag families: proximity (<50 ft or >50 ft), occupation (farmer or farmworker), and applicator or nonapplicator status. One-way analysis of variance (ANOVA) of $\log_{10}$-transformed data revealed significant differences between the categories of all three variables for parathion ($p < 0.001$ for proximity, occupation, and applicator status). No statistical differences were seen between categories of these variables for the other pesticides. Two-way ANOVAs with parathion concentrations of dust showed that the variables "proximity" and "applicator status" were not interactive and that each explained a significant component of variability in OP dust levels between the groups (proximity: $p = 0.002$, applicator: $p = 0.004$, proximity*applicator: $p = 0.82$). When two-way ANOVAs included the variable "occupation," the difference in levels of OPs between farmers and farmworkers varied whether looking at applicators or nonapplicator status, or living <50 ft or >50 ft from an orchard; i.e., when the occupation was paired with either applicator status or proximity, there was interaction, and the variables could not be considered independent in predicting OP household dust level.

Surface Loading and Track-in

Surface loading levels are defined as mass per unit surface area, in this case micrograms of OP pesticide per square meter of carpet. On average, a larger surface was sampled in the reference family homes than in the Ag family homes (6.1 m^2 vs. 4.1 m^2), suggesting differences in dust concentrations. Average (± SD) dust loadings across the three study groups were 8.2 ± 6.4 $\mu g/m^2$ for farmer, 14.9 ± 13.4 $\mu g/m^2$ for farmworker, and 4.4 ± 2.9 $\mu g/m^2$ for reference families. OP loading levels are summarized in Table 5. Loading levels across groups follow the same patterns as described previously for OP concentrations in household dust. Ag families were again divided into applicators and nonapplicators to determine if mass loading levels differed between the two groups. Significant differences between the two groups were observed for chlorpyrifos and parathion (applicators>nonapplicators; Mann-Whitney U Test: $p = 0.04$, and $p = 0.002$, respectively).

Questions pertaining to variables affecting pesticide loading in homes, including track-in behavior, cleaning activities, and orchard proximity, were answered as indicated in Table 6. No significant differences in OP loading levels were found for any of these questionnaire variables, even after adjusting for the number of days since participants had last vacuumed (Mann-Whitney U test: $p>0.05$). Multiple regression analysis of these variables also failed to show any significant relationships.

Discussion

This study reports residential levels of agricultural chemicals in a farming region across both agricultural and nonagricultural households. The sample population included both farmers and farmworkers, most of whom lived on orchard property, where OP pesticides are sprayed frequently. As such, the study population would appear to approximate a "maximally exposed" group, at least in the tree fruit regions of North America. This study had a potential for selection bias because participation was voluntary and self-selected. Studies which focus on health and safety often attract participants with concerns for these issues. However, we have no evidence to suggest that the study families were unrepresentative of families in the region.

As expected, significantly higher levels of OPs were found in homes of Ag families than in those of reference families. Much higher levels of pesticides were found in household dust, where chemicals are not degraded or dispersed by environmental factors such as rain, sun, and soil microbial activity. These results are consistent with other reports of the persistence of pesticides in indoor environments (*12,13,20,22*).

Despite low pesticide concentrations in soil, significant correlations were observed between paired outdoor and indoor levels, suggestive of common sources for pesticide contamination of soil and household dust. In

Table 5. Organophosphorus mass loading results (μg pesticide/m² carpet)[a]

Pesticide	Ag families[b] (n = 48)	Reference families (n = 11)	Farmers (n = 26)	Farmworkers (n = 22)	Applicators (n = 28)[c]	Nonapplicators (n = 20)
Azinphosmethyl						
Mean	16.6	1.4	16.6	16.7	19.3	13.7
Median	9.9	0.83	10.7	8.0	14.4	5.8
Range	0.8–878	0.39–3.18	0.8–88	1.1–51	0.8–88	1.3–51
Phosmet						
Mean	27.1	0.91	18.4	36.1	26.8	27.5
Median	3.0	0.94	2.1	8.4	5.2	2.5
Range	<MLOQ–289	0.21–1.93	<MLOQ–289	0.2–222	<MLOQ–289	<MLOQ–164
Chlorpyrifos						
Mean	4.8	0.59	4.1	5.4	5.7	3.5
Median	1.9	0.47	1.62	2.0	2.7*	1.2*
Range	<MLOQ–27.7	<MLOQ–1.62	<MLOQ–25	0.09–28	<MLOQ–24.7	0.12–27.7
Ethyl parathion						
Mean	3.9	0.35	5.2	2.4	5.1	2.2
Median	1.2	<MLOQ	2.5	0.57	2.7*	0.05*
Range	<MLOQ–20.4	<MLOQ–2.43	<MLOQ–20	<MLOQ–17	<MLOQ–20.4	<MLOQ - 17.0

[a]Mass loading (μg/m²) = concentration of pesticide (ng/g) × grams of dust collected/m² carpet × 1 μg/1000 ng. Method limits of quantitation (MLOQ) in dust (ng/g): azinphosmethyl, 40; phosmet, 12; chlorpyrifos, 17; ethyl parathion 11; values <MLOQ assigned one-half MLOQ for statistical analysis.
[b]Ag families group combines the data from the farmers and farmworkers groups.
[c]Applicators and nonapplicators are groups within the Ag family group, based on whether orchard workers were engaged in pesticide handling (mixing, loading, application).
*Significant differences across groups: chlorpyrifos, $p = 0.04$; parathion, $p = 0.002$ (Mann-Whitney U test).

Table 6. Behavioral variables related to pesticide track-in

Question (n = 59; agricultural and reference families)	Positive response (%)
Do family members remove shoes at the door?	28
Are there walk-off mats outside main entries?	69
Is there a pet that goes in and out of the house?	33
How frequently are children's indoor play areas vaccuumed?	
>Weekly	40
Weekly	45
<Weekly	16
How far is the house from a commercial orchard? (n = 48 agricultural families)	
<50 ft	69
50–200 ft	15
>200 ft	17

contrast to trends for lead and arsenic contamination in these same samples (*35*), soil OP levels appear to be poor predictors of the magnitude of dust OP contamination due to degradation in the outdoor environment.

Pesticide concentrations in reference homes were much lower than those in Ag homes, yet it was surprising how frequently agricultural OP compounds were detected in dust samples for reference families. Due to the prevalence of orchards in the Wenatchee region, it was difficult to find volunteers who met the reference family inclusion criteria for reference homes. Although all reference families did live >0.25 mile from an orchard, many were within 0.5 mile. It therefore appears likely that those who reside in an agricultural region such as Wenatchee will have measurable pesticide residues in their homes regardless of personal pesticide use.

The significant relationship between proximity to orchards and concentration of azinphosmethyl in dust for the Ag families may reflect the fact that azinphosmethyl was the most recently sprayed and most commonly used OP compound (reported by 83% of farmers). Azinphosmethyl was the most frequently detected insecticide in household dust (100%) and soil (21%) among all the residences, including the reference homes. Chlorpyrifos had been applied 2–3 months previously by 57% of the surveyed farmers, but elevated soil residues were not found on these farms. The small number of subjects in each proximity category limits interpretation of these results. Phosmet was used by only 22% of farmers, limiting the possibility of detecting differences between groups with respect to proximity.

The interrelationship of three possible categories of the Ag family participants—homesite orchard proximity, occupation, and applicator status—complicated our analysis. The finding that the proximity and applicator status variables were not interactive for parathion levels in household dust suggests that either of these variables is predictive for elevated OP concentrations in dust.

Previous studies have suggested that toxicants carried or tracked into the home accumulate and may concentrate in dust, particularly in carpeted homes (*13,35,37*). Although the data presented here demonstrate substantial accumulation of pesticides in agricultural family homes, we were unable to identify specific exposure pathways such as track-in on shoes or by pets in this study. Pathway identification may have been confounded by variables such as type of carpeting, type of vacuum cleaner, composition of dust, or recall bias in self-reported information.

Dust sampling was conducted with the HVS-3 vacuum, a relatively new tool for environmental sampling. This method has been used to demonstrate that certain interventional measures can reduce the mass loading of contaminants in carpeted homes (*36*), but it is unclear whether the loading values obtained with the HVS-3 are representative of residues available to young children. A previous report in nine homes compared the HVS-3 technique with a polyurethane foam roller weighted to simulate the pressure applied to a surface by a crawling child (*13*). Mass loading results obtained by the two methods were correlated, but the levels from the HVS-3 were 4–12 times higher than those obtained by the foam roller. Methods used by other investigators in studies demonstrating correlations between mass loading and children's exposures have included wipe sampling and the use of a low-flow,

hand-held suction device (*37–39*). The more powerful suction of the HVS-3 may render results obtained with this technique more susceptible to confounding by carpet age, vacuum type, and frequency of cleaning. Further studies are needed to determine the representativeness of surface sampling techniques for estimating children's exposures.

The highest pesticide concentration found in any sample was 17 ppm (17,100 ng/g; phosmet in household dust), and the greatest total OP concentration measured in dust was 21.5 ppm (21,549 ng/g; sum of four OP compounds without regard to relative toxicity). A hazard evaluation was conducted for acute health risks among children living in study homes and indicated that acute intoxications from OP pesticide exposure through soil and contact with dust were unlikely. The hazard evaluation included use of toxicity data for the four OP compounds studied, a standard EPA soil contact transfer factor of 200 mg/day for children 1–6 years old (*18*), and the total OP dust concentration values from this study. A more detailed analysis of potential exposure to multiple OP compounds in these residential environments will be reported elsewhere.

Conclusions

Investigations of environmental and occupational health hazards normally proceed through the steps of recognition, evaluation, and control. This study has identified a potential hazard for young children residing in homes on or near sites of agricultural pesticide use by documenting environmental concentrations of four OP pesticides. In particular, it appears that children are likely to be exposed simultaneously to several pesticides that are not registered for residential use and that have the same mechanism of toxicity. Additional work is needed to evaluate children's exposure to agricultural pesticides in these settings, and, if necessary, to develop appropriate interventions to mitigate exposures. Carefully designed longitudinal or interventional studies will be needed to more adequately identify risk factors associated with the introduction of contaminants into the home. Biological monitoring based on urine sample collection may serve as an appropriate and noninvasive means of sampling exposure among small children.

Proximity to spray areas appears to have been the predominant, though not the only, factor responsible for elevated pesticide concentrations in household dust in this study. A number of variables still need to be assessed before it is possible to accurately estimate children's exposure from the dust/soil pathway, such as track-in, children's activity patterns, surface-to-skin contact/transfer rates for pesticides, dust/soil ingestion rates, and percutaneous uptake. Further investigation is warranted to address cumulative exposure to the multiple OP compounds found in these environments, rather than the traditional approach of focusing on a single compound for regulatory purposes.

Several strategies are available to reduce the risk potential of pesticide contamination in the home. A high percentage of participants in this study reported the use of full protective equipment while spraying and indicated that they did not bring this equipment into the home. These prudent work practices should be encouraged. Furthermore, programs designed to assist families with preventing or reducing indoor contaminants have been implemented in urban areas, especially for lead, and can be implemented in rural areas as well. Recommendations to reduce residential contaminants include improved home hygiene and personal hygiene measures, such as removal of shoes at the door, use of door mats, improved vacuuming techniques, and frequent washing of children's hands. The use of greater precautions when applying pesticides close to homes and a change in the practice of situating homes within orchard spray regions might also be considered. Finally, a change at the policy level to reduce the use of pesticides in the home and in surrounding agricultural areas would represent a strategy of primary prevention of pesticide exposure. The Environmental Protection Agency and the U.S. Department of Agriculture have recently proposed a Pesticide Use Reduction Initiative, which has as one of its goals the establishment of integrated pest management on 75% of active agricultural lands in 5 years. Policies such as this are very likely to affect pesticide contamination in the home, thereby reducing potential exposure to children and other family members.

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World Health Organization

International Agency for Research on Cancer (IARC)

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Applications from women are encouraged.

Gestational and Lactational Exposure of Rats to Xenoestrogens Results in Reduced Testicular Size and Sperm Production

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This study assessed whether exposure of male rats to two estrogenic, environmental chemicals, 4-octylphenol (OP) and butyl benzyl phthalate (BBP) during gestation or during the first 21 days of postnatal life, affected testicular size or spermatogenesis in adulthood (90–95 days of age). Chemicals were administered via the drinking water at concentrations of 10–1000 µg/l (OP) or 1000 µg/l (BBP); diethylstilbestrol (DES; 100 µg/l) and an octylphenol polyethoxylate (OPP; 1000 µg/l), which is a weak estrogen or nonestrogenic *in vitro*, were administered as presumptive positive and negative controls, respectively. Controls received the vehicle (ethanol) in tapwater. In study 1, rats were treated from days 1–22 after birth; in studies 2 and 3, the mothers were treated for approximately 8–9 weeks, spanning a 2-week period before mating, throughout gestation and up until 22 days after giving birth.

With the exception of DES, treatment generally had no major adverse effect on body weight: in most instances, treated animals were heavier than controls at day 22 and at days 90–95. Exposure to OP, OPP, or BBP at a concentration of 1000 µg/l resulted in a small (5–13%) but significant ($p<0.01$ or $p<0.001$) reduction in mean testicular size in studies 2 and 3, an effect that was still evident when testicular weight was expressed relative to body weight or kidney weight. The effect of OPP is attributed to its metabolism *in vivo* to OP. DES exposure caused similar reductions in testicular size but also caused reductions in body weight, kidney weight, and litter size. Ventral prostate weight was reduced significantly in DES-treated rats and to a minor extent in OP-treated rats. Comparable but more minor effects of treatment with DES or OP on testicular size were observed in study 1. None of the treatments had any adverse effect on testicular morphology or on the cross-sectional area of the lumen or seminiferous epithelium at stages VII–VIII of the spermatogenic cycle, but DES, OP, and BBP caused reductions of 10–21% ($p<0.05$ to $p<0.001$) in daily sperm production. Humans are exposed to phthalates, such as BBP, and to alkylphenol polyethoxylates, such as OP, but to what extent is unknown. More detailed studies are warranted to assess the possible risk to the development of the human testis from exposure to these and other environmental estrogens. *Key words*: butyl benzyl phthalate, daily sperm production, diethylstilbestrol, 4-octylphenol, Sertoli cell number, spermatogenesis. *Environ Health Health Perspect* 103:1136–1143 (1995)

The report by Carlsen et al. (*1*) that mean sperm counts in some men had declined by around 40–50% over the past 50 years was greeted with a mixture of concern (*2*) and skepticism (*3*). The most recent data from a number of countries, which have charted changes in sperm counts in semen donors over the past 20–25 years, have, however, all reported a marked and significant downward trend (*4–6*). The most comprehensive of these studies, in Paris, concluded that sperm counts in fertile men have declined by around 2% per year over the past 23 years (*6*). Moreover, two of the cited studies (*5,6*) identified that the temporal decline in sperm counts appears to apply to men born from around 1950 onwards.

Two years ago, we hypothesized (*7*) that the reported decline in sperm counts might be related to an increasing incidence of other disorders of development of the male reproductive system (e.g., testicular cancer) and that this could have arisen because of increased exposure of the developing fetus to estrogens. One potential source of this increased estrogen exposure was via environmental estrogenic chemicals, or "xenoestrogens," the release of which has more or less coincided with the decline in sperm counts (*8,9*). Concern about such hormonally active pollutants, such as chlorinated pesticides, has been voiced for 10–20 years (*10*), but has become more acute recently because of the discovery of a range of new xenoestrogens, including bisphenol-A (*11*), certain alkylphenolic chemicals (*12–14*), certain phthalates (*15*), as well as a number of pesticides (*16*). Most of these estrogenic chemicals are ubiquitous in the environment, and humans are exposed to them daily by a number of routes (*9*). However, the risk to humans from these chemicals is currently theoretical because there are no data to show that these chemicals can cause any disorder of reproductive development or function in animals.

Pathways via which exposure of the developing male fetus or neonate to estrogenic chemicals could result in reduced testicular size and sperm production in adult life have been identified (*2,7,9*), but there is no direct evidence to confirm whether this hypothesis has any factual basis. It is known that some phthalates are passed from the mother both across the placenta (*17*) and via milk (*18*), although comparable data on the transfer of alkylphenolic chemicals are lacking. The aim of the present studies was to evaluate whether exposure of the male rat fetus/neonate to either of two environmental estrogenic chemicals has any effect on testicular size and spermatogenesis in adult life.

Material and Methods

Animals and treatments. All rats used in these studies were of the Wistar strain and were bred in our own animal facility. They were maintained under standard, controlled conditions and had free access to food and water. Administration of chemicals was via the drinking water, which was provided in a bottle per cage. A stock solution of each dose of chemicals was made by dissolving a weighed amount in ethanol such that addition of 0.5 ml of this stock to 5 l of tapwater resulted in the test dose; control animals had 0.5 ml ethanol/5 l added to their drinking water.

Study design. The most likely mechanism via which estrogenic chemicals could cause an irreversible reduction in testicular size and sperm production is by decreasing the number of Sertoli cells. In adult life, the number of Sertoli cells determines testicular size and sperm production in all animals that have been studied (*19*). In the male rat, Sertoli cells begin to proliferate soon after testicular differentiation (about day 15 of gestation) and continue until around day 15 of postnatal life, with perhaps minor proliferation until around day 21; after this time, no further Sertoli cell proliferation can occur (*19*). Thus, by day 22, the ultimate size to which the testis will grow in adulthood (90–95 days of age) has been predetermined (*19–22*).

Our studies were designed such that animals were exposed to chemicals for

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either the postnatal period (i.e., days 1–22 after birth) of Sertoli cell proliferation (study 1; see Fig. 1) or for the complete period of Sertoli cell proliferation (studies 2 and 3; see Fig. 1). In the latter two studies, treatments were administered to adult female rats for 2 weeks before mating with a sexually experienced male, throughout mating, throughout gestation, and up until day 22 after giving birth (Fig. 1). This protocol of exposure was used to assess the possible effects of bioaccumulation. In all three studies, exposure of the male offspring to the test chemicals was thus largely indirect, via the placenta or milk.

In study 1, in which there was no prenatal exposure to the test chemicals, litters were culled to eight pups on the day of birth (day 1) by culling excess females. The same was done in study 2. In study 3, the full litter size was maintained from birth through day 22. The female offspring of test litters were not evaluated. Adult females used for mating in studies 2 and 3 were the same: when offspring of these females were weaned at the completion of study 2, the mothers were maintained on the same treatment for 2 weeks, then mated and exposed until the weaning of study 3 offspring (Fig. 1).

Test chemicals and doses. Three chemicals were selected for study based on the results of *in vitro* investigations suggesting that they were estrogenic (*13–15*). Of the three, 4-*tert*-octylphenol (OP; Aldrich Chemical Co., Gillingham, UK) and butyl benzyl phthalate (BBP; Chem Service, West Chester, Pennsylvania) were both estrogenic *in vitro*, whereas an octylphenol polyethoxylate with a side-chain of five ethoxylate groups (OPP; Igepal CO-520; Aldrich) was essentially devoid of estrogenicity *in vitro*. In study 1, nonylphenoxycarboxylic acid (NP1EC, K & K Labs, Cleveland, Ohio), which is approximately 10-fold less estrogenic *in vitro* than OP (*14*), was also assessed at a single dose. Diethylstilbestrol (DES; Sigma Chemical Co., Poole, Dorset, UK), which is a potent nonsteroidal estrogen, was included as a positive control.

