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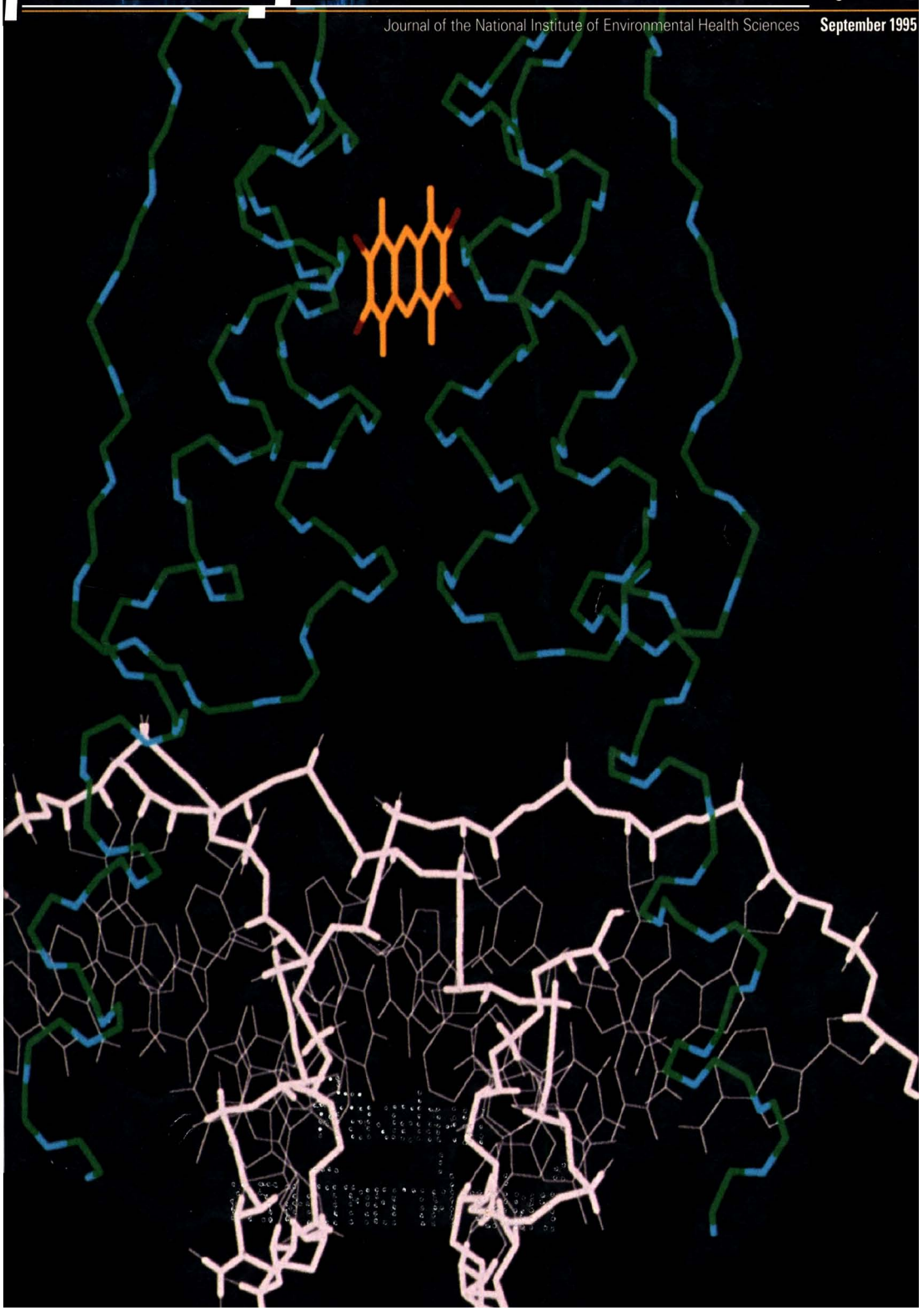

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Environmental Health perspectives
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Perspectives

Environews

Research

Environmental Health perspectives
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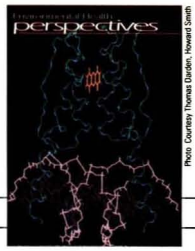
On The Cover: A molecular model of dioxin bound to the Ah receptor. DeVito et al. (p. 820) compare body burdens of dioxins that cause effects in animals to those that cause effects in animals to humans.



Environmental Health Perspectives

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