Little is known about the degree of exposure of humans to the chemicals used in the present studies, but concentrations of alkylphenolic compounds in the aquatic environment reportedly range up to hundreds of micrograms per milliliter (*23,24*), whereas human intake of phthalates is reportedly as high as 15 mg per day (200–300 µg/kg/day) (*25*). Therefore, we chose doses that would be mildly estrogenic based on *in vitro* analyses (*14,15*), but which remained within an order of magnitude of the possible environmental/human intake level. We tested OP at 1000 and 100 µg/l in all three studies (and also at 10 µg/l in study 1), whereas OPP and BBP were tested only at the single dose of 1000 µg/l in studies 2 and 3. DES was tested at 100 and 10 µg/l in study 1 and at 100 µg/l in studies 2 and 3. No formal confirmation that the test chemicals (or their metabolites) actually reached the male offspring, or in what amounts, was obtained, as the objective was simply to establish whether or not the chemicals exerted any biological effects. However, water intake, and thus the nominal intake of chemical/day, was assessed in some of the treatment groups in study 3 by weighing water bottles every 48 hr.

Body and organ weights, litter size and composition. In studies 2 and 3, we recorded litter size and composition at birth. In all three studies, the male offspring were weighed at weaning (day 22), which was the day that treatment ceased. The male offspring were then maintained in their litters under standard conditions until 90–95 days of age, when they were killed by inhalation of CO_2, followed by cervical dislocation. Body weight was recorded and the right testis, left kidney, and ventral prostate were dissected out and weighed; the epididymis, seminal vesicles, and left testis were also inspected macroscopically for any obvious abnormalities. We recorded kidney weight because the kidney varies according to body weight but was not expected to be affected by the experimental manipulations. The kidney was thus used as an internal "organ control" for the specificity of any effects observed on testis size.

Testicular morphology. At 90–95 days of age, representative animals from the various treatment groups were fixed with 3% glutaraldehyde in 0.2 M cacodylate buffer by perfusion via the dorsal aorta, as described elsewhere (*26*). The fixed testes were then cut transversely with a razor blade into 2-mm-thick sections and then into small blocks (1–2 mm^2). After postfixation for 12–16 hr in the same fixative, the blocks were processed and embedded in plastic as described previously (*26*). Semithin sections (0.5 µm) were then cut, stained with toluidine blue, and examined using a Zeiss photomicroscope.

To provide a preliminary quantitative assessment of spermatogenesis, seminiferous tubules at stages VII–VIII of the spermatogenic cycle were subjected to image analysis (Cue-2, Olympus) to determine the cross-sectional area of the seminiferous tubule and seminiferous epithelium (*27*). We analyzed 10 round cross-sections for two or three rats per treatment group and calculated the mean and standard deviation for each animal. Stages VII–VIII were chosen for analysis because they contain representative germ cells from all steps of development (*19*).

Daily sperm production. In some of the animals from study 3, daily sperm production was determined by counting homogenization-resistant spermatids, using minor modifications of the techniques of Johnson et al. (*28,29*). A 500–700 mg portion of testicular tissue recovered at autopsy was immersion-fixed in 2% glutaraldehyde in 0.2 M cacodylate buffer and kept at 4°C until used for daily sperm production determination within the next 6 weeks. The tissue was then removed, blotted, and

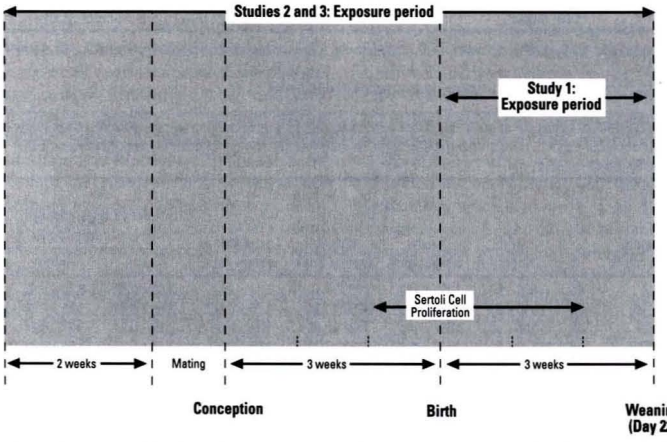

Figure 1. Experimental design of the present studies, indicating the periods of treatment in relation to the time of normal proliferation of Sertoli cells. After cessation of treatment (day 22 after birth) animals were maintained under normal conditions until they were killed at the age of 90–95 days.

weighed and two 50-mg portions cut with a scalpel, weighed, and homogenized separately in 5 ml 0.15 M NaCl, 0.05% Triton-X100, 0.025% sodium azide, using a Polytron homogenizer (PT-K/PCU-8; Kinematica AG, Luzern, Switzerland) at speed 5 for 60 sec (these conditions had previously been validated and optimized). Using a hemocytometer, homogenization-resistant step 18 and 19 spermatids were then counted separately in 3 aliquots of each of the 2 homogenates per sample and the mean of the 6 measurements calculated; the coefficient of variation for these replicates averaged 7% for all samples. This value was then corrected for sample weight and overall testis weight, and transformed to the daily sperm production by dividing by the appropriate time divisor (4.61) based on the proportional duration of stages VI–VIII in days, according to Leblond and Clermont (*30*). We confirmed that only step 18 and 19 spermatids were being counted by applying the same procedures to known lengths of seminiferous tubule isolated from normal adult rats by transillumination-assisted microdissection (*31*) at stages II–V (containing step 16 and 17 spermatids) and VI–VIII (containing step 18 and 19 spermatids).

Statistical analysis. In each of the three studies, each parameter in the different treatment groups was subjected to analysis of variance to determine whether there were significant effects of treatment. Where these were indicated, subgroup comparisons between means for the control and each treatment group were made using the variance from the study as a whole as the measure of error. All data were normally distributed, so no transformations were made, and results are all reported as means ± SD.

Results

Litter size and composition at birth were not evaluated in study 1, as there was no prenatal treatment of the mothers. In studies 2 and 3, in which the mothers were treated prenatally, there was no effect of treatment with OP, OPP, or BBP, on litter size or composition. Exposure to DES (100 µg/l) reduced average litter size by nearly half in study 3 and had a more minor effect in study 2 (Table 1). Curiously, the proportion of male offspring was increased significantly in the DES-exposed group in study 3 (Table 1).

At weaning, which corresponded to the cessation of treatment in all three studies, body weight of DES-exposed offspring was reduced significantly in studies 1 and 2 but was increased significantly in study 3, perhaps because of the much smaller litter sizes (Table 1). Otherwise, exposure to any of the test chemicals had either no effect or, more commonly, resulted in a significant increase in body weight on day 22 (Table 1).

In study 1, mean body weight was generally higher in treatment groups, compared with controls, but only in the case of OP (100 µg/l) did this reach statistical significance (Table 2). Average testis weight was reduced marginally, but significantly, in animals exposed to DES (100 µg/l) and OP (1000 µg/l), and relative testis weight (i.e., relative to body weight or to kidney weight) was reduced significantly in these two groups and in animals exposed to the intermediate concentration (100 µg/l) of OP (Table 2). Kidney weight was increased markedly in animals exposed to the two highest doses of OP and, although this may have been due to some extent to the greater average body weight of the animals, a significant difference in kidney weight relative to body weight was still evident (Table 2).

In study 2, mean body weight in animals exposed to DES or OPP was reduced when compared to controls, whereas animals exposed to either dose of OP were increased in size by 8% or more (Table 3). Except for animals exposed to the lower dose of OP (100 µg/l), all other treatment groups exhibited a highly significant decrease in absolute testis weight and in the ratio of testis/kidney size (Table 3); all treatment groups showed a significant decrease in relative testis weight. There were some minor, but significant, changes in absolute and relative kidney weight in some of the treatment groups. Ventral prostate weight was reduced by 16% in DES-treated animals, though this effect largely disappeared when the size relative to body weight was evaluated (Table 3).

Table 1. Litter size and composition at birth and body weight on day 22 (means ± SD)

Study no.	Treatment group (µg/l)	Litter size (no. of litters)	% Males at birth	Body weight (g) of males, day 22 (*n*)
1	Control	ND	ND	60 ± 8 (70)
	DES (100)	ND	ND	55 ± 7**(46)
	DES (10)	ND	ND	54 ± 5†(48)
	OP (1000)	ND	ND	60 ± 5 (56)
	OP (100)	ND	ND	65 ± 6† (35)
	OP (10)	ND	ND	65 ± 9**(34)
	Nonylphenol (100)	ND	ND	ND
2	Control	10.0 ± 2.5 (5)	61 ± 8	53 ± 5 (29)
	DES (100)	8.2 ± 1.7 (6)	64 ± 14	46 ± 8† (30)
	OP (1000)	12.0 ± 1.1 (6)	46 ± 14	61 ± 8† (30)
	OP (100)	10.8 ± 3.5 (6)	53 ± 14	66 ± 5† (33)
	OPP (1000)	10.6 ± 3.2 (5)	63 ± 15	54 ± 7 (39)
	BBP (1000)	11.6 ± 3.4 (5)	64 ± 13	59 ± 7**(38)
3	Control	12.7 ± 2.2 (6)	55 ± 12	50 ± 10 (36)
	DES (100)	6.2 ± 3.3 (5)**	80 ± 20*	57 ± 8** (27)
	OP (1000)	11.2 ± 1.8 (5)	64 ± 7	52 ± 8 (39)
	OP (100)	10.8 ± 2.1 (6)	57 ± 13	54 ± 7*(36)
	OPP (1000)	13.8 ± 0.4 (6)	45 ± 11	47 ± 4 (34)
	BBP (1000)	13.4 ± 1.5 (5)	57 ± 3	57 ± 8** (38)

Abbreviations: DES, diethylstilbestrol; OP, octylphenol; OPP, octylphenol polyethoxylate; ND, not determined.
*$p<0.05$, ** $p<0.01$, †$p<0.001$, compared to respective control value.

Table 2. Effect of exposure of male rats, from birth to day 22, to diethylstilbestrol, octylphenol, or nonylphenoxyacetic acid added to the drinking water (µg/l) on body weight, testis, and kidney weight (means ± SD) at age 90–95 days (study 1)

Treatment group (µg/l)[a]	*N*	Body weight (g)	Testis weight (mg)	Kidney weight (mg)	Testis/kidney weight ratio	Relative organ weight (mg/g body weight)	
						Testis	Kidney
Control	65	504 ± 66	1968 ± 163	1739 ± 172	1.14 ± 0.14	3.85 ± 0.38	3.39 ± 0.32
DES (100)	36	516 ± 33	1894 ± 218*	1797 ± 124	1.06 ± 0.16**	3.69 ± 0.49*	3.49 ± 0.20
DES (10)	44	506 ± 40	1961 ± 147	1732 ± 131	1.16 ± 0.09	3.89 ± 0.34	3.37 ± 0.24
OP (1000)	49	518 ± 34	1898 ± 130*	1883 ± 223†	1.02 ± 0.12†	3.68 ± 0.23**	3.64 ± 0.38**
OP (100)	29	556 ± 37†	1990 ± 126	1968 ± 165†	1.02 ± 0.10†	3.60 ± 0.34**	3.54 ± 0.23*
OP (10)	27	511 ± 39	1940 ± 132	1776 ± 164	1.10 ± 0.11	3.82 ± 0.38	3.48 ± 0.27
NPA (100)	33	522 ± 45	1955 ± 203	ND	ND	3.75 ± 0.26	ND

Abbreviations: DES, diethylstilbestrol; OP, octylphenol; NPA, nonylphenoxyacetic acid; ND, not determined.
[a]Litters were culled to eight pups on the day of birth.
*$p<0.05$, **$p<0.01$, †$p<0.001$ compared to respective control value.

Table 3. Effect of exposure of male rats, throughout gestation and until postnatal day 22, to diethylstilbestrol, octylphenol, octylphenol polyethoxylate, or butyl benzyl phthalate added to drinking water (μg/l) on body weight and the weights of the testis, kidney, and ventral prostate (means ± SD) at age 90–95 days (study 2)

Treatment group (μg/l)[a]	N	Body weight (g)	Organ weights (mg)			Testis/kidney weight ratio	Relative organ weight (mg/g body weight)		
			Testis	Kidney	Ventral prostate		Testis	Kidney	Ventral prostate
Control	26	489 ± 32	2014 ± 155	1837 ± 118	468 ± 79	1.10 ± 0.08	4.12 ± 0.26	3.76 ± 0.19	0.96 ± 0.14
DES (100)	26	445 ± 41**	1750 ± 180†	1759 ± 158*	393 ± 44**	1.00 ± 0.09†	3.94 ± 0.34*	3.96 ± 0.24*	0.89 ± 0.11
OP (1000)	27	530 ± 53†	1899 ± 123**	1915 ± 160*	428 ± 88	0.99 ± 0.07†	3.61 ± 0.32†	3.63 ± 0.32	0.82 ± 0.19**
OP (100)	29	534 ± 41†	2042 ± 179	1880 ± 112	442 ± 82	1.09 ± 0.10	3.84 ± 0.38**	3.53 ± 0.23**	0.84 ± 0.18*
OPP (1000)	32	461 ± 32*	1783 ± 137†	1796 ± 159	476 ± 103	1.00 ± 0.11†	3.88 ± 0.30**	3.90 ± 0.35	1.03 ± 0.20
BBP (1000)	35	476 ± 28	1809 ± 126†	1830 ± 116	454 ± 84	0.99 ± 0.09†	3.81 ± 0.34†	3.85 ± 0.24	0.96 ± 0.19

Abbreviations: DES, diethylstilbestrol; OP, octylphenol; OPP, octylphenol polyethoxylate; BBP, butyl benzyl phthalate.
[a]Litters were culled to eight pups on the day of birth.
*$p<0.05$, **$p<0.01$, †$p<0.001$ compared to respective control value.

Table 4. Effect of exposure of male rats, throughout gestation and until postnatal day 22, to diethystilbestrol, octylphenol, octylphenol polyethyoxylate, or butyl benzyl phthalate added to drinking water (μg/l) on body weight and the weights of the testis, kidney, and ventral prostate (means ± SD) at age 90–95 days (study 3)

Treatment group (μg/l)[a]	N	Body weight (g)	Organ weights (mg)			Testis/kidney weight ratio	Relative organ weight (mg/g body weight)		
			Testis	Kidney	Ventral prostate		Testis	Kidney	Ventral prostate
Control	36	479 ± 26	1954 ± 118	1749 ± 169	522 ± 84	1.13 ± 0.14	4.09 ± 0.28	3.66 ± 0.33	1.08 ± 0.16
DES (100)	23	466 ± 34	1847 ± 157**	1792 ± 183	387 ± 83†	1.04 ± 0.16**	3.99 ± 0.45	3.85 ± 0.30*	0.84 ± 0.18†
OP (1000)	37	474 ± 34	1696 ± 140†	1621 ± 121**	461 ± 63**	1.05 ± 0.07**	3.57 ± 0.23†	3.42 ± 0.24†	0.98 ± 0.11**
OP (100)	34	461 ± 22*	1838 ± 114**	1699 ± 121	470 ± 76*	1.09 ± 0.09	4.00 ± 0.31	3.69 ± 0.22	1.03 ± 0.20
OPP (1000)	34	460 ± 32*	1810 ± 110†	1710 ± 160	495 ± 65	1.07 ± 0.11*	3.95 ± 0.35*	3.72 ± 0.22	1.08 ± 0.12
BBP (1000)	35	477 ± 24	1819 ± 119†	1830 ± 133	486 ± 63	1.01 ± 0.08†	3.82 ± 0.26†	3.79 ± 0.20	1.03 ± 0.12

Abbreviations: DES, diethylstilbestrol; OP, octylphenol; OPP, octylphenol polyethoxylate; BBP, butyl benzyl phthalate.
[a]Litters not culled to a standard size at birth.
[b]Litter size, etc., also affected by treatment (see Table 1).
*$p<0.05$, **$p<0.01$, †$p<0.001$ compared to respective control value.

Relative weight of the prostate was reduced significantly in animals exposed to either dose of OP.

In study 3, animals were noticeably smaller on average, both at weaning and at 90–95 days of age, than animals in studies 1 and 2 (Table 4). Under this regimen, no treatment group in adult life had a larger mean body weight than the control group, and two of the groups (OP at 100 μg/l and OPP) showed a small but significant decrease in body weight relative to controls. In all treatment groups, testis weight was reduced significantly when compared to controls, though when expressed relative to body weight this difference disappeared for the groups exposed to DES or the lower dose of OP (Table 4). Kidney weight was reduced noticeably in animals exposed to 1000 μg OP/l, a difference still evident when expressed relative to body weight; however, the testis/kidney weight ratio in this group was still significantly lower than that observed in the control group (Table 4). As in study 2, ventral prostate weight was reduced significantly in DES-exposed animals but, in study 3, a significant reduction was also obvious in animals exposed to OP, particularly at the higher dose (Table 4).

Although exposure to the test chemicals throughout gestation and neonatal life resulted in fairly consistent reductions in testis size as adults, these decreases were only on the order of 5–13%. However, plotting the data for testis weight against body weight for four of the treatment groups from studies 2 and 3 (i.e., DES, OP at 1000 μg/l, BBP) shows that the treated animals have a different distribution from controls (Fig. 2). This is most evident by noting how few of the values for treated animals lie above the linear regression line plotted for the control group. Although a similar trend was evident in the DES-exposed animals, testicular weights were far more variable in this treatment group, probably because of the confounding effects of this treatment on litter size, etc.

Testicular morphology was indistinguishable in animals from the control and treatment groups, and no obvious abnormalities in the seminiferous tubules, interstitium, or vasculature were evident (Fig. 3). Image analysis confirmed this impression by demonstrating no adverse effect of treatment on the cross-sectional parameters of stage VII–VIII seminiferous tubules; indeed, for the most part, these parameters tended to be higher for the treated animals than for the controls, though this is based on a small sample size (Table 5).

Daily sperm production in control animals from study 3 averaged 24.9 ± 3.6 × 10^6 per testis per day (mean ± SD, $n = 12$), which agrees closely with that determined morphometrically by Wing and Christensen (*32*). Animals exposed during fetal and neonatal life to DES, OP (1000 μg/l), or BBP in study 3 all showed significant reductions of 10–21% in the mean daily sperm production (Fig. 4), which were proportionately similar to the decrease in testis weight (Table 4); tissue from animals exposed to OPP was not evaluated.

The nominal intake of chemical was assessed in study 3 for animals in two of the treatment groups (OP at 1000 μg/l, BBP) based on water intake, and ranged from around 125 μg/kg/day in the first 2 days after birth to 370 μg/kg/day just before weaning (Table 6). As these calculations take no account of spillage, adsorption, or degradation of the chemicals (which were not evaluated), these values for intake can be viewed as overestimates of the actual intake. The level of water intake was not affected by treatment (Table 6).

Discussion

The purpose of the present studies was to assess whether exposure of male rats to known estrogenic, environmental chemicals during gestation or neonatal life had any adverse effect on testicular size and spermatogenesis when these animals

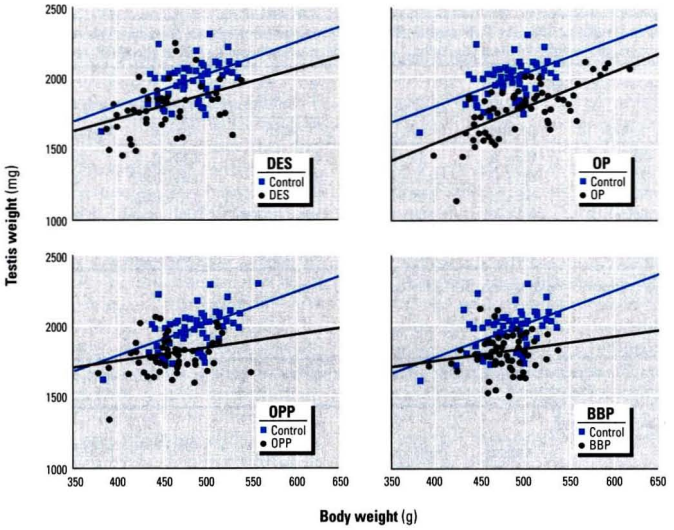

Figure 2. Scatter plots of testicular size versus body weight for animals from studies 2 and 3 combined. The data for controls are shown in each of the panels in comparison to that for animals exposed to diethylstilbestrol (DES), 1000 µg octylphenol/l (OP), octylphenol polyethoxylate (OPP), or butyl benzyl phthalate (BBP). Linear regression lines for the control and each treatment group are shown to aid comparison. Mean values for studies 2 and 3 are given separately in Tables 3 and 4.

reached adulthood. The results are unequivocal in showing that exposure to such chemicals does cause a reproducible and consistent decrease in ultimate testicular size and daily sperm production in rats, an effect which cannot be attributed to any obvious overt toxicity (judged by body weight and kidney weight). Although the chemical-induced decrease in testicular size and daily sperm production only ranged from 5% to 21%, this effect occurred during a relatively short period of treatment and after exposure to relatively low levels of the chemicals. Previous data involving a similar protocol of exposure of rats to the estrogenic pesticide methoxychlor also reported a small reduction in adult testicular size and sperm counts (*33*), and a recent study in trout (*34*) has demonstrated inhibition of testis growth *in vivo* after exposure to estrogenic alkylphenolic chemicals.

Ultimate testicular size in all mammals that have been investigated is determined by the number of Sertoli cells present in the testis, despite the fact that it is the germ cells, rather than the Sertoli cells, which constitute the bulk of the testis (*19,21*). Each Sertoli cell can only support a fixed number of germ cells through their development into spermatozoa, and hence the more Sertoli cells present, the more germ cells present, and thus the larger the testis. The number of Sertoli cells can be increased or decreased experimentally in various animals by a number of treatments, with corresponding changes in testicular size and daily sperm production, and the same relationship appears to apply to men (*19*). Usually, when Sertoli cell number is altered, there is little change in the cross-sectional appearance or size of the seminiferous tubules because Sertoli cell number affects primarily the length, not the breadth, of the tubules (*20,21*). The number of Sertoli cells per testis is determined by the rate and duration of their proliferation, which usually occurs during a precisely timed period that begins in fetal life (shortly after testicular differentiation) and continues into neonatal life for a period that varies according to the species (*19*).

In the present studies, rats were exposed to estrogenic chemicals for either part (study 1) or all (studies 2 and 3) of the period when Sertoli cell proliferation occurs (Fig. 1). If these treatments had reduced the rate of Sertoli cell proliferation, we would expect that, in adult life, the testes would be smaller and daily sperm production will be reduced, but the cross-sectional appearance of the seminiferous tubules would probably be unchanged. Our findings are consistent with the treatments having reduced Sertoli cell number, though morphometric determination of Sertoli cell number will be necessary to determine whether this interpretation is correct; as the expected change in Sertoli cell number would only be of the order of 2–4%, measurements in large cohorts of animals will be necessary to demonstrate this. However, an earlier study (*35*)

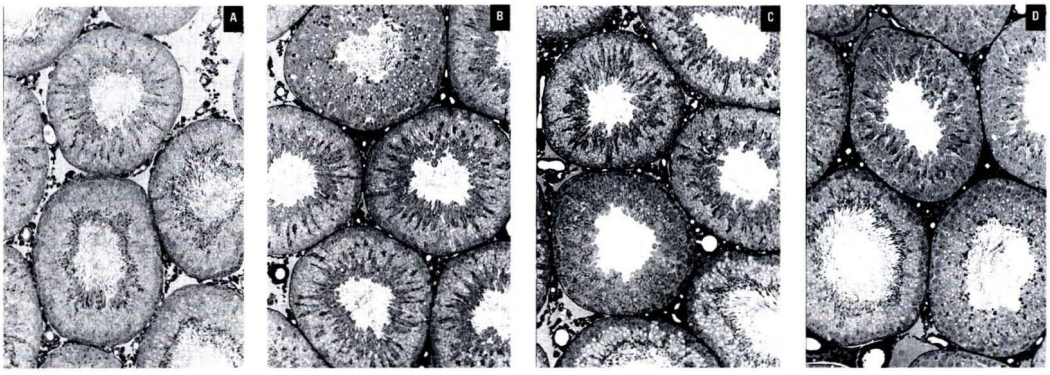

Figure 3. Representative testicular morphology in a control animal (A) and in rats exposed during fetal/neonatal life to diethylstilbestrol (B), 1000 µg octylphenol/l (C), or butyl benzyl phthalate (D) in study 3. (A–D) × 62.

Table 5. Quantitative analysis of the cross-sectional area of seminiferous tubules and the seminiferous epithelium at stages VII–VIII of the spermatogenic cycle in animals from study 3[a]

Treatment group (µg/l)	Animal no.	Seminiferous tubule area ($\times 10^3$ µm^2)	Seminiferous epithelium area ($\times 10^3$ µm^2)
Control	1	77 ± 9	56 ± 6
	2	71 ± 9	51 ± 6
DES (100)	1	94 ± 11	69 ± 6
	2	89 ± 12	68 ± 8
OP (1000)	1	79 ± 7	58 ± 5
	2	74 ± 6	56 ± 5
	3	76 ± 9	61 ± 6
OPP (1000)	1	83 ± 9	61 ± 8
	2	86 ± 12	63 ± 8
BBP (1000)	1	86 ± 11	62 ± 8
	2	86 ± 9	64 ± 7

Abbreviations: DES, diethylstilbestrol; OP, octylphenol; OPP, octylphenol polyethoxylate; BBP, butyl benzyl phthalate.
[a]Rats were exposed to chemical via drinking water throughout gestation and until postnatal day 22. Data are the means ± SD for 10 seminiferous tubules per animal and were based on analysis of perfusion-fixed tissue. Because of the small numbers of animals, no statistical analysis of these data was attempted.

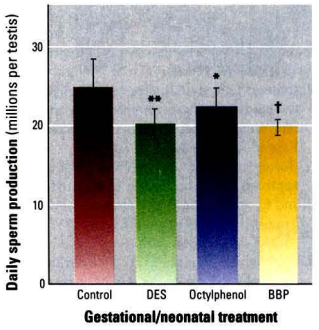

Figure 4. Daily sperm production (means ± SD) in representative control animals (n =12) and in rats exposed during fetal/neonatal life to diethylstilbestol (DES; n = 7), octylphenol (1000 µg/l; n = 18) or butyl benzyl phthalate (BBP; n = 7) in study 3. *p<0.05, **p<0.01, †p<0.001 compared to control.

demonstrated that exposure of rats to di(2-ethylhexyl)phthalate for 5 days during neonatal life resulted in a reduction in Sertoli cell number and some reduction in testis size and sperm production in adulthood; however, the level of phthalate exposure in this study was at least 1000-fold higher than in the present studies.

When animals are exposed to test chemicals, it is possible that toxic effects on organs (e.g., the liver) other than the testis or reproductive axis could lead to a reduction in testicular size and thus daily sperm production as a result of nonspecific effects. Although this possibility cannot be excluded completely, the present data on body weight and kidney weight provide little evidence for any such effect—indeed, in most instances treated animals were larger than the controls. The exception was animals exposed to DES (positive controls), which showed consistent evidence of somewhat lower body weights. The explanation for this effect is not clear from the present studies, but it is likely that lactation may have been impaired by DES (*36*). If this is the case, it is somewhat puzzling why there was no evidence for such effects in animals exposed to any of the estrogenic chemicals, as these caused equal, or even larger, reductions in testicular weight than did DES exposure. This discrepancy could reflect differences in the pharmacokinetics of the chemicals compared with DES.

Table 6. Estimation of the nominal intake of octylphenol (OP) and butyl benzyl phthalate (BBP) between birth and day 21 of postnatal life, based on water consumption in study 3 (means ± SD, N = 6)

Treatment group (µg/l)	Days postnatal[a]	Water intake (ml/48 hr)	Nominal mean intake of chemical (µg/kg/day)[b]
Control	1 + 2	75 ± 22	—
	10 + 11	182 ± 55	—
	20 + 21	243 ± 72	—
OP (1000)	1 + 2	90 ± 36	129
	10 + 11	216 ± 42	309
	20 + 21	257 ± 69	367
BBP (1000)	1 + 2	88 ± 24	126
	10 + 11	192 ± 64	274
	20 + 21	256 ± 43	366

[a]Measurements of water intake were made every 48 hr; hence values are for 2 successive days.
[b]Assumes a body weight of 350 g for lactating females.

Although the estrogenic chemicals tested in the present studies exerted similar effects on testis size and daily sperm production, no evidence is provided that these effects resulted specifically from the estrogenicity of these compounds. The fact that treatment with OPP caused a similar reduction in testicular size as did treatment with OP, despite the fact that OP is nonestrogenic *in vitro* (*14*), could be interpreted as evidence against estrogenicity per se being a common causal mechanism. However, it is possible that, when ingested, OPP is metabolized such that the five ethoxylate groups are cleaved, resulting in the formation of OP. This interpretation is supported by the observation that, whereas short-chain alkylphenol polyethoxylates (such as OPP) do not bind to the estrogen receptor in cell-free systems (*14*), they are estrogenic in cell-based *in vitro* assays (*13,14*) and *in vivo* (*34*). It will be important in future studies to establish unequivocally whether only estrogenic chemicals are able to reduce testicular size and daily sperm production in the manner described here.

Irrespective of whether the reduction in testis size and daily sperm production caused by developmental exposure to OP or BBP resulted from their estrogenicity, the key question is whether these effects have relevance to humans. This is a complex issue which requires detailed dose–response data and measurement of the actual levels of the administered chemicals in the male rats. It would be appropriate to consider whether the nominal level of exposure of rats to OP and BBP in the present studies (which is presumed to be an overestimate of actual exposure levels) bears any relationship to the equivalent level of human exposure. There are little or no data specifically for OP, but there is some information on the environmental levels of the class of compounds to which OP belongs, namely, alkylphenol polyethoxylates, several of which have been shown to be estrogenic (*14*). Reported levels of these compounds in river water vary from the low micrograms per liter (*37*) to tens and hundreds of micrograms per liter (*23,24*), which approach the nominal levels of exposure in the present study. Even tapwater has been reported to contain estrogenic degradation products of both nonylphenol ethoxylate and octylphenol ethoxylate (*38*), although the combined concentration was only about 1 µg/l. However, as alkylphenol polyethoxylates are used widely in industrial and some household detergents and cleaners, in certain plastics, and in many other ways, human exposure via routes other than drinking water are likely.

In the case of BBP, there is more evidence for concern about the possible risk to human health. BBP and other phthalates are the most ubiquitous of all environmental contaminants, primarily because of their use as plasticizers, and human exposure is likely to be high (*25,39,40*). For example, a recent study reported levels of BBP alone as high as 47.8 mg/kg in some foil-wrapped butters (*41*), which would mean that ingestion of 50 g/day of such butter by a 60-kg

woman would lead to an intake of approximately 40 µg/kg/day, which approaches the nominal intake values in the present study. As the levels of total phthalates in other dairy produce can exceed 50 mg/kg (*42,43*), and there are many other possible sources of human exposure to these compounds, the present findings suggest that further studies of the estrogenicity of phthalates should be a priority. There is already a huge literature on the toxicity of phthalates, including their testicular toxicity, but few of the published studies have been able to detect developmental effects similar to those reported here. This is borne out by the reported no-observed effect level (NOEL) of BBP for testicular toxicity in rats of 125–150 mg/kg/day (*44,45*). The fact that, in the present studies, nominal intake of 300-fold lower amounts than the NOEL resulted in around a 10% decrease in testicular weight in two separate studies with a commensurate fall in daily sperm production, argues further that the cause of these decreases differs from the previously reported toxic effects of these compounds on the testis.

The present data do not provide direct evidence of a link between human exposure to environmental estrogens and falling sperm counts in men. However, the findings do provide some preliminary, indirect evidence that exposure of rats to certain environmental estrogenic chemicals during gestation or neonatal life can result in reduced testicular size and sperm production in adulthood. As these effects occurred in rats after only 3–9 weeks of exposure, whereas in men the corresponding window of development (and Sertoli cell proliferation) spans several years, there is at least the theoretical possibility that similar effects in men might be of larger magnitude than those described here for the rat. However, considerably more work, particularly in establishing the likely level of human exposure to estrogenic chemicals, will be necessary if the risk to man from such exposure is to be assessed with any accuracy.

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Effects of Residential Mobility on Individual Versus Population Risk of Radon-related Lung Cancer

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The U.S. Environmental Protection Agency (EPA) does not consider the effects of normal patterns of residential mobility in estimating individual radon-related lung cancer risks. As a consequence, the EPA's population risk estimates may have little bearing on individual risks, and remediation of high-radon homes may have only small health benefits for the individuals who remediate their homes. Through a simulation analysis, we examine the effects of residential mobility on radon exposure and lung cancer risk. Given normal mobility, only 7% of eventual radon-related mortality among current 30 year olds will occur in the 5% currently living in homes above 4 pCi/l (the EPA's action level for remediation), in contrast with an estimate of 31% of deaths when mobility is ignored. Above 10 pCi/l, the no-mobility assumption implies 10.3% of deaths, compared to only 0.4% when mobility is taken into account. We conclude that knowledge of one's current radon exposure is not necessarily a useful guide to one's risk, especially for residents of the high-radon homes targeted for remediation by the EPA. The risk of such individuals is likely to be substantially lower than that implied in the EPA's risk charts. If people currently living in high-radon homes remediate their houses, the majority of the resulting health benefits will accrue to future occupants of their homes. *Key words*: computer simulation, lung cancer, radon, residential mobility, risk analysis. *Environ Health Perspect* 103:1144–1149 (1995)

Models that extrapolate mortality from the high-dose radon exposures of miners to the low-dose exposures typically experienced in homes imply that radon-222 and its decay products cause from 7,000 to 30,000 lung cancer deaths annually in the United States (*1,2*). Although this extrapolation is controversial (*3–6*), the U.S. Environmental Protection Agency (EPA) has labeled radon "probably the biggest public health problem we have" and has called for the testing of every home for radon, with remediation of all homes found to exceed the EPA action level of 4 pCi/l. The EPA believes that full compliance would avoid thousands of radon-related deaths annually (*1*).

The EPA has mounted an aggressive and controversial risk communication program intended to achieve this objective on a voluntary basis (*3,7*). The "Citizen's Guide to Radon" (*8*) includes a table that estimates the risk of lung cancer associated with living in homes with each of several levels of radon exposure. Risks are posed both in terms of lifetime risk of lung cancer per 1,000 people and in comparison with other familiar and more dramatic risks. For example, living in a home with 4 pCi/l is equated to "100 times the risk of dying in an airplane crash" for smokers and "the risk of drowning" for never-smokers.

The EPA's estimates for individual risk depend on the premise that individuals always have lived and always will live in their current residences, or at least that all of their residences will expose them (on average) to the same level of radon (*8,9*). This assumption deviates significantly from actual experience, however. This is particularly important for the group the EPA targets as being at highest risk and who, therefore, according to the EPA, should remediate their homes: the 5% of individuals living in residences estimated to have radon exposures of 4 pCi/l or greater. [The EPA estimates that 7% of homes have radon concentrations of 4 pCi/l or more (*1*). These homes house only 5% of the population, however, reflecting a small negative correlation between population density and radon levels.] Precisely because these homes fall in the upper tail of the distribution, other homes these individuals have occupied previously and will occupy in the future are not likely to expose them to comparably high levels of radon. Rather, on average their past and future homes will be closer to the mean of the distribution, 1.25 pCi/l. Since the average American moves 10–11 times over a lifetime (*10*), exposure to the current high levels of radon will occur during only a small fraction of that lifetime. Thus, typical persons currently exposed to high levels of radon will experience cumulative lifetime exposure reflecting a much lower average rate of exposure. As a consequence, the risk of radon-induced lung cancer for such persons will fall well below that estimated in the EPA's risk charts. Similarly, typical persons currently exposed to low levels of radon will experience cumulative lifetime exposure reflecting a higher average rate of exposure, meaning that their risk will exceed that found in the EPA's charts, although still falling below average.

Mobility, and its consequences for assessment of individual risk, does not alter the EPA's conclusion about the aggregate mortality burden associated with radon (assuming the validity of the model used by the EPA to estimate the relationship between cumulative radon exposure and lung cancer risk, as we do throughout this analysis). Rather, mobility implies that the distribution of individuals' cumulative radon exposure is clustered much more around the mean than is the distribution of residential radon itself. In the model employed in this study, the variance of people's actual average exposures to radon is less than 30% of the variance of radon exposures among residences. As a consequence, the number of Americans at very high risk of radon-related lung cancer is dramatically smaller than would be inferred from a model that does not allow for mobility.

To assess the differences between typical individual risks and those presented in the EPA's risk charts, we used a simulation model that incorporates realistic patterns of residential mobility into the standard radon risk model. Although we produce specific quantitative estimates, our purpose is to develop a sound qualitative understanding of the relative importance of mobility in defining radon-related risk. Other analysts have recognized that mobility complicates determination of the relationship between cumulative exposure to radon and the incidence of lung cancer (*11,12*), but no one has explicitly studied the implications of mobility for individual versus collective risk.

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Methods

Model

To estimate lifetime residential radon exposure and hence lung cancer risk, we developed a model that links three component models: 1) a residential mobility model that describes Americans' typical patterns of movement over a lifetime, 2) a residential radon exposure model that describes the distribution of radon throughout homes in the United States, and 3) a model relating radon exposure to lung cancer risk.

Residential mobility model. For a person of a given age, the residential mobility model uses age-specific mobility rates from Long (*13*), derived from 1980 U.S. Census data, to compute the likelihood of moving from one's current residence to another residence in a given year. There are three possible destinations for a move, in decreasing order of probability: 1) the same county, 2) the same state, but a different county, 3) another state.

Conditional on moving to another state, we use a gravity model (*14*), estimated with 1990 Census data, to evaluate the likelihood of an individual's moving to any particular state within the continental United States. The gravity model yielded the following equations, which estimate the number of people who migrate each year from state i to state j (M_{ij}) (adjusted R^2 = 0.80):

$$\log(M_{ij}) = -15.50 + 0.92\log(Pop_i) + 0.87 \log(Pop_j) - 0.52\mathrm{Log}(Dist_{ij})$$

if i and j are not contiguous states, and

$$\log(M_{ij}) = -3.09 + 0.47\log(Pop_i) + 0.50 \log(Pop_j) - 0.31\log(Dist_{ij})$$

if i and j are contiguous states. Pop_i represents the population of state i and $Dist_{ij}$ the distance between the most populous cities of states i and j.

Conditional on making an interstate move, we then compute the probability of moving from state i to state j, as:

$$P_{ij} = \frac{M_{ij}}{\sum_k M_{ik}}$$

The probability of moving to a specific county, given a target state, was taken to be the proportion of the population of the state living in that county.

Intrastate moves fall into two categories: intracounty and intercounty. Intracounty moves are assigned the age-specific probability of such moves from Long (*13*). Within counties, movers are assumed to move at random with respect to the distribution of residential radon in the county. For intercounty moves, estimated destination probabilities are proportionate to each county's share of the state's 1990 population.

Note that mobility-induced changes in radon levels for an individual may be less random than is implied by our model. For example, apartment dwellers may tend to move to other apartments. The highest radon exposures are typically found in basements and first floors.

Radon distribution model. The distribution of radon levels in homes across the United States was assumed to follow a lognormal distribution, consistent with previous research (*15*). To estimate the geometric mean and standard deviation of the distribution, we used data from the EPA State Residential Radon Surveys (SRRS) (*16*) and the EPA National Residential Radon Survey (NRRS) (*17*).

The SRRS were conducted in 42 states and 6 Indian lands to characterize the distribution of radon in the lowest livable area of owner-occupied homes and to identify areas within the states with elevated levels of radon. The SRRS contain short-term radon screening measurements in over 63,000 randomly selected houses during the winter heating season. The EPA grouped counties within states into areas based on the geology of the states to identify zones of homogeneous radon levels. The EPA claims that the SRRS results provide an accurate representation of the distribution of radon at the state and substate (area) levels, but the estimates from the survey cannot be used to assess health risks directly because winter screening measurements can be up to 3 times higher than annual average measurements (*18*).

The NRRS was designed to provide an estimate of the frequency distribution of annual average radon concentrations in all lived-in levels of residences for each of the 10 EPA national regions. The NRRS contains information collected in 5,694 housing units used by the EPA to assess potential health risks associated with radon (*18*).

Combining the information contained in the two surveys, we estimated parameters to describe the annual average radon concentrations over all lived-in housing levels, at the state and area level. We transformed the EPA data as follows. First, we eliminated 9,169 observations from the SRRS representing negative radon readings (which we considered to be errors), observations above the second floor, and readings from Indian reservations and the states of Alaska and Hawaii. Then we normalized all basement readings in the SRRS to an equivalent first-floor reading, using the average ratio of radon readings between basement and first floor for each EPA national region. Finally, we normalized the resulting radon readings of the SRRS to the average radon levels by EPA national region obtained from the NRRS.

With the transformed data, we estimated the parameters of the lognormal radon distribution for each intrastate area in each of the states included in the survey. To the states that did not participate in the SRRS, we assigned the radon geometric mean of the EPA national region to which they belong and the national geometric standard deviation, both estimated from the NRRS.

Lung cancer risk model. To evaluate the risk at each age a due to radon exposure, $r(a)$, we used the model developed by the Committee on Biological Effects of Ionizing Radiation (BEIR IV) of the National Research Council (*19*), which is the model used by the EPA. A recent study concluded that, as time since exposure increases, the influence of radon likely diminishes somewhat more than is reflected in the BEIR IV model (*2*). The use of an alternative model incorporating this phenomenon and others discussed in the study would change the quantitative details of our results. For the purposes of this research, however, differences in the results produced by BEIR IV and such alternative models are qualitatively indistinguishable, as confirmed by the senior author of the new study (J. Lubin, personal communication). We used BEIR IV to make our work directly comparable to that of the EPA. Thus, we accept all of the EPA's other assumptions, including implicit assumptions about time spent at home and exposure outside the home. The BEIR IV model expresses risk as a linear function of cumulative exposure to radon, subject to adjustments for current age and time since exposure, as given in the following equation.

$$r(a) = r_o(a)[1 + 0.025\,\gamma(a)(W_1 + 0.5\,W_2)]$$

where $r_o(a)$ is the age-specific background lung cancer mortality rate from all causative agents; $\gamma(a)$ is 1.2 when age (a) is less than 55 years old, 1.0 when 55–64 years old, and 0.4 when 65 years or older; W_1 is the radon exposure expressed in working level months (WLM) incurred between 5 and 15 years before this age; and W_2 is WLM incurred 15 years or more before this age. A WLM is defined as the total exposure derived from a radon concentration of 1 working level (WL) for 1

working month (170 hr). A WL is the concentration of radon daughters in 1.0 l of air that results in the ultimate release of 1.3×10^5 MeV of α energy during complete decay. Under typical indoor conditions, a concentration of 200 pCi/l of radon-222 produces the α emission of 1 WL (*20*). Thus, for example, living in a house at 4 pCi/l for 1 year produces exposure over the year of about a quarter of a WLM. Because presentations to the U.S. public about the dangers of radon are usually expressed in picocuries per liter, throughout our paper we have converted WLM into the equivalent constant picocuries per liter.

The impact on radon-related risk of cigarette smoking, the principal cause of lung cancer, is estimated in the equation through differences in the background lung cancer mortality risks, $r_0(a)$, for smokers, former smokers, and never smokers.

Analysis

Using the small-area distributions of radon generated by our model, for each of 100,000 individuals, and for every year of life to age 80, we simulated radon exposure and geographic area of residence. The number of people originally assigned to each geographic area was proportional to the 1990 population of the area. Then, for each location and every year of life, the distribution of cumulative radon exposure was estimated. Finally, combining the estimated cumulative radon distribution, the BEIR IV model, and the distribution of the U.S. population by location and age, we computed radon-induced lung cancer rates by age and region.

This "mobility model" generates distributions of cumulative radon exposures and lung cancer deaths, by age and location. We compare these to distributions of the same variables generated using exactly the same initial conditions, but applying the effective assumption in the EPA's risk tables that there is no mobility: exposures and deaths are the same as if people never move from the residences in which they are born. Hence we refer to this as the "no-mobility model."

Results

The means of the distributions of exposure (and mortality) generated by the two models are approximately the same, the equivalent of residing permanently in a home with 1.18 pCi/l in the case of the no-mobility model and 1.22 for the mobility model. The small difference reflects the fact that people show a slight tendency to move toward higher-radon areas in the mobility model. This is consistent with cross-sectional data from the 1990 Census, which show a slight positive correlation between age and average radon by area of residence. (These estimates differ slightly from the mean for houses, 1.25 pCi/l, due to population-weighting of the housing stock in the models.)

Although the means of the exposure distributions are nearly identical, the variance of the no-mobility model distribution is dramatically larger than that of the mobility model distribution, 3.5 and 1.0 pCi/l, respectively. Given the lognormal distribution, the substantial reduction in variance due to mobility means that a much larger proportion of lifetime exposures clusters within any given interval around the mean value. In the no-mobility model, 5% of lifetime exposures equal or exceed a lifetime of being exposed to the EPA's action level of 4 pCi/l, compared to 2% in the mobility model. Similarly, the very high-risk population with an average exposure equivalent to living permanently at or above 10 pCi/l is 0.7% of all people in the no-mobility model, but only 0.1% in the mobility model.

To illustrate how this occurs, Table 1 presents the lifetime mobility and radon exposure experience of a single individual from our simulation who has the following three traits: a residence with radon exposure of 10 pCi/l at age 30, close to the average number of lifetime moves (10.4), and lifetime radon exposure close to that of the average person in the model exposed to 10 pCi/l at age 30 (the equivalent of living permanently in a home with 2.5 pCi/l). This typical high-exposure individual (at age 30) thus has a lifetime exposure equaling a quarter of that of someone who always lived at 10 pCi/l. The individual's effective lifetime exposure rate falls well below that which would be experienced by living permanently in a house with a radon concentration equal to the EPA's action level. Furthermore, if the individual had followed the EPA's recommendations and successfully mitigated the 10 pCi/l exposure down to 2 pCi/l when he or she first occupied the house, the person's cumulative lifetime exposure would have been the equivalent of living permanently at 2.18 pCi/l, a very modest reduction from the rate of 2.56 pCi/l without mitigation.

Table 1. Residential mobility and radon exposure history of a simulated individual in a home with 10 pCi/l at age 30

Age	Location	Radon exposure (pCi/l)
1	Erie, Pennsylvania	0.64
10	Newark, New Jersey	0.03
15	Somerset, Kentucky	0.70
18	Paducah, Kentucky	2.28
21	Bowling Green, Kentucky	0.40
28	Fargo, North Dakota	10.02
32	Fargo, North Dakota	1.52
36	Des Moines, Iowa	5.44
38	Cedar Rapids, Iowa	5.75
57	Cedar Rapids, Iowa	1.15
Effective average lifetime exposure		2.56

Tables 2 and 3 translate the exposure differences under the two models into differences in the distribution of radon-associated lung cancer deaths, employing a cohort longitudinal perspective (Table 2) and a national cross-sectional perspective (Table 3).

Table 2 shows the relationship between radon readings at age 30 for a cohort of 100,000 individuals, half male and half female, and expected lifetime lung cancer deaths due to radon. The no-mobility model finds that nearly one-third (30.8%) of all radon-related deaths in this cohort will occur in people currently (and in that model, permanently) residing in homes with radon readings at or exceeding 4 pCi/l. This proportion of radon-related deaths is six times the percentage of 30 year olds living in such high-radon homes. In contrast, the mobility model concludes that only 6.8% of deaths will be experienced by persons who are residents of these homes at age 30, representing less than 1.4 times their percentage of the cohort.

Table 2. Lifetime lung cancer mortality attributable to radon in a cohort of 50,000 males and 50,000 females, by exposure at age 30

	Radon, pCi/l				
	≤0.5	0.5–4	>4	>10	Total
No-mobility model	40 (8.7%)	277(60.5%)	141 (30.8%)	47 (10.3%)	458 (100%)
Mobility model	158 (34.9%)	264 (58.3%)	31 (6.8%)	2 (0.4%)	453 (100%)

Table 3. Annual U.S. lung cancer mortality attributable to radon in a typical year, by exposure at time of death

	Radon, pCi/l				
	≤0.5	0.5–4	>4	>10	Total
No-mobility model	1,294 (9.8%)	7,990 (60.3%)	3,970 (29.9%)	1,232 (9.3%)	13,254 (100%)
Mobility model	4,379 (32.3%)	7,683 (56.7%)	1,495 (11.0%)	356 (2.6%)	13,557 (100%)

Even more dramatic, the no-mobility model predicts more than one-tenth of all radon-associated deaths (10.3%) in persons who live, at age 30, in residences with radon concentrations greater than or equal to 10 pCi/l. The mobility model predicts one-twenty-fifth as many radon-related deaths for this group (0.4%). The reason in both instances is that, followed to age 80, more than 90% of 30 year olds living at or above the EPA's action level will have experienced lifetime radon exposure less than they would have if they never moved from their residences at age 30. Table 1 illustrates this phenomenon. This analysis illustrates why it is difficult to find correlations between lung cancer deaths and the radon levels of decedents' homes. Even if cumulative radon exposure is an important cause of lung cancer, the correlation between radon level in decedents' final residences and their lifetime exposures may be too small to observe the underlying relationship.

Because the aggregate number of radon-related deaths is essentially the same in both the mobility and no-mobility models, the differences in deaths for people who experience high exposures at age 30 imply that a much larger proportion of total radon-related deaths is accounted for by people whose residences at age 30 have relatively low radon concentrations. According to the mobility model, more than one-third (34.9%) of eventual radon-related deaths will occur in people in residences below 0.5 pCi/l at age 30, compared to less than one-tenth (8.7%) in the static no-mobility model. In both models, close to 60% of deaths will occur in people currently residing above this minimal level of radon but below the EPA's action level.

We selected age 30 for this cohort analysis to illustrate how mobility affects radon-related risk in young adults, who have the greatest opportunity to reduce cumulative lifetime exposure to radon. The qualitative findings of this analysis hold for all age groups, although with less dramatic quantitative differences for the middle-aged and the elderly. For example, a cohort analysis of 45 year olds finds that 13.6% of eventual radon-related lung cancer deaths would occur among people currently living in homes registering above 4 pCi/l, compared with 6.8% for the 30 year olds (30.8% in the no-mobility model). For individuals living at or above 10 pCi/l, the share of radon-related deaths is 1.8% for the 45 year olds, compared to 0.4% for the 30 year olds (10.3% in the no-mobility model).

Table 3 shows the distributions of lung cancer deaths attributable to radon in a single year for the entire nation, by current level of exposure. Both models predict essentially the same total mortality [slightly in excess of 13,000 deaths, consistent with previous estimates by the EPA (*1*)]. Compared to the mobility model, however, the no-mobility model estimates nearly three times as many deaths occurring in people currently residing in homes above the EPA's action level (29.9% in the no-mobility model, 11% in the mobility model). Above 10 pCi/l, the no-mobility model finds 9.3% of deaths, while the mobility model indicates only 2.6%. In contrast, at exposures below 0.5 pCi/l, the mobility model implies three times more mortality than does the no-mobility model (32.3% and 9.8%, respectively).

The proportional differences in deaths between the no-mobility and mobility models in the cross-sectional analysis in Table 3, though still large, are relatively smaller than in the cohort analysis in Table 2. This results because Table 3 presents a cross-sectional view of the current radon exposures of all people who die in a given year as a result of their cumulative radon exposures over their lifetimes. Current radon exposure at the end of life is much more highly correlated with cumulative exposure than is exposure at age 30 (the subject of the cohort analysis presented in Table 2), because as people age, and hence become more vulnerable to lung cancer, their residential mobility declines.

Mobility affects estimates of mortality by geographic region in a similar manner, causing state and area death rates to cluster more closely around the national mean than does the geographic distribution of radon per se. In analyses not shown here, we found that the no-mobility model overestimated the number of deaths in the five highest-radon states by 25–50%, while underestimating mortality in the lowest five by 11–42%. The differences are proportionately larger for areas within states, reflecting the much larger number of areas, their greater range and variance of radon readings, and the greater mobility among areas than among states.

Figure 1 further illustrates how mobility influences cumulative radon exposure. Each line shows the expected cumulative exposure of an individual of the indicated age, given the individual's location in a state and an area within that state, and given one of four assumptions about how cumulative exposure is generated. Each of the three straight lines shows what would happen to an individual subject to constant (lifetime) exposure at the national, state, or area average. The violet line shows what happens, on average, to residents of an area who have been subject to normal patterns of lifetime mobility. Consistent with the smaller variance of the mobility model, mobility-affected cumulative exposures tend more toward the state and national averages (i.e., away from the area-specific average). The state average is more important than the national in influencing any individual's exposure because intrastate mobility is much more common than interstate mobility.

The two graphs in Figure 1 illustrate phenomena that occur consistently. On average, residents of an area that has a radon reading exceeding the state average in a higher-than-average radon state will experience less cumulative radon exposure with mobility than without it, as seen in Figure 1, which depicts a radon "hot spot" in Pennsylvania, a modestly above-average radon state. For the typical resident of this area, cumulative exposure at any age is approximately two-thirds that of a permanent resident of the area. Because exposure in this hot spot is so high (an average of 4.0 pCi/l) and, specifically, so much above that of the state (1.8 pCi/l) and nation (1.18 pCi/l), normal patterns of mobility decrease cumulative exposure from the hot spot average toward the state and national averages.

Figure 1 shows the implications of living in a low-radon area in a lower-than-average radon state, in this case Louisiana (state average exposure of 0.5 pCi/l). With normal mobility, cumulative exposure will average 1.5 times that of the never-moving resident of the area. In this instance, interstate mobility is sufficiently influential to increase average exposure well above the state's average.

Discussion

This analysis has important implications for both individual and collective societal responses to the hazard posed by radon. To deal with radon, the EPA and many state environmental agencies have emphasized voluntary remedial action based on individual interest in one's own health. Given the effects of normal residential mobility on cumulative radon exposure, however, we find that the EPA's assessment of risk, as conveyed to the public in its risk charts, greatly exaggerates the actual risk faced by most residents of high-radon homes. This conclusion does not depend on the strengths or weaknesses of our specific mobility model. Any reasonable model that incorporates residential mobility will demonstrate a similarly dramatic reduction in the variance of lifetime cumulative exposures and hence the variance of radon-related mortality.

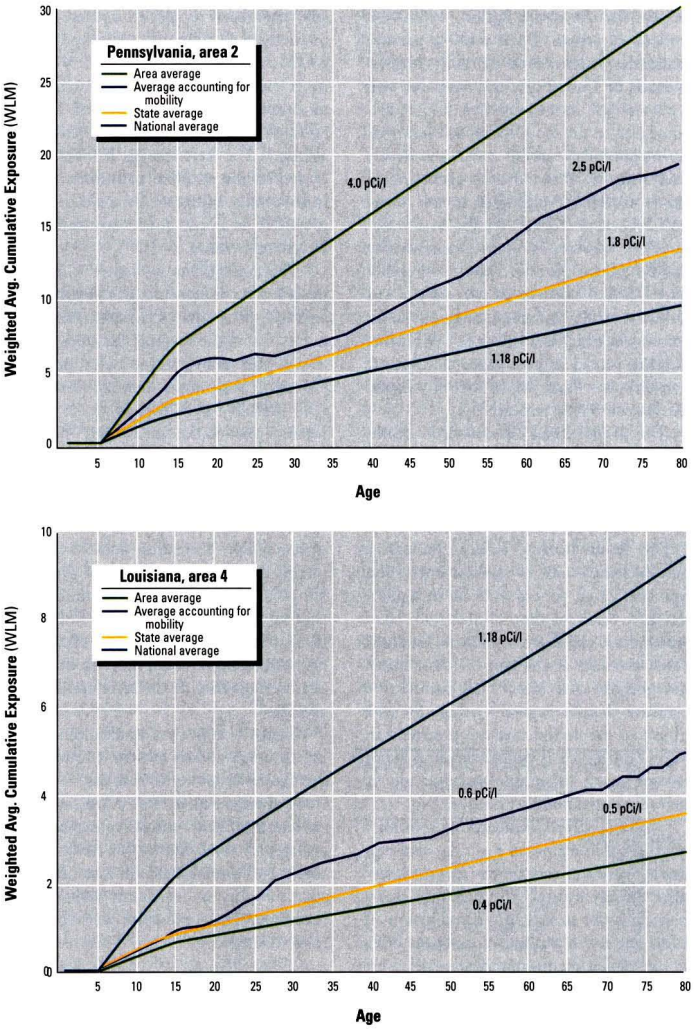

Figure 1. Influence of mobility on cumulative radon exposure, by age, for residents of a high-radon area in Pennsylvania (a high-radon state) and for residents of a low-radon area in Louisiana (a low-radon state). Each of the three straight lines shows what would happen to an individual subject to constant (lifetime) exposure at the national, state, or area average. (The turn in these lines at age 15 reflects the fact that the BEIR IV model applies a weight of 50% to the cumulative exposure incurred 15 years or more before the age at risk, effective after age 15.) The violet line shows what happens, on average, to residents of an area who have been subject to normal patterns of lifetime mobility.

Assuming the validity of the basic risk model (*19*), the results from our mobility model concur with those of the EPA concerning the aggregate mortality burden of radon. Thus, if Americans voluntarily followed the EPA's guidelines, remediating all homes above the action level, thousands of lives could be saved annually, just as the EPA states. However, most of the lives saved would not be those of the people who undertook the effort and expense of remediation. Using our model, for example, we find that if all houses with exposures in excess of 4 pCi/l were remediated today to 2 pCi/l, the level the EPA believes attainable on average (*1*), only between a quarter and a third of the reduction in mortality would occur among those who occupied the homes at the time of the remediation.

Thus, a policy directed at voluntary individual behavior effectively asks current homeowners to subsidize improved health for others. If people truly understand their actual risk and respond in a self-interested manner, only a small fraction of the lives that could be saved through complete compliance with the EPA's preferred approach will be saved. Private, voluntary action is unlikely to have much success dealing with radon.

One might expect that real estate markets would generate widespread testing and mitigation of radon levels, without public intervention. Just as pollution is reflected in the market value of a home (*21*), radon might be expected to affect market value, with low-radon homes selling or renting at a premium. Thus, quite independent of the effects on their own health, homeowners would remediate to the point that the cost equaled the increase in house value.

Generally, however, profit-seeking behavior in real estate markets will have only small effects on testing and mitigation. Increases in house value arising from mitigation will be determined by the discounted valuations that future residents of the house place on the radon level, and those valuations are highly uncertain. They depend on the size of each future household, the age of the residents, the length of time that each future resident will stay in the house, and the residents' preferences for risk reduction. Equally important are the future supply and demand for housing with different radon levels in each local housing market. Where there is an abundant supply of low-radon housing relative to demand, as will be typical, remediation of a given high-radon house will not be profitable, because the equilibrium premium for low radon will be small. Moreover, the equilibrium premium can never exceed the cost of testing and mitigation, because any future owner can choose to test and remediate. Thus, all the financial risk is on the downside. The best possible outcome for current owners who test and remediate is that they will recover much of their radon investment in higher property values. Given the many sources of uncertainty in determining future valuations, however, as well as the abundant supply of low-radon homes, the vast majority of remediators are likely to recover only a small fraction of their investment, if anything at all.

The policy implication is strong, if likely difficult for the EPA and perhaps the public to accept: assuming, as the evidence suggests, that radon constitutes a genuine threat to health, the effects of mobility make it primarily a public health problem, rather than an individual risk problem. Despite formidable political obstacles (*3,22,23*), collective action may well be the only appropriate and effective approach to

dealing with radon. Short of legal requirement of universal near-term testing and mitigation, collective action could (and in a few jurisdictions does) take such forms as revision of building codes to ensure minimal radon exposure and requirement of radon testing and, where appropriate, mitigation at the time a residence is sold. These more moderate policies, although more politically feasible, would take more time to achieve a given reduction in mortality than would policies with broader coverage.

Ultimately, the decision of whether or not to adopt radon testing and mitigation policies, at either the individual or societal level, must rest on consideration of the costs of testing and mitigation, as well as the health benefits. To date there have been only a handful of cost–benefit evaluations of radon intervention strategies (*1,24,25*); we are examining the issue in ongoing research. It is virtually certain, however, that there are health, economic, and technological circumstances under which formal regulatory policies would pass a cost–benefit test, while reliance on individual voluntary action would consistently fail.

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Hormone Replacement Therapy May Reduce the Return of Endogenous Lead from Bone to the Circulation

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Hormone replacement therapy (HRT) in postmenopausal women suppresses the increase in bone resorption expected as circulating levels of endogenous estrogen decline. We tested the hypothesis that bone lead content might remain elevated in women on HRT. Fifty-six women who at recruitment were on average 3.5 years postmenopausal were placed on calcium supplementation. Six months later, 33 of these women were prescribed either low dose or moderate dose hormone replacement in addition to the calcium supplementation. After approximately 4 years of hormone replacement, lead content was measured at the tibia and calcaneus by *in vivo* fluorescence excitation, and lead concentrations were measured in serum, whole blood, and urine. Women not taking hormones had significantly lower lead concentrations in cortical bone compared to all women on HRT ($p = 0.007$). Tibia lead content (mean ± SD) for women on calcium only was 11.13 ± 6.22 μg/g bone mineral. For women on HRT, tibia bone lead was 19.37 ± 8.62 μg/g bone mineral on low-dose HRT and 16.87 ± 11.68 μg/g bone mineral on moderate-dose HRT. There were no differences between groups for lead concentrations measured in trabecular bone, whole blood, serum, or urine. Hormone replacement maintains cortical bone lead content. In women not on HRT, there will be a perimenopausal release of lead from bone. *Key words*: bone resorption, endogenous lead, estrogen, hormone replacement, menopause. *Environ Health Perspect* 103:1150–1153 (1995)

The concentration of lead in bone apparently increases steadily throughout life. By the time a woman reaches the age of menopause she can expect to have a bone lead content of about 12 μg/g mineral in cortical bone, with somewhat higher levels in trabecular bone (*1*). Because about 95% of body lead resides in the skeleton, a typical endogenous lead burden for a menopausal woman will be 30 mg.

Endogenous lead has access to the circulation through the normal processes of mineral exchange and bone turnover. The concentration of lead in bone can decrease if the rate of lead transfer from bone to the circulation during mineral exchange and bone turnover is greater than the rate of lead transfer from the circulation to bone. Such a situation can best be achieved by reducing the rate of ingestion of lead such that the concentration of lead in newly formed bone will fall and the amount of lead available for exchange from the circulation is reduced.

Recent declines in blood lead levels achieved by reducing environmental lead exposures (*2*) should also produce reductions in bone lead content by decreasing uptake of lead into bone. Superimposed on this effect, the increase in bone resorption associated with menopause should increase the release of lead from bone. Since hormone replacement therapy (HRT) opposes the increase in both bone resorption and mineral exchange, it would be expected that HRT would maintain bone lead concentration and suppress the transfer of endogenous lead to the circulation. Women on HRT should have higher concentrations of bone lead and lower concentrations of plasma lead. To test this possibility, we compared the concentrations of lead in bone, serum, urine, and whole blood for subjects who were either on calcium supplementation or on HRT plus calcium supplementation.

Materials and Methods

The subjects who volunteered for these measurements were participants in a study of the impact of HRT on bone mass. The protocol for that study, which has been described previously (*3*), was approved by the Research Advisory Committee of the Faculty of Health Sciences, McMaster University. White women, who were typically between 1 and 5 years postmenopause, were recruited from the local community and placed on calcium supplementation (500 mg/day). Six months later each woman chose either to add HRT to the calcium supplementation or to remain on calcium alone. Those given HRT received either a low-dose, continuous or a moderate-dose, cyclical regime. The low-dose regime consisted of 0.3 mg/day equine estrogen (Premarin) and 2.5 mg/day medroxyprogesterone (Provera). The moderate-dose, cyclical regime was 0.625 mg/day Premarin for days 1–25 and 5 mg/day Provera for days 16–25 of a monthly cycle. After 2 years, the moderate-dose, cyclical regime was changed to a moderate-dose, continuous regime which consisted of 0.625 mg/day Premarin and 2.5 mg/day Provera.

Longitudinal measurements of bone mass were made at the one-third radius and lumbar spine using photon absorptiometry (*3,4*). These sites were selected as representative of cortical and trabecular bone. There were no statistically significant differences in age, weight, height, years since menopause, or bone-mass variables between the three groups at entry into the study (*4*). There were also no differences between groups for variables such as smoking, drinking, diet, or parity (*5*). A questionnaire designed to identify occupational, recreational, and environmental exposure to lead was administered to each subject at the time of bone lead measurement. No differences in lead exposure could be detected between groups.

The calcium-only group consisted of 23 women, 2 of whom originally chose HRT but declined their assigned therapy and continued on calcium only. One additional subject was transferred to the calcium-only group after less than 3 months on low-dose HRT. There were 33 women who chose calcium supplementation plus HRT. Sixteen of these were on low-dose, continuous HRT and 11 were on moderate-dose HRT. The mean duration of HRT for subjects on the low-dose regime was 4.10 years (SD = 0.22 years). For the subjects on moderate-dose HRT, the mean duration was 3.90 years (SD = 0.37 years). A fourth group of six women did not comply with their assigned HRT regime between the times of enrollment and the measurement of lead concentrations. Their mean duration of exposure to hormones was 2.15 years (SD = 0.49 years).

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The lead concentrations of bone, blood, serum, and urine were measured during the fourth or fifth year of HRT. Bone lead was measured at the mid-tibia and the right calcaneus in all subjects using the instrument described by Gordon et al. (*6*). These sites are considered to be representative of cortical and trabecular bone. Briefly, the bone was exposed to a beam of 88-keV photons emitted from a ^{109}Cd source. Characteristic X-rays emitted from any lead present in the bone are detected by a high-purity germanium detector. At the same time, 88-keV photons coherently scattered by bone mineral into the detector are monitored. With appropriate calibration, the system yields a measurement of bone lead content in units of micrograms of lead per gram of bone mineral. The standard deviation of repeated *in vivo* measurements is typically between 2 and 5 μg Pb/g mineral at the tibia (*7*). For calcaneal measurements the uncertainty is greater (typically 8 μg Pb/g mineral) because a smaller mass of bone is measured and because the overlying tissue is thicker at the calcaneal site compared to the tibial site.

Serum and urine lead was measured using a technique developed by Bowins and McNutt (*8*). A tracer quantity of enriched ^{204}Pb was added to each serum or urine sample, which was acidified with nitric acid. Aliquots were then volatilized in a graphite furnace and introduced into an inductively coupled plasma mass spectrometer. The technique yields a standard deviation of 0.006 μg/dl at a lead concentration of 0.03 μg/dl.

Blood lead was measured by flameless atomic absorption spectrophotometry. The coefficient of variation from measurements repeated over a 3-month period for a control sample with a lead concentration of about 60 μg/dl was 4%.

We used one-way analysis of variance to search for differences between groups in the concentrations of lead in blood, serum, and urine as well as for the bone lead content of the tibia and calcaneus. Two-sample *t*-tests were used to test for significant differences between subjects who had never taken hormones and those who had complied fully with their regime. Only those subjects with complete data sets were included in the analysis, and differences associated with a p-value <0.05 were considered significant.

Results

As reported elsewhere (*3*), moderate-dose HRT increased bone mineral mass at the spine, while low-dose HRT eliminated a rate of loss of 0.3 g mineral/year observed on calcium alone. HRT produced no differences in rates of change of mineral mass at the radius.

Complete results for whole blood and serum lead concentrations, 24-hr urine excretion of lead, and the lead content of the tibia and calcaneus were obtained in 22 subjects in the calcium-only group, 15 in the low-dose HRT group, 11 in the moderate-dose HRT group, and 6 subjects in the partial HRT group. Two serum samples, one from the calcium-only group and one from the low-dose HRT group, were lost to analysis due to a technical failure of the mass spectrometer. The mean values and standard deviations for the measured variables are given in Table 1 for each group of subjects. One-way analysis of variance showed that tibia lead content was significantly lower in the group of subjects not taking hormones ($p = 0.049$). No other statistically significant difference existed between groups. The mean tibia lead content is shown for each group in Figure 1.

If the subjects are assigned to two groups, one of which is composed of subjects who had never taken hormones ($N = 22$), the other of those subjects who fully complied with their hormone replacement regime ($N = 26$), a two-sample *t*-test shows a significant difference in tibia bone lead ($p = 0.007$) but no difference in calcaneal bone lead ($p = 0.47$).

Discussion

These results suggest that postmenopausal women who are on HRT will have a greater skeletal lead burden than women not on hormones. The excess lead is located within cortical bone, rather than trabecular bone, probably because the retention time in cortical bone is at least twice that of trabecular bone (*9*). This is consistent with measures of blood lead made during the second National Health and Nutrition Examination Survey (NHANES II) (*10*). In 849 women between 40 and 60 years of age, blood lead concentration postmenopause was 13.0 μg/dl, whereas in premenopausal women it averaged 11.9 μg/dl. The increased blood lead is thought to be due to release of lead from bone as a consequence of menopause-related increases in bone turnover. In our study, hormone replacement did not produce a significant difference in blood lead concentration. The average concentrations in our subjects were approximately one-third those observed in NHANES II but were similar to the values reported in NHANES III (*11*). Hormone replacement prevents the menopause-associated increase in bone turnover, and lead would be expected to remain in the skeleton. The menopause-related increase in blood lead is greater for white than for black women because the increase in bone turnover is greater for white women (*12*). Especially at risk for increased blood lead after menopause are white women who have had no children. This is thought to be because skeletal lead burdens would have been reduced by a postpartum increase in bone turnover in lactating women (*13,14*), resulting in less lead being available for mobilization after menopause (*15*).

The transfer of calcium between bone and the circulation takes place through the processes of mineral exchange and bone turnover. The purpose of exchange and turnover is to release minerals to the circulation, repair damaged bone, replace effete

Table 1. Mean values (SDs) for the measured variables

Variable	Hormone replacement therapy[a] None ($N = 22$)	Low dose ($N = 15$)	Moderate dose ($N = 11$)	Partial HRT ($N = 6$)
Blood lead (μg/dl)	4.60 (1.59)	4.08 (1.60)	5.22(3.36)	3.35 (1.32)
Serum lead (μg/dl)	0.074 (0.048)	0.106 (0.065)	0.061 (0.025)	0.086 (0.057)
Urine lead (μg/day)	2.77 (1.70)	2.23 (0.90)	2.93 (2.20)	2.10 (1.78)
Tibia lead (μg/g)	11.13 (6.22)	19.37 (8.62)	16.80 (11.68)	14.66 (8.41)
Calcaneus lead (μg/g)	21.12 (13.55)	24.02 (10.88)	23.83 (14.18)	17.63 (16.09)

[a]Low dose consisted of 0.3 mg/day estrogen and 2.5 mg/day medroxyprogesterone; moderate dose consisted of 0.625 mg/day estrogen on days 1–25 and 5 mg/day progesterone on days 16–25 for 2 years, then 0.625 mg/day estrogen and 2.5 mg/day progesterone for 2 years; partial therapy included women who did not comply with their assigned regime. See Materials and Methods for details.

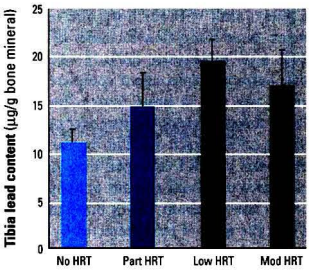

Figure 1. Mean tibia lead content and standard error of the mean for postmenopausal women who have never used hormone replacement therapy (No HRT) and those who partially complied (Part HRT) or fully complied with a low dose (Low HRT) or moderate dose (Mod HRT) hormone replacement regime.

bone and reorganize bone in response to altered mechanical environments. It has been estimated that each day the mass of calcium involved in exchange is about 10 times the mass of calcium involved in turnover (*16*). In recently proposed compartmental models of lead metabolism, the assumption is made that these same processes are responsible for the movement of lead between the circulation and bone (*17,18*). Lead on bone surfaces is considered to be rapidly exchangeable, whereas lead distributed throughout the bone volume is in exchange with bone surface lead. In reality, not all bone lead will be equally exchangeable with circulating lead, but there is likely to be a spectrum of accessibility ranging from freely exchangeable to non-exchangeable. The latter will correspond to lead fixed within bone crystals and available only after osteoclastic resorption of bone (*19*).

The exchange of calcium between bone and the circulation is under the influence of parathyroid hormone (*20*). The sensitivity of postmenopausal bone to the effects of parathyroid hormone is reduced by estrogen replacement. That is, for the same parathyroid hormone concentration, the estrogen replete woman will have lower serum calcium (*21,22*). The rate of resorption of bone is decreased by estrogen through a reduction in the activation frequency of new remodeling cycles with an increase in the mean age of the bone (*23–25*). It is known that elements that are bone-volume seekers such as lead and the alkaline earth elements are retained to a greater extent in older bone than in newly formed bone (*26*).

The relative contributions of exchange and turnover to the reduction in bone lead content after menopause can be estimated from our data. In premenopausal women the rate of bone turnover is about 150 mg Ca/day, and, without hormone replacement, bone resorption increases by about 20% after menopause (*27*). If we assume that hormone replacement completely prevents any rise in bone resorption, then HRT should prevent the turnover of about 30 mg calcium each day. During the course of 4 years of HRT, the total mass of calcium protected could amount to about 44 g of calcium or about 100 g bone mineral. Thus, the mass of lead protected by a hormone-induced reduction of bone turnover could amount to almost 2 mg. The observed difference in concentration at the tibia between the calcium-only group and the HRT groups is about 6 μg/g bone mineral or, for a bone mass of 2000 g, a total mass of lead of 12 mg. Thus, about 10 mg of lead remains in bone because of a hormone-induced reduction in exchange. It should be noted that this estimate neglects the lead present in trabecular bone and also any differences in bone mass produced by HRT.

This study is an observational report on a convenience sample of subjects. It is limited by its cross-sectional nature, and it is conceivable that differences in bone lead may have existed before HRT was started. The fraction of bone lead released after menopause may be greater in some women than indicated by this work. All women in this study took calcium supplementation, and it is possible that in subjects with calcium deficiency, the mass of bone involved in exchange and turnover processes may be even greater. In addition, hormone replacement did not start immediately after endogenous estrogen levels fell. The mean time after menopause that women started HRT was 3.5 years (*4*). Consequently, all subjects in this study suffered a period without HRT and therefore will have lost some lead from the bone. The woman who is likely to release the most lead from her skeleton is the woman who has accumulated considerable lead from her environment, who suffers a significant perimenopause increase in bone turnover and mineral exchange, and who may have a poor calcium intake.

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January

5–11 January, Fri–Thu. Integrins and Signaling Events in Cell Biology and Disease, Keystone, Colorado. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

5–11 January, Fri–Thu. Molecular and Developmental Biology of the Extracellular Matrix, Keystone, Colorado. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

5–11 January, Fri–Thu. Small GTP-binding Proteins and Growth Factor Signaling Pathways, Tamarron, Colorado. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

5–11 January, Fri–Thu. Exploring and Exploiting Antibody and Ig Superfamily Combining Sites, Taos, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

8–14 January, Mon–Sun. Oxidant Stress: From Molecules to Man, Santa Fe, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

11–17 January, Thu–Wed. The Cell Cycle, Taos, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

15–21 January, Mon–Sun. Blood Stem Cell and Bone Marrow Transplants, Keystone, Colorado. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

17–23 January, Wed–Tue. Molecular Biology of HIV, Taos, New Mexico. Information: Keystone Symposia Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

23–29 January, Tue–Mon. Hepatitis C and Beyond, Burlington, Vermont. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

23–29 January, Tue–Mon. Tissue Engineering, Taos, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

23–29 January, Tue–Mon. Wound Repair in Context, Taos, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

29 January–4 February, Sun–Sat. The Molecular Biology of the Cardiovascular System, Keystone, Colorado. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

29 January–4 February, Sun–Sat. Breast and Prostate Cancer: Basic Mechanisms, Taos, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

February

1–7 February, Thu–Wed. Cell Polarity, Lake Tahoe, California. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

4–10 February, Sun–Sat. Ion Channels as Therapeutic Targets, Tamarron, Colorado. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

4–10 February, Sun–Sat. Gene Therapy for Hematopoietic Stem Cells in Genetic Disease and Cancer, Taos, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

4–10 February, Sun–Sat. Cell Migration, Santa Fe, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

8–13 February, Thu–Tue. AMSIE '96, Baltimore Convention Center, Baltimore, Maryland. Information: Stephenie Brooks, American Association for the Advancement of Science, 1333 H Street, NW, Washington, DC 20005, (202) 326-6711

8–14 February, Thu– Wed. Neural Peptides, Lake Tahoe, California. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

8–14 February, Thu–Wed. Inductive Interactions during Vertebrate Embryogenesis, Hilton Heald Island, South Carolina. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

10–16 February, Sat–Fri. Molecular Mechanisms in DNA Replication and Recombination, Taos, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

10–16 February, Sat–Fri. Cell Biology of Virus Entry, Replication and Pathogenesis, Santa Fe, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

13–14 February, Tue–Wed. Environmental Excellence Conference, New York City. Information: Arthur D. Little, Inc., Corporate Marketing and Communications, Acorn Park, Cambridge, MA 02140-2390, (617) 498-5896, FAX (617) 498-7161

16–22 February, Fri–Thu. Molecular Regulation of Platelet Production, Taos, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

16–22 February, Fri–Thu. The Hematopoietic Microenvironment, Taos, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

17–21 February, Sat–Wed. Biophysical Society Annual Meeting, Baltimore Convention Center, Baltimore, Maryland. Information: FASEB, Office of Scientific Meetings and Conferences, 9650 Rockville Pike, Bethesda, MD 20814-3998, (301) 530-7010, FAX (301) 530-7014

22–28 February, Thu–Wed. Exploring and Exploiting Antibody and Ig Superfamily Combining Sites, Taos, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

22–28 February, Thu–Wed. Molecular Helminthology: An Integrated Approach, Santa Fe, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

March

1–7 March, Fri–Thu. Molecular Approaches to the Function of Intercellular Junctions, Lake Tahoe, California. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

1–7 March, Fri–Thu. Viral Genome Replication Tamarron, Colorado. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

4–6 March, Mon–Wed. Building Energy, (1st International Solar Electric Buildings Conference, 12th Annual Quality Building Conference, and RENEW'96), Copley Plaza Hotel, Boston, Massachusetts. Information: Northeast Sustainable Energy Association, 50 Miles Street, Greenfield, MA 01301, (413) 774-6051, FAX:(413) 774-6053

8–14 March, Fri–Thu. The Extracellular Matrix of Plants: Molecular, Cellular and Developmental Biology, Tamarron, Colorado. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

10–14 March, Sun–Thu. Society of Toxicology, Anaheim Convention Center, Anaheim California.

How to Reach Us

If you have a Calendar, Fellowships, Grants, & Awards, or Position Announcements item you would like included, follow the instructions below.

For a Calendar Event—Please provide the name of the event, the dates, location, and who to contact for further information including FAX number and BITNET/Internet address if possible. The entries in this section are brief. If you would like us to advertise additional information about your event, such as an overview of the contents and speakers, we have limited space available for public service advertising.

Fellowships, Grants, & Awards or Position Announcements—Please provide a concise description of the fellowship, grant, or award requirements or the position announcement including the application address and deadline. Send an electronic version via electronic mail or disk if possible.

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How to Submit Material to EHP—For camera-ready copy, computer disk, or text submissions, send your material to EHP Announcements, NIEHS/EHP, MD WC-01, PO Box 12233, Research Triangle Park, NC 27709. FAX (919) 541-0273 BITNET/Internet address: burton_l@niehs.nih.gov

Information: Trish Strong Society of Toxicology, 1767 Business Center Drive, Suite 302, Reston, VA (703) 438-3115, FAX: (703) 438-3113

10–16 March, Sun–Sat. Posttranscriptional RNA Processing, Hilton Head Island, South Carolina. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

11–17 March, Mon–Sat. Molecular Basis for Drug Resistancein Bacteria, Parasites and Fungi, Park City, Utah. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

15–21 March, Fri–Thu. Signaling in Neuronal Development, Differentation and Degeneration, Tamarron, Colorado. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

17–23 March, Sun–Sat. Steroid/Thyrid/Retinoic Acid Gene Family, Lake Tahoe, California. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

17–23 March, Sun–Sat. Transcriptional Mechanisms, Taos, New Mexico. Information: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

20–26 March, Wed–Tue. Lymphocyte Activation, Hilton Head Island, South Carolina. Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

25–31 March, Mon–Sun. Proteolytic Enzymes and Inhibitors in Biology and Medicine, Keystone, Colorado. Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

26 March–1 April, Tue–Mon. Immunopathogenesis of HIV Infection, Hilton Head Island, South Carolina. Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

27 March–2 April, Wed–Tue. Signal Transduction through Tyrosine Kinases, Taos, New Mexico. Keystone Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

31 March–3 April, Sun –Wed. American Society of Mechanical Engineers Solid Waste Processing Division Seventeenth Biennial Conference, Trump Regency Hotel, Atlantic City, New Jersey. Information: Richard Will, The Coordinate Group, Inc., Box 3356, Warrenton, VA 22186-1956 (800) 627-8913, FAX (703) 349-4540

April

14–17 April, Sun–Wed. Experimental Biology '96, Washington, DC Convention Center, Washington, DC. Information: FASEB Office of Scientific Meetings and Conferences, 9650 Rockville Pike, Bethesda, MD 20814-3998, (301) 530-7010, FAX (301) 530-7014

21–23 April, Sun–Tue. American Association for Cancer Research, Washington, DC Convention Center, Washington, DC. Information: AACR Public Ledger Building, Suite 816, 150 South Independence Mall West Philadephia, PA 19106-3483 (215) 440-9300, FAX: (215) 440-9313

26 April–May 3, Fri–Fri. American Occupational Health Conference, San Antonio Convention Center, San Antonio, Texas. Information: Nancy Kay Olson, Director of Conferences & Meetings, American Occupational Health Conference, 55 W. Seegers Road, Arlington, Heights, IL 60065, (708) 228-6850, ext. 156, FAX (708) 228-1856

May

4–7 May, Sat–Tue. Annual Meeting of the Council of Biology Editors, Portland Hilton, Portland, Oregon. Information: Cindy Clark, Council of Biology Editors, 11 South LaSalle Street, Suite 1400, Chicago, IL 60603-1210, (312) 201-0101, FAX (312) 201-0214

6–10 May, Mon–Fri. 1996 International Conference on Incineration and Thermal Treatment Technologies, Savannah, Georgia. Information: Lori Barnow,Office of Environment Health, and Safety, University of California, Irvine, CA 92717-2725 (714) 824-5859

19–22 May, Sat–Wed. Fourth International Symposium on Metal Ions in Biology and Medicine, Tarragona/Barcelona, Catalonia, Spain. Information: Mercedes Gómez, Laboratory of Toxicology and Biochemistry, School of Medicine, c/San Lorenzo 21, 43201 REUS, Spain 34 77 759 376, FAX 34 77 759 322

20–22 May, Mon–Wed. International Symposium on Work in the Information Society, Marina Congress Center, Helsinki, Finland. Information: Work in the Information Society, Finnish Institute of Occupational Health, Topeliuksenkatu 41 a A, FIN-00250 Helsinki, Finland, FAX: 358 0 4747 548, e-mail: slil@occuphealth.fi

June

2–6 June, Sat–Thu. ASBMB/ASIP/AAI Joint Meeting, Ernest N. Morial Convention Center, New Orleans, Louisiana. Information: FASEB Office of Scientific Meetings and Conferences, 9650 Rockville Pike, Bethesda, MD 20814-3998. (301) 530-7010, FAX (301) 530-7014

July

14–17 July, Sun–Wed. The Coastal Society, Fifteenth International Conference, Seattle, Washington. Information: The Coastal Society 15th International Conference, c/o Washington Sea Grant Program, 3716 Brooklyn Avenue, NE, Seattle, WA 98105, (206) 685-1108.

August

12–14 August, Sat–Mon. Occupational Health and Safety in Progress; Northern-Baltic-Karelian Regional Symposium, Lappeenranta, Finland. Information: Secretariat, Occcupational Health and Safety in Progress, c/o Finnish Institute of Occupational Health, Anneli Vartio, Topeliuksenkatu 41 a A, FIN-00250 Helsinki 358 0 4747 345, FAX 358 0 4747 548, e-mail: avar@occuphealth.fi

September

11–13 September, Wed–Fri. Biological Monitoring in Occupational Environmental Health, Espoo, Finland. Information: Biological Monitoring, c/o Finnish Institute of Occupational Health Symposium Secretariat, Topeliuksenkatu 41 a A FIN-00250 Helsinki, Finland, 358-0-47-471, FAX 35804747548

15–20 September, Sat–Fri. International Congress of Occupational Health, Stockholm, Sweden. Information: Arne Wennberg, Secretary General ICOH'96, National Institute of Occupational Health, S-171 84 SOLNA, Sweden, (+46) 8 730 91 00, FAX (+46) 8 82 05 56

October

20–24 October, Sun–Thu. Second World Congress on Alternatives and Animal Use in the Life Sciences, Utrecht, The Netherlands. Information: World Congress Alternatives 1996, FBU Congress Bureau, P.O. Box 80.125, 3508 TC Utrecht, The Netherlands 31.30.53.5044/2728 FAX 31.30.53.3667, e-mail: l.donkers@pobox.ruu.nl

December

7–11 December, Sat–Wed. Sixth International Congress on Cell Biology/Thirty-Sixth American Society for Cell Biology Annual Meeting, Moscone Convention Center, San Francisco, California. Information: The American Society for Cell Biology, 9650 Rockville Pike, Bethesda, MD 20814-3992, (301) 530-7153, FAX (301) 530-7139, e-mail: ascb.info@ascbfaseb.org

1997

August

24–29 August, Sun–Fri. Seventeenth International Congress of Biochemistry and Molecular Biology 1997 Annual Meeting American Society for Biochemistry and Molecular Biology, Moscone Convention Center, San Francisco,California. Information: Congress Secretariat, 17th International Congress for Biochemistry and Molecular Biology, 9650 Rockville Pike, Bethesda, MD 20814-3996, FAX (301) 571-1824, e-mail: 171UBMB@asbmb.faseb.org.

Fellowships, Grants & Awards

Postdoctoral Fellowships in Toxicology/Epidemiology

Postdoctoral fellowships are available in a unique NIH-sponsored training program in toxicology/epidemiology of respiratory tract disease caused by environmental agents. Conducted jointly by the Inhalation Toxicology Research Institute (ITRI) and the Department of Medicine, University of New Mexico (UNM), the program provides training focus in either laboratory or epidemiology-based research with cross-training in the other discipline. The program develops research skills for investigative careers, incorporating interdisciplinary laboratory-human extrapolation. ITRI-based participants will undertake postdoctoral laboratory research and receive lecture and field cross-training in epidemiology and toxicology jointly with UNM-based fellows in epidemiology. Programs are tailored to individuals. Laboratory research or pathogenesis of disease can focus on one of several disciplinary areas, including cell biology, molecular biology, biochemistry, immunology, pathology, physiology, toxicology, radiobiology, aerosol science, or mathematics modeling, depending on interests and qualifications. Annual stipend of $30,800 plus health insurance, tuition and travel costs.

Contact: Dr. David E. Bice, Education Coordinator Inhalation Toxicology Research Institute, PO Box 5890, Albuquerque, NM 87185, or call (505) 845-1257 for application materials. We are an Equal Opportunity Employer.

European Cancer Centre Two-Year Fellowships for Oncologists

The European Cancer Centre was founded in Amsterdam in 1991. Its major goal is to improve oncologic care by developing an international research network through collaborative research. The ECC focuses on organizing early clinical research, placing emphasis on translating basic laboratory research into clinical phase I and phase II studies.

The ECC invites young clinical specialists with a proven interest in research to apply for the ECC Fellowship Programme, which is funded by trade and industry. A substantial part of this two-year fellowship will be spent in the laboratory, performing basic research. The fellows work in the Amsterdam oncologic centres participating in the European Cancer Centre under the supervision of the principal investigator of the study.

Eligibility Criteria: Candidates must meet the following conditions:

- Maximum age 35 years
- Medical degree with specialization in oncology
- Proven research skills
- At least two publications with first authorship in the international peer reviewed literature
- Guaranteed position in home institute after completion of the fellowship.

It is recommended to support an application with letters of reference from present and former supervisors and/or mentors.

Application Procedures: The Research Groups of the European Cancer Centre submit their research proposals and request for a fellow. The ECC Scientific Board, chaired by Professor H.M. Pinedo, MD, PhD, evaluates the proposal on scientific value and innovative importance. After approval of the project, fellowship candidates can be recommended by members of an ECC Research Group. Those interested can also request information about available projects and send in their application.

To apply, candidates must submit: 1) a letter of application with the completed ECC Fellowship Programme Application Form, 2) a short curriculum vitae listing at least three specialists/scientists willing to supply a reference, 3) no more than five relevant full publications, 4) a letter stating a guaranteed permanent position at the home institute upon return.

Selection Procedure: Twice a year, on March 1 and September 1, the applications are reviewed by a selection committee, considering the aforementioned criteria. Selected fellows are then informed of the available research projects best suiting their curriculum and are introduced to the principal investigators.

They will also be invited for interviews with the selection committee and to give a presentation of their work. After the second deliberation round, the selected fellows will be invited to start their two-year fellowship in Amsterdam within a foreseeable time.

Salary and Stipend: A salary and stipend are provided which include all costs of housing and living. The Board encourages the home institute to provide additional funding.

Contact: European Cancer Centre, PO Box 7057, NL-1007 MB Amsterdam, The Netherlands, 31 20 644 4500/4550, FAX 31 20 644 4551.

Earthwatch Field Grants

The Center for Field Research invites field biologists to apply for an Earthwatch field grant. The Center for Field Research encourages and evaluates proposals for support by its international affiliate Earthwatch. Earthwatch is a private, nonprofit organization established in 1971 to fund field research, promote communication between scholars and the public, improve science education, and enhance public understanding of pressing environmental and social problems.

Through its system of participant funding, Earthwatch supports both basic and applied research. Proposals are welcome for field studies on almost any life science topic, in any country, by advanced scholars of any nationality. The research must have scientific merit and feasibly and constructively involve nonspecialist Earthwatch volunteers in the research tasks.

Earthwatch field grants average $20,000. These funds are derived from the contributions of Earthwatch members who enlist for the opportunity to join scientists in the field and assist with data collection and other tasks. On average, each volunteer contributes $600–900 towards the field grant and spends 12–16 days in the field. A typical Earthwatch project employs 4–8 volunteers each on 3–5 sequential teams. To be economically feasible for Earthwatch, the total number of Earthwatch volunteers participating on a project in one year is usually at least 20.

Earthwatch field grants cover the costs of maintaining volunteers and principal researchers in the field. They also help with other project expenses, except principal investigator salaries, capital equipment, overhead, and preparation of results for publications. Applying for grants is a two-stage process. Preliminary proposals are submitted to The Center for Field Research at least 13 months in advance of anticipated field dates. Full proposals are invited upon review of preliminary materials. Proposals are accepted and reviewed year round.

Contact: Dee Robbins, Life Sciences Program Director, The Center for Field Research, 680 Mt. Auburn Street, Watertown, MA 02172, (617) 926-8200, FAX (617) 926-8532.

American Honda Foundation

Grants to support science education projects for youths are available from the American Honda Foundation. Eligible applicants include colleges and universities (including community colleges), elementary and secondary schools, and trade schools.

Projects should seek to improve the human condition; address complex cultural, educational, scientific or social concerns currently facing American society; involve foresightful programs that look to the future; be innovative and broad in scope; and represent an urgent priority for funding. National programs pertaining to academic or curriculum development that emphasize innovative educational methods would be an example.

Grants generally range from $40,000–$80,000 per year. Grants are awarded quarterly, with approximately 8 awards made per quarter out of about 400 applications. Application deadlines include February 1, and May 1. For application forms and more information, send a self-addressed mailing label to: AHF: Grant Application Request, P.O. Box 2205, Torrance, CA 90509-2205, (310) 781-4090, FAX (310) 781-4829.

U.S. Grants Available for Training Environmental Experts in NIS

The U.S. Department of Commerce (DOC) is announcing the availability of funds for the Special American Business Internship Training Program (SABIT), which is designed to train business executives and scientists from the New Independent States (NIS) of the former Soviet Union. Although experts in many fields are eligible, special attention is being paid to environment specialists, including those working on cleanup of defense facilities. The DOC's International Trade Administration (ITA) established SABIT in September 1990 to help the former Soviet Union's transition to a market economy. SABIT has matched many NIS business executives and scientists with U.S. firms that provide them with three to six months of training. The estimated amount of financial assistance available for the program in $1.4 million. Under the SABIT program, qualified U.S. firms will receive funds through a cooperative agreement with ITS to help defray the cost of hosting interns. ITA will interview and recommend eligible interns to companies.

Interns may be from any of the following independent states: Armenia, Azerbaijan, Belarus, Georgia, Kazakhstan, Kyrgyzstan, Moldova, Russia, Tajikistan, Turkmenistan, Ukraine, and Uzbekistan. The U.S. firms will be expected to provide the interns with a hands-on, non-academic, executive training program designed to maximize their exposure to management or commercially oriented scientific operations. At the end of the training program, interns must return to the NIS. Applications will be considered on a rolling basis as they are received, subject to the availability of funds. Companies that wish to sponsor an intern by themselves through SABIT can do so but must pay all costs. Contact: SABIT Acting Director Liesel Duhon, HCHB Room 3319, 14th Street and Constitution Ave., NW, Washington, DC 20230; (202) 482-0073, FAX (202) 482-2443.

Senior Scientist Awards

Applications for Senior Scientist Awards, which provide five years of salary support to outstanding scientists who have demonstrated a high level of produc-

tivity, should be submitted to the National Institutes of Drug Abuse, Mental Health, or Alcoholism and Alcohol Abuse by February 1.

Under this program, NIH institutes identify and support exceptionally talented investigators who are well established in their fields, as a means of enhancing those investigators' skills and dedication to their areas of research.

For copies of the program announcement, contact: Dr. Ernestine D. Vanderveen, PhD, NIAAA, 6000 Executive Blvd., Suite 402/MSC 7003, Bethesda, MD 20892-7003. (301) 443-1273, FAX (301) 594-6043, e-mail: tvanderv@willco.niaa.nih.gov. Reference: PA-95-051.

Great Lakes Protection Fund Call for Preproposals

To assist potential applicants in planning and coordinating grant requests, the Great Lakes Protection Fund announces adoption of two fixed dates for submission of preproposals–January 2 and July 1. The fund may also issue a limited call for preproposals to target a specific topic or topics within one of the fund's four goals.

The Fund's priority applicants are nonprofit agencies; however, individuals and proprietary entities may apply if a clear public benefit can be demonstrated and if financial benefits stemming from the proposed work accrue to the public good. Successful applicants must maintain open access to project data, records and financial information. Results must be disseminated so that they are readily accessible to others.

The two-page preproposal is the first of two steps in the fund's proposal review process. The second step is an invitation to submit a full proposal based upon favorable evaluation of the preproposal.

Preproposals are evaluated strictly against the fund's mission and must address one of the fund's four goals. Proposed projects must be appropriately collaborative among the private, public and independent sectors. The fund seeks to support projects which are supplemental and non-duplicative of other efforts. For multiyear projects, the fund may issue challenge grants to encourage supplemental contributions.

Staff reviews the preproposals and makes recommendations to the fund's grant making committee of the Board of Directors. Preproposals are not sent to outside technical reviewers. Full proposals, however, are sent to at least three independent technical reviewers.

Preproposals must be received in the office by 5:00 pm Central Time, January 2, 1996. Preproposals received after that date will be considered with preproposals submitted for the July 1, 1996 deadline. *There are no exceptions to these deadlines.*

The fund also supports efforts to promote collaboration, coordination and regional action through planning and discretionary travel grants. For more information on these grants, please contact the fund:

Preproposal Application, Great Lakes Protection Fund, 35 East Wacker Drive, Suite 1880, Chicago, IL 60601.

Magnetic Fields and Breast Cancer

Studies to evaluate the potential of 50 Hz magnetic fields to promote mammary gland carcinogenesis in rats treated with dimethylbenzanthracene (DBMA) are needed over a 17-month performance period. This work will seek to replicate previously reported studies that magnetic fields increase breast cancer rate in DMBA-treated rats. For solicitation copy and more information, reference RFP NIH-ES-95-31 and contact: Jo Ann Lewis, Contracts and Procurement Management Branch, NIEHS, 79 T.W. Alexander Dr., Bldg. 4401 Research Commons, PO Box 12874, Research Triangle Park, NC 27709, (919) 541-7893, FAX (919) 541-2712.

Metabolic Engineering

Research that will expand the conceptual and experimental basis of metabolic engineering is sought by the National Institute of Diabetes and Digestive and Kidney Diseases.

This initiative invites: (1) basic research that contributes to a quantitative understanding of the integration and control of genetic, catalytic and transport processes that comprise metabolism; and (2) research to create techniques that facilitate the exploitation of metabolic processes for biomedical uses.

Examples of more specific topics include: (1) determinants of flux for metabolic pathways; (2) in-vivo behavior of metabolic enzymes and regulatory molecules; (3) such genetic tools as selective markers, reporter genes, vectors and regulatory molecules to introduce targeted synthetic or regulatory capacity into host cells; (4) creation of a broader range of host cells for introducing heterologous genes; (5) databases and computational tools for pathway analysis and quantitative modeling of metabolism; (6) determinants of small molecule transport; and (7) systems (e.g., plants, microbes) that show potentially novel metabolic capacity. Program deadlines include February 1 and June 1.

For copies of the program announcement, contact: Warren Jones, Division of Pharmacology, Physiology and Biological Chemistry, NIGMS, 45 Center Drive, MSC 6200, Bethesda, MD 20892-6200. (301) 594-5938, FAX (301) 480-2802, e-mail: JONESW@gml.nigms.nih.gov or Catherine McKeon, Division of Diabetes, Endocrinology and Metabolic Diseases, NIDDKD, 45 Center Drive, Room 5AN-18B—MSC 6600, Bethesda, MD 20892-6600. (301) 594-8810, FAX (301) 480-3503, e-mail: McKEONC@ep.niddk.nih.gov. Reference PA 95-087.

American Association for the Advancement of Science Announces 1996–1997 Fellowships for Scientists and Engineers

The American Association for the Advancement of Science invites applications for one-year public policy fellowships, which bring scientists and engineers to Washington, DC, to work in Congress, the U.S. Department of State, the U.S. Agency for International Development, the U.S. Environmental Protection Agency (EPA), and the RAND Critical Technologies Institute. Additional fellowships at EPA are for 10 weeks in the summer. Applicants should be postdoctoral to midcareer scientists and engineers, from any physical, biological, or social science or any field of engineering. The programs are designed to provide each Fellow with a unique public policy learning experience and to make practical contributions to the more effective use of scientific and technical knowledge in the U.S. government. Stipends vary by program. Deadline for receipt of applications is January 15, 1996. For further information and application instructions call 202-326-6600, FAX: 202-289-4950, e-mail: science_policy@aaas.org

The National Institute of Environmental Health Sciences Announces Request for Applications: Endocrine-Disrupting Chemicals and Women's Health Outcomes

Purpose

Research on the health effects of chemicals and other exposures which are suspected to disrupt the normal activity of the endocrine system is a high priority of the National Institute of Environmental Health Sciences (NIEHS). Exposure to these chemicals may have broad-based systemic effects and may increase a human risk to hormonal cancers. Accordingly, the goal of this Request for Applications (RFA) is to encourage toxicologic, basic science and epidemiologic research on the human health effects of exposure to chemicals which mimic, antagonize or indirectly alter the activity of hormones. Of particular interest is the health effects associated with exposure to women, since such exposures may affect both the woman herself and future offspring. Research is encouraged to determine the endocrine potential of a variety of chemicals, understand their biological activity, and understand the biologic consequences during early development, the reproductive period and later life. Research on the offspring of exposed women is also needed to understand the transgenerational effects of these exposures.

Research Goals

The goals and scope of this initiative are twofold. The first is to encourage and support mechanistically based research on the health effects of endocrine disruptors at concentrations that are commonly found in the environment. Experimental work on the cellular, molecular, genetic and systemic effects of exposures are appropriate. The second area of emphasis is to examine emerging hypothesis in human populations that complement the recent findings in the laboratory and in wildlife. Emphasis should be placed on development and validation of methods to precisely measure these exposures in human populations.

Mechanisms of Support

This RFA will use the NIH individual research grant (R01), and First Independent Research Support and Transition Awards (FIRST/R29). The costs and project period for R01 applications submitted in response to the RFA may not exceed $100,000 (direct costs)/per year.

Funds Available

This RFA is supported jointly by NIEHS and the NIH Office of Research on Women's Health. The expected number of awards is 8 to10.

Application Deadline: January 18, 1996

Inquiries

A complete copy of this RFA can be found in the NIH Guide to Grants and Contracts. Written and telephone inquiries concerning this RFA are encouraged. The opportunity to clarify any issues or questions from potential applicants is welcomed.

Direct inquiries to:

For Human Studies: Gwen W. Collman, Ph.D., Program Administrator, Chemical Exposures and Molecular Biology Branch, Division of Extramural Research and Training, National Institute of Environmental Health Sciences, P.O. Box 12233 Research Triangle Park, NC 27709, (919) 541-4500, FAX: (919)541-2843, e-mail: collman@niehs.nih.gov

For Animal Studies:

Jerrold Heindel, Ph.D., Program Administrator, Organ and Systems Toxicology Branch, Division of

Extramural Research and Training, National Institute of Environmental Health Sciences, P.O. Box 12233 Research Triangle Park, NC 27709, (919) 541-4500, FAX: (919) 541-2843, e-mail: heindel_j@niehs.nih.gov

Research Development Awards

Applications under the National Institute of Health's Extramural Associates Research Development Program should be submitted by January 19.

Eligibility is limited to domestic academic institutions that have a significant enrollment of minorities or that are women's colleges. This program enables participating institutions to establish or enhance an office of sponsored research as well as to meet other research infrastructure needs.

For more information: Dr. Matthew A. Kinnard, Office of Extramural Programs, NIH, Bldg. 31, Room 5B38, Bethesda, MD 20892-2182. (301) 496-9728, FAX: (301) 496-7060, e-mail: KINNARDM@NIHOD31.NIH.GOV Please reference: RFA OD-96-001

Blastocyst Implantation

Investigators are sought by the National Institute of Child Health and Human Development to participate in an ongoing multisite project on markers of uterine receptivity for blastocyst implantation. Up to six 4-year grants are anticipated. This research will identify markers indicative of the state of uterine receptivity, characterize how these markers are involved in the process of blastocyst implantation, and develop noninvasive methods for measuring markers.

For solicitation copy, contact: Koji Yoshinaga, Ph.D., Reproductive Sciences Branch, NICHD, Bldg. 61E, Rm. 8B01, Bethesda, MD 20892-7510. (301) 496-6515, FAX: (301) 496-0962, e-mail: yoshinak@hd01.nichd.nih.gov Please reference: RFA HD-95-018. Letters of intent are requested by January 5, proposals will be due February 8.

Prostate Cancer

Proposals for molecular epidemiologic studies on the etiology of prostate cancer are invited under a joint program of the National Cancer Institute, the National Institute of Diabetes and Digestive and Kidney Diseases, and the National Institute of Environmental Health Sciences.

A major program emphasis is the use of biochemical and molecular markers for identifying and assessing risk factors of prostate cancer that could lead to effective prevention strategies. Upcoming program deadlines include February 1 and June 1.

For the program announcement, contact: Dr. Kumiko Iwamoto, Division of Cancer Etiology, NCI, Building Executive Plaza North, Room. 535, Bethesda, MD 20892-7395, (301) 496-9600, FAX: (301)402-4279,
e-mail: Jasonc@EPNDCE.NCI.GOV.
Reference: PA-95-084

Alexander Hollaender Distinguished Postdoctoral Fellowship Program

Program Description

The U.S. Department of Energy (DOE), Office of Health and Environmental Research (OHER), established the Alexander Hollaender Distinguished Postdoctoral Fellowship Program in 1986 in memory of the late Dr. Alexander Hollaender, 1983 recipient of DOE's prestigious Enrico Fermi Award. Research conducted under his direction was instrumental in making DOE's biomedical research programs among the most prominent in the world. This program continues the tradition of excellence that was characteristic of all of Dr. Hollaender's activities in research and education.

These fellowships are tenable at DOE laboratories having substantial research programs supportive of the OHER mission. This mission is directed at understanding the health and environmental effects associated with energy technologies, and developing and sustaining basic and applied research programs at the frontiers of biomedical and environmental sciences in which DOE has responsibilities or unique capabilities. OHER's research mission includes atmospheric, marine, and terrestrial systems; global change; molecular and subcellular mechanisms underlying human somatic and genetic proceses; human genome; nuclear medicine; structural biology; and development of instrumentation necessary to achieve programmatic success.

Eligibility

Applicants must have received a doctoral degree (PhD, MD, DVM, or equivalent) in an appropriate discipline (or completed all internship or residency requirements) after April 30, 1994, or must complete all such requirements prior to starting the appoint ment. The starting date must be between May 1 and September 30, 1996.

This program is open to all qualified U.S. citizens and permanent resident aliens without regard to race, age, gender, religion, color, national origin, mental or physical disability, or status as a disabled veteran or a veteran of the Vietnam war era.

Program Provisions

Fellows receive an annual stipend of $37,500 the first year and $40,500 the second year. Inbound travel and moving expenses (limit $2,500) are reimbursed according to the ORISE Travel and Moving Policies. Participants are eligible for limited reimbursements to cover the cost of medical insurance. Fellowships are for one year, are renewable for a second year upon recommendation of the laboratory at which the fellowship is held, and are subject to the availability of funds.

Application Information

Completed applications and all supporting materials must be received by January 15, 1996. Fellowship offers will be made in May 1996. For more information and application material, contact:
Hollaender Postdoctoral Fellowships
Science/Engineering Education Division
Oak Ridge Institute for Science Education
PO Box 117
Oak Ridge, TN 37831-0117
(615) 576-9975

Position Announcements

Public Health Scientist

The Natural Resources Defense Council, a national nonprofit public interest organization, seeks a Senior Scientist to bring scientific analysis and knowledge to advocacy in various forums for the prevention of adverse health and ecological effects of toxic chemical pollution. A PhD or MD/MDH is required, with several years of experience in environmental or public health, or a related field. Candidates should be knowledgeable about cutting-edge toxics issues such as disproportionately impacted subpopulations, endocrine disruption, and other noncancer endpoints, and emerging issues regarding carcinogenesis. The position requires the established ability to keep abreast of scientific advances and work with the public health and academic communities. The ability to conduct outreach activities to build bridges with persons affected by toxics problems is also very important. The salary is $30,000 to $50,000, commensurate with experience. Send resume to: Public Health Program, NRDC, 1350 New York Avenue, NW, Suite 300, Washington, DC 20005. Equal Opportunity Employer.

Open Rank Faculty Position Announcement—Occupational and Environmental Exposure Assessment

University of Michigan invites applications for an open rank, tenure-track faculty position in Occupational and Environmental Exposure Assessment. The primary appointment will be in the School of Public Health, Department of Environmental and Industrial Health and will be at a rank and salary commensurate with experience.

Desired candidates will hold wither a PhD in industrial hygiene, epidemiology, environmental health, molecular genetics or other relevant disciplines or an MD with experience in such disciplines. Candidates should have an active interest in innovative and interdisciplinary solutions to theoretical and applied problems in exposure assessment in environmental and occupational settings. Examples of areas of interest include the application of environmental and occupational exposure assessment to exposure-response modeling and risk estimation, and the integration of measures of target organ dose in exposure modeling. Successful candidates will have a demonstrated ability to attract competitive external funding, to publish original research in the peer reviewed literature, and to teach at the graduate level including doctoral level students or medical students. The University of Michigan actively encourages interest from women and minorities and is an Equal Opportunity/Affirmative Action Employer.

Letters of application, accompanied by a curriculum vitae, statement of research and teaching interest, and the names and addresses of three references should be sent to: Thomas Robins, MD, MPH, Associate Professor, The University of Michigan School of Public Health, Department of Environmental and Industrial Health, 1420 Washington Heights, Ann Arbor, Michigan 48109-2029, e-mail: trobins@umich.edu, FAX (313) 763-8095.

Public Health Scientist

The San Francisco office of the Natural Resources Defense Council, a national nonprofit public interest organization, seeks a senior scientist with a Ph.D. or M.D. and relevant work experience to promote the prevention of adverse health effects from exposure to toxic chemicals. We will also consider an individual with a Masters Degree and highly relevant work experience.

The position involves bringing scientific analyses and knowledge to advocacy in various forums. Candidates should have expertise in cutting-edgetoxics issues, such as the special vulnerability of children or other disproportionately exposed subpopulations to some toxins, endocrine disruption, or other non-cancer endpoints. The ability to keep abreast of scientific advances, to translate technical issues into simple lay language, and to conduct outreach to persons affected by toxics as well as the scientific and medical communities is required. Salary is commensurate with experience. Send resume with salary requirements to:

Public Health Program
DR, NRDC, 71
Stevenson, #1825
San Francisco, CA 94105.
Equal Opportunity Employer.
People of color are encouraged to apply.

Postdoctoral Research Opportunities at the National Institute of Environmental Health Sciences

Listed below are outstanding opportunities to conduct research with leading scientists in Research Triangle Park, North Carolina.

To apply, please send a cover letter, curriculum vitae, bibliography, and names of three references to the hiring scientist at the maildrop and laboratory listed using the following address: NIEHS, PO Box 12233, Research Triangle Park, North Carolina 27709. In your cover letter, list the position title and the HNV number.

Minorities, women and handicapped individuals are encouraged to apply. All applicants receive consideration without regard to race, religion, color, national origin, sex, physical or mental handicap, political affiliation, age (with statutory exceptions) or any other nonmerit factor. Positions are open until filled.

Molecular Mechanisms of DNA Repair (HNV88)

Mechanisms of DNA repair in *Drosophila* are being investigated with focus on the *in vivo* and *in vitro* functions of Rrp1 (recombination repair protein 1). This protein is potentially important in DNA repair and homologous recombination. Future studies will include enzymatic, physical, and genetic characterization of Rrp1.

Contact: Miriam Sander, (919) 541-2799, Laboratory of Molecular Genetics, Maildrop D3-04.

Molecular Neurobiology (HNV94)

The signal transduction pathways regulating the expression of neuropeptide and cytokine genes in neural and glial systems are being investigated. Studies on the effects of neuropeptides on the biosynthesis and release of cytokines in microglial cells and potential roles of cytokines in neurodegeneration will be conducted. Applicants should have experience in neuropharmacology, neurochemistry or molecular biology.

Contact: J.S. Hong, (919) 541-2358, Laboratory of Environmental Neurosciences, Maildrop E1-01.

Ion Homeostasis and Cell Injury (HNV95)

Changes in ion transport and homeostasis appear to be involved in apoptotic cell death. Studies focus on measuring changes in intracellular calcium, pH, sodium and magnesium in isolated cells using fluorescent indicators in cells stimulated to undergo apoptosis. Alterations in signal transduction pathways which are responsible for the ionic alternations are also under study. Applicants must have experience in ion measurements using fluorescent indicators or experience with cell culture or molecular biology.

Contact: Elizabeth Murphy, (919) 541-3873, Laboratory of Molecular Biophysics, Maildrop 17-05.

Molecular Dosimetry and Epidemiology (HNV96)

Knowledge and techniques in molecular biology are applied to investigations designed to determine effects of low-dose exposures to environmental agents. Animal models, cell systems and human samples are used. Studies encompass mutation analysis and signal transduction elements.

Contact: George W. Lucier, (919) 541-3802, Laboratory of Biochemical Risk Analysis, Maildrop A3-02.

Molecular and Cellular Biology (HNV97)

The action and function of several nuclear (orphan) receptors in the regulation of gene expression and differentiation are being investigated. Studies involve characterization of response elements, interaction with other transcriptional factors and gene knockouts. Applicants must have training in molecular biology techniques.

Contact: Anton Jetten, (919) 541-2768, Laboratory of Pulmonary Pathobiology, Maildrop D2-01.

Mechanisms by Which Organisms Produce Mutations (HNV99)

Studies are aimed at understanding the mechanisms by which organisms produce mutations. Specific projects involve the isolation and molecular characterization of *antimutator* mutants in the bacterium *E. coli*; the genetic and biochemical analysis of DNA replication fidelity in this organism; and a structure-function analysis of the *dnaE* and *dnaQ* genes (encoding, respectively, the DNA polymerase and exonucleolytic proofreading activity).

Contact: Roel M. Schaaper, (919) 541-4250, Laboratory of Molecular Genetics, Maildrop E3-01.

Mechanisms of DNA Replication (HNV100)

The regulation and mechanism of human DNA polymerases involved in the replication of nuclear and mitochondrial DNA is being investigated. Attention is on the mutation rate of the mitochondrial and nuclear genome by understanding the enzymology of the mitochondrial and nuclear DNA polymerases. Future studies will include the regulation of these essential enzymes in the cell.

Contact: William Copeland, (919) 541-4792, Laboratory of Molecular Genetics, Maildrop E3-01.

Reproductive Biology and Toxicology (HNV104)

The molecular events underlying the abnormal development of the reproductive system associated with exposure to xenobiotic estrogens such as diethylstilbestrol (DES) are being investigated. Particular interest is the biochemical and molecular analysis of transient and permanent alterations in the estrogen-responsive (e.g., lactoferrin) and metabolizing (e.g., sulfotransferase) genes and the implications for human health disease.

Contact: Masahiko Negishi, (919) 541-2404, Laboratory of Reproductive and Developmental Toxicology, Maildrop E4-07.

Cell Adhesion in Metastasis (HNV105)
The molecular mechanisms by which cancer cells metastasize are being studied, focusing on the roles of cell surface receptors and cell adhesion. Special interests include the effects of swainsonine, an inhibitor of protein glycosylation, on tumor cells and the hematopoietic system. Candidates should have expertise in cancer biology, molecular biology and biochemistry.
Contact: John Roberts, (919) 541-5023, Laboratory of Molecular Carcinogenesis, Maildrop C2-14.

Molecular Mechanisms of Respiratory Diseases (HNV110)
This is a tenure track position to develop an independent research program in cellular and molecular mechanisms of respiratory biology and diseases. Extensive postdoctoral experience in molecular biology, developmental biology, signal transduction or biochemical mechanisms of inflammation is required.
Contact: Paul Nettesheim, (919) 541-3540, Laboratory of Pulmonary Pathobiology, Maildrop D2-01.

Epitope Mapping (HNV111)
Mass spectrometry combined with proteolytic foot printing is being used to determine conformational epitopes of recombinant HIV proteins towards monoclonal antibodies. Candidates should have primary experience in protein chemistry, including affinity techniques and proteolytic techniques.
Contact: Kenneth Tomer, (919) 541-1966, Laboratory or Molecular Biophysics, Maildrop 6-01.

Molecular Biology and Fatty Acid Biochemistry (HNV112)
Novel human cytochrome P450 enzymes that metabolize fatty acids are cloned and expressed, and the catalytic properties of the recombinant, purified proteins are evaluated by HPLC and GC/MS. The P450 enzymes are localized to specific cell types by immunohistochemistry and *in situ* hybridization and the regulation of P450 gene expression is studies using Northern blot analysis, RT-PCR and protein immunoblotting. Applicants should have a strong background in cell and molecular biology.
Contact: Darryl Zeldin, (919) 541-1169, Laboratory of Pulmonary Pathobiology, Maildrop D2-01.

Laboratory of Reproductive and Developmental Toxicology (HNV114)
An independent program of basic research in the field of developmental biology relative to studies in reproductive biology, developmental toxicology, hormone mechanisms, signal transduction, cell growth and differentiation, apoptosis, gene regulation and cancer biology will be initiated. Applicants with the potential for creative research in developmental biology who are studying cellular and molecular mechanisms of mammalian development desired.
Contact: Kenneth Korach, (919) 541-3512, Laboratory of Reproductive and Developmental Toxicology, Maildrop B3-02.

Gametogenesis (HNV116)
Genes with stage-specific expression during spermatogenesis are studied to define intrinsic and extrinsic mechanisms regulating development and function of male gametes. We use transgenic mice to dissect promoter regions and gene knockout mice to define the roles of gene products in meiotic and post-meiotic processes. Strong background in cell and molecular biology required.
Contact: E.M. Eddy, (919) 541-3015, Laboratory of Reproductive and Developmental Toxicology, Maildrop C4-01.

Signal Transduction (HNV117)
Studies include receptor mechanisms, G-proteins, inositol phosphates, calcium signaling, ion channels, cell growth and differentiation, apoptosis, gene regulation and cancer biology. Priority will be given to applicants utilizing cellular and molecular approaches to study intermediate steps in signal transduction pathways such as phosphorylation-dephosphorylation cascades.
Contact: James Putney, (919) 541-1420, Laboratory of Cellular Molecular Pharmacology, Maildrop 19-01.

Molecular Biology of Renal Transport (HNV118)
Renal organic anion and cation secretion mediate elimination of toxic chemicals. Current projects use cultured epithelium, membrane vesicles and imaging to examine control of secretion and coordination of intracellular and membrane events during secretion. Expression cloning of secretory transport proteins has begun. A molecular biologist desired.
Contact: John B. Pritchard, (919) 541-4054, Laboratory of Cellular and Molecular Pharmacology, Maildrop 19-01.

Molecular Biomarkers of Risk (HNV119)
Molecular epidemiologic studies of gene-environment interaction. Development and application of methods for detecting somatic mutation and germline polymorphism in genes that modulate exposure, DNA damage and disease in human population studies. Candidates should have molecular biology experience.
Contact: Douglas A. Bell, (919) 541-7686, Laboratory of Biochemical Risk analysis, Maildrop C3-03.

Ion Channel Physiology and Modulation (HNV120)
Ligand-gated (serotonin 5-HT_3 and glutamate) and voltage-gated calcium channels are studied in neurons and cell lines, as well as channels expressed in mammalian cells or *Xenopus* oocytes. Structure-function aspects of theses channels are investigated, as well as how intracellular signal transduction pathways modulate the physiological properties of these channels. Applicants must have electrophysical (preferably patch-clamp) experience. Experience in molecular biological techniques would be a great asset.
Contact Jerrel L. Yakel, (919) 541-1407, Laboratory of Cellular and Molecular Pharmacology, Maildrop 19-04.

Environmental HEALTH FOUNDATION

Finding Treatments for Environmentally Related Diseases

The advancement of global technology during the past fifty years has provided many benefits. This progress has brought about a higher standard of living, an abundant food supply and state-of-the-art technology. Unfortunately, mankind must now bear an unforeseen burden.

Advanced technology has introduced new pollutants and toxins into our environment. Scientific and medical communities agree that environmental pollutants are adversely affecting human health. Many toxins and pollutants have been linked to birth defects, cancer, asthma, emphysema, allergies, neurological disorders, autoimmune syndromes and other medical conditions. The incidence of many chronic diseases continues to rise significantly. New and unexplainable disorders are debilitating us in our homes, schools, and workplaces. The environment's impact on human health has been grossly underestimated.

More research must be done. More funding is needed. There is a need to know how pollutants affect mankind. There is a void of where to turn for answers and help. Yet, existing governmental support for this type of research is inadequate due to an already strained federal budget. But, there is hope that soon answers will be found because people care.

The Environmental Health Foundation (a not-for-profit organization) is dedicated to saving lives and improving the health of people affected by environmental toxins. It is the only organization of its kind in America solely dedicated to finding treatments and cures for environmentally related diseases. It is not a special interest group, has no political agenda, and strives to enhance "environmental health equity" for all Americans. The Foundation supports unbiased scientific research at major medical institutions, educates people about environmentally related diseases and shares scientific information with the public.

For more information, please call or write our national office:

Environmental Health Foundation
1760 East River Road
Tucson, Arizona 85718-5876
(520) 577-5225
Fax: (602) 577-5180

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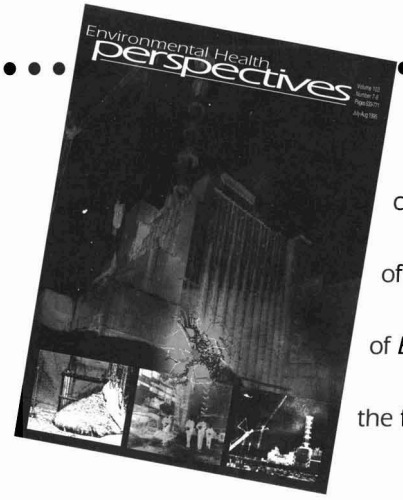

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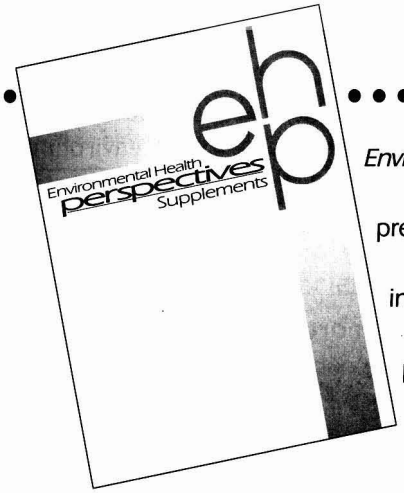

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Editorial Policy

Environmental Health Perspectives is intended to be a forum for the discussion of issues in environmental health, and several formats have been devised for that purpose. In addition, several formats are available for the publication of scientific articles and scientific discussion. All scientific articles are subject to rigorous peer review. The primary criteria for publication are environmental significance and scientific quality.

Environmental science is made up of many fields, and therefore we are prepared to consider scientific progress in all of them. Cross-fertilization and serendipity have proven to be extremely important processes in the advance of science in general, and this must hold true for the science of environmental health. We will consider for publication articles ranging from the most basic molecular biology to environmental engineering. We particularly encourage those researchers concerned with mechanisms of toxic action and new approaches for detecting and/or remedying environmental damage.

Opinions and ideas based on scientific observation and argument are welcome. While the expression of opinions may lead to debate and disagreement, such reactions are healthy and can lead to new research and discoveries. Presentations of ideas and opinions will be promoted, but our policy will be to strive for objectivity and balance.

In addition to scientific articles and discussion, we publish news of the environment. We will consider factual articles about issues that affect the environment and human health. We summarize legislative and regulatory developments, grant information from NIEHS and other granting agencies, new research areas, environmental problems, technological advances, and information about the National Toxicology Program and other important programs. Presentations of news strives for objectivity and balance and is based on the strength of scientific evidence.

Our current policy is to give the corresponding author of each published article 200 free reprints.

Perspectives

The journal is a forum for the expression of ideas and opinions. Opinions and ideas should be carefully considered and based on scientific principles. Three formats are offered:

EDITORIAL statements are published by our editors, members of our editorial boards, and occasional guest editors. These statements are intended to focus attention on important or neglected areas of environmental health, offer opinions and ideas, and stimulate discussion.

COMMENTARIES are up-to-date articles that may present commentaries offering perspective and insight on a particular topic. Commentaries are subject to peer review.

CORRESPONDENCE is encouraged. Opinions, perspectives, and insight are welcome. Comments on articles published in *Environmental Health Perspectives* are also welcome, but criticism will always be balanced by the opportunity for defense and clarification.

Research

To ensure fairness in the review process, we routinely seek opinions from three reviewers. Suggestions for reviewers of manuscripts will be considered. The research portion of the journal consists of four formats:

RESEARCH ARTICLES are original manuscripts reporting scientific research and discovery in the broad field of environmental health. Research articles may come from any field of scientific research, from the most basic molecular biology and biochemistry to atmospheric physics, ecology, and engineering. The criteria for publication are weighted toward scientific quality and environmental significance. The work will be assessed according to its originality, scientific merit, and experimental design; the manuscript will be evaluated based on its conciseness, clarity, and presentation. We also attempt to address certain ethical problems during the review process. We require assurances that all human and animal subjects have been treated humanely and with due regard for the alleviation of suffering. Manuscript review also considers scientific integrity as part of the process.

RESEARCH ADVANCES are concise articles intended to address only the most recent developments in a scientific field. Clarity of presentation is of primary importance because these articles are intended to be educational though targeted to the expert audience.

REVIEWS are narrowly focused articles that emphasize recent developments in a particular field of research. Lengthy historical perspectives are not appropriate.

MEETING REPORTS are short summaries of conferences, symposia, or workshops in which the scientific objectives and achievements of a meeting are described.

Environews

The news section provides up-to-date information on important issues in environmental health covering a variety of areas including policy, legislative, and regulatory actions; innovative technological and conceptual research advances; conference and meeting summaries; and emerging environmental problems. The news section consists of several components:

FORUM articles are brief reports on matters of potential environmental health significance such as chemical spills and contamination episodes. Brief reviews of recent scientific advances are also included.

NIEHS NEWS summarizes significant activities or accomplishments at NIEHS and the National Toxicology Program.

FOCUS articles are substantive news items about important issues in environmental health. Examples include reports on risk assessment, risk management dilemmas, women's health initiatives, environmental equity, relevance of animal models to toxicity testing, and structure–activity approaches to toxicity evaluation.

SPHERES OF INFLUENCE is a legal/regulatory column that presents reports on significant events and decisions involving the executive branch, Congress, and regulatory agencies. Examples include new directions of White House policies, impact of Clean Air Act legislation, and coverage of congressional hearings on environmental health issues.

INNOVATIONS presents emerging opportunities in environmental health based on new discoveries or approaches in biology, chemistry, engineering, or information sciences. Examples include the use of transgenic animals in toxicity testing, new advances in molecular biology, development of more rapid and efficient methods for clean-up of hazardous wastes, and methods for early detection of environmental damage and environmentally mediated diseases.

ANNOUNCEMENTS includes a calendar of upcoming events such as conferences, workshops, and public hearings. Appropriate listings are made for industrial, academic, regulatory, and legal activities. This section also includes listings of fellowship and grant announcements and positions available.

Environmental Health Perspectives Supplements

During the last 20 years, we focused on the development of a series of monographs that have generally arisen from symposium or conference proceedings. Monographs are now published as supplements to the main journal. Six to eight supplements are published per year: four to seven of these consist of conference, workshop, or symposium proceedings, and one issue is dedicated to solicited and unsolicited comprehensive reviews on environmental health. Conference manuscripts must be of the highest scientific quality and are subject to rigorous peer review. Manuscripts that do not meet *EHP* standards are not published.

Each supplement resulting from a conference should address a specific area of concern, a research problem, or a particular scientific issue. Supplements are, in general, dedicated to scientific issues and not to programmatic themes. Each supplement should form a landmark statement for a particular subject and must be an up-to-date, balanced source of reference material for researchers, teachers, legislators, and the informed public. Publication of conference proceedings in *EHP Supplements* requires the submission of a proposal as described in Instructions to Authors.

SUPPLEMENT ARTICLES from conferences are generally the result of research investigations, reviews, or a combination of both; however, brief reports and commentaries are also appropriate.

PERSPECTIVE REVIEWS are targeted to the one or two specific issues of *EHP Supplements* set aside for the publication of reviews in environmental health sciences. Perspective reviews are in-depth, comprehensive articles that address developments in specific areas. Perspective reviews must not be simply a compilation of the literature but should be scholarly, landmark statements offering a complete and balanced perspective as well as insight into the environmental significance of the research.

Instructions to Authors

Environmental Health Perspectives covers all disciplines engaged in the broad field of environmental health. Authors should therefore write in a clear and simple manner, avoiding unnecessary technical jargon, so that the article is understandable to readers in other disciplines.

All submitted manuscripts are acknowledged upon receipt and subjected to three independent peer reviews. Submit four copies of the manuscript, along with three sets of publication-quality figures. Authors may suggest reviewers when submitting a manuscript, although suggested reviewers may not be chosen. Peer review is generally completed within four weeks and authors are notified of necessary revisions or rejection of the manuscript. Revisions are requested within three weeks of notification. Authors must submit two copies of the revised manuscript, a letter responding to reviewer's comments, and a diskette containing the revised manuscript. Articles are generally published three months after receipt of revisions. Corresponding authors are sent 200 free reprints of their article upon publication.

Manuscript Preparation

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Titles should not exceed 20 words and should generally not contain abbreviations or numerical values. The title page should also list authors (first or second names spelled out in full), full address of the institution where the work was done, and affiliation of each author. Indicate author to whom galley proofs and reprints should be sent (include complete address for express mail service, telephone and FAX numbers).

Place a **running title**, not to exceed 50 characters and spaces, on the second page of the manuscript. Also on this page, list 5–10 **key words** for indexing purposes, list and define all abbreviations, and include **acknowledgments** and grant information, not to exceed 50 words. Nomenclature and symbols should conform to the recommendations of the American Chemical Society or the International Union of Pure and Applied Chemistry (IUPAC).

All articles except meeting reports and commentaries must include an **abstract**, not to exceed 250 words, which should be placed on the third page of the manuscript. Do not include details of materials and methods or references in the abstract.

Text should begin on the fourth page. For research involving human subjects, include a statment that informed consent was obtained. For animal subjects, include a statement that care and treatment was conducted in accordance with established guidelines. Concise headings (not to exceed 8 words) may be used to designate major sections. Recommended headings, where appropriate, are "Materials and Methods," "Results," and "Discussion" or "Conclusion." **References** must be listed by number, in order of citation. Reference numbers should be italicized, if possible, and placed in parentheses in the text.

The **reference list** should begin on a separate page. Personal communications, unpublished observations, manuscripts in preparation, and submitted manuscripts should not be included in the reference list, nor should explanatory text (footnotes). Such references should be inserted at appropriate places in the text, in parentheses, without a reference number. "In press" articles should be included in the reference list. Abbreviate journal names according to *Index Medicus* or *Serial Sources for the BIOSIS Previews Database*. List all authors and editors; do not use et al. in the bibliography. Include the title of the journal article or book chapter and inclusive pagination. For reports, include the authoring organization, report number, "publisher" and location, and year of publication. Some examples are shown below:

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1. Canfield RE, O'Connor JF, Birken S, Kirchevsky A, Wilcox AJ. Development of an assay for a biomarker of pregnancy in early fetal loss. Environ Health Perspect 74:57–66 (1987).

Book Chapter:

2. Lohman AHM, Lammers AC. On the structure and fiber connections to olfactory centers in mammals. In: Progress in brain research: sensory mechanisms, vol 23 (Zotterman Y, ed). New York:Elsevier, 1967;65–82.

Report:

3. U.S. EPA. Status of pesticides in reregistration and special review. EPA 738-R-94-008. Washington, DC:Environmental Protection Agency, 1994.

Each **table** must be on a separate page. Tables should be numbered with Arabic numerals, followed by a brief title (not to exceed 25 words). General footnotes to tables should be indicated by lowercase superscript letters beginning with *a* for each table. Footnotes indicating statistical significance should be identified by asterisks (*, **) and daggers (†, ‡). Type footnotes directly after the table. Tables should contain no more than three layers of column headings, and the entire table should fit on one journal page.

Figure legends should be typed, double-spaced, on a separate page. Legends should be as brief as possible without compromising explanation of the figure. Use Arabic numerals to number figure legends. Define any abbreviations in the legend on first mention.

Three sets of publication-quality **figures** must be submitted. Electronic versions of figures are encouraged, but should be submitted in addition to, not in lieu of, hard copies of the figures. Dot matrix computer drawings are not acceptable as original art. The style of figures should be uniform throughout the paper. Identify all figures on the back with the authors' names and figure number and indicate orientation. Label axes of graphs clearly and define all symbols used.

Material suitable for inclusion as **on-line documentation**, such as kinetic studies, is welcome. Contact the *EHP* office for instructions regarding submission.

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Environmental Health Perspectives Supplements

Supplement Manuscripts result from conferences, symposia, or workshops and may take several forms. 1) Manuscripts reporting original research should be formatted as described for Research Articles, 2) opinions and discussion about a particular topic should be formatted as described for Commentaries, 3) manuscripts reviewing a topic or reporting a combination of review and original research should be formatted as described below for Perspective Reviews.

Perspective Reviews are in-depth, comprehensive reviews of a specific area. They should begin with a title and second page as described for research articles. Introduction and presentation of information should be continuous with specific items and discussion indentified by using subheadings. Abstracts, references, abbreviations, figures, and tables should also be handled as described for research articles.

Proposals for the publication of conference, symposium, and workshop proceedings will be considered; however, space is limited. We turn away many excellent proposals simply because we do not have space to publish them.

All proposals are reviewed and examined with a number of specific questions in mind. In developing a proposal, consider the following: Proposals are assessed according to their originality and scientific merit. Is the supplement needed? Is the subject matter timely and potentially useful to workers in the field? What is the environmental significance of the topic being addressed? Is the proposed supplement a complete representation of the field? Are there other aspects that should be included? Does the proposal contain sufficient information for evaluation? Is the presentation clear? Can the organizers integrate the participants into a cohesive unit? Are the contributors appropriate for the topic listed and do they have scientific credibility?

The source of funding is also considered. Scientific objectivity is extremely important, and it must be clear that organizers are not being used to present a bias favored by the funding body. Contributions from an interested party to a conference need not disqualify a proposal, but

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All proposals must be submitted at least six months in advance of the conference. In the publication of conference proceedings, timeliness is essential. Because it takes at least six months to publication, no proposal will be considered after the conference has been held.

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In your covering letter please provide assurances that the manuscript is not being considered for publication elsewhere and that all animals used in the research have been treated humanely according to institutional guidelines, with due consideration to the alleviation of distress and discomfort. If the research involved human subjects then a statement must be made to the effect that participation by those subjects did not occur until after informed consent was obtained.

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Finally, a statement must be made indicating that all authors have read the manuscript and are in agreement that the work is ready for submission to a journal and that they accept the responsibility for the manuscript's contents.

Inquiries may be made by calling (919) 541-3406 or by FAX at (919) 541-0273.

Submission of News Information

Environmental Health Perspectives welcomes items of interest for inclusion in the Environews, Calendar of Events, and Announcements sections of the journal. All items are published subject to the approval of the Editors-in-Chief. All submission for these sections should be sent to the attention of:

News Editor
Environmental Health Perspectives
National Institute of Environmental Health Sciences
PO Box 12233
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Research Triangle Park, NC 27709 USA

Items submitted for inclusion in the Forum section must not exceed 400 words. Items may be edited for style or content, and by-lines are not attached to these articles. If possible, items should be submitted on computer disk using WordPerfect or Microsoft Word, in straight text without formatting.

Items received for the Calendar of Events will be published in as timely a manner as possible, on a space-permitting basis. Submissions should include all relevant information about the subject, date, time, place, information contact, and sponsoring organization of the event.

Position announcements will be limited to scientific and environmental health positions and will be run on a space-permitting basis. Although we seek to publish all appropriate announcements, the timeliness of publication cannot be guaranteed.

Public information advertisements will be run free-of-cost as space becomes available. All ads are run subject to their appropriateness to the editorial format of the journal. Submissions of advertisements should include full-page, half-page, and quarter-page formats if available. Ads should be camera-ready, black and white positives.

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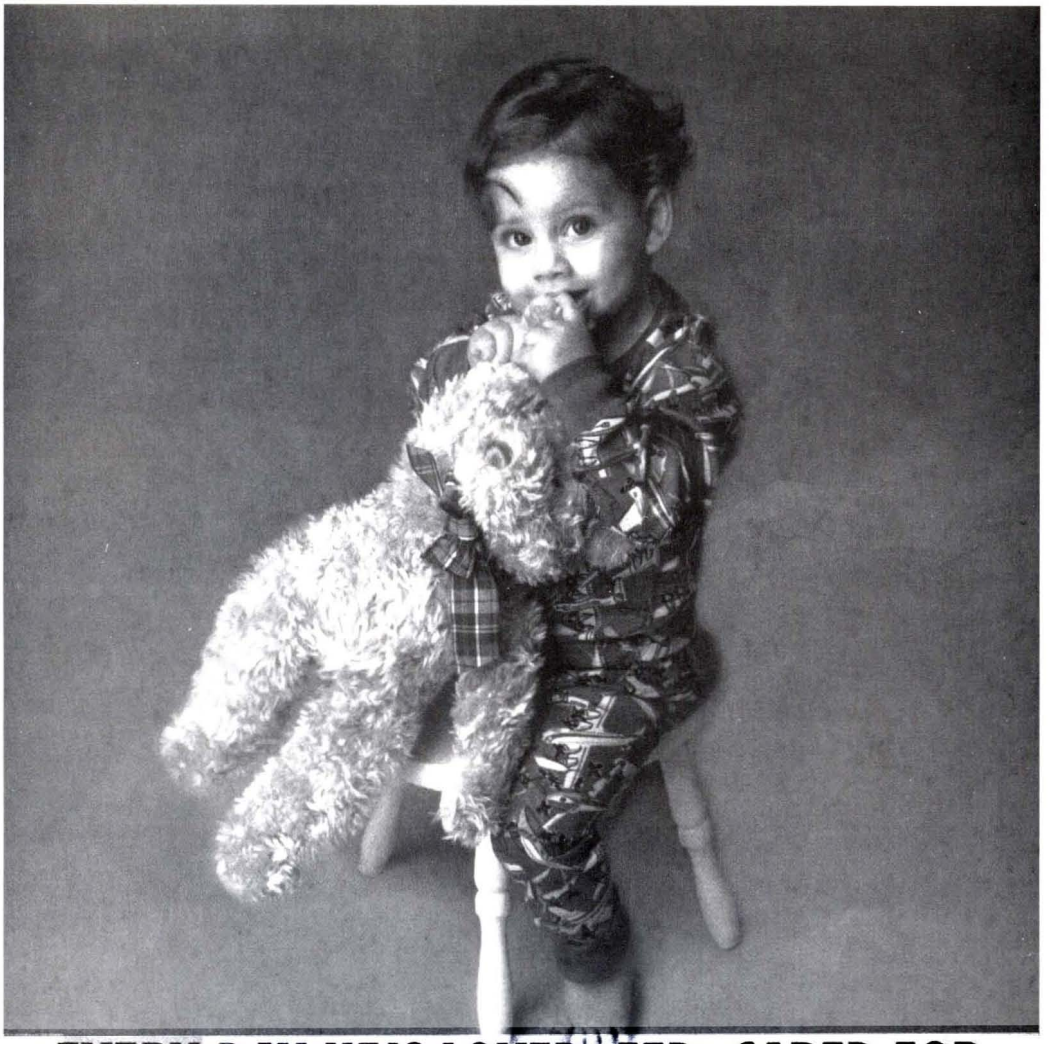

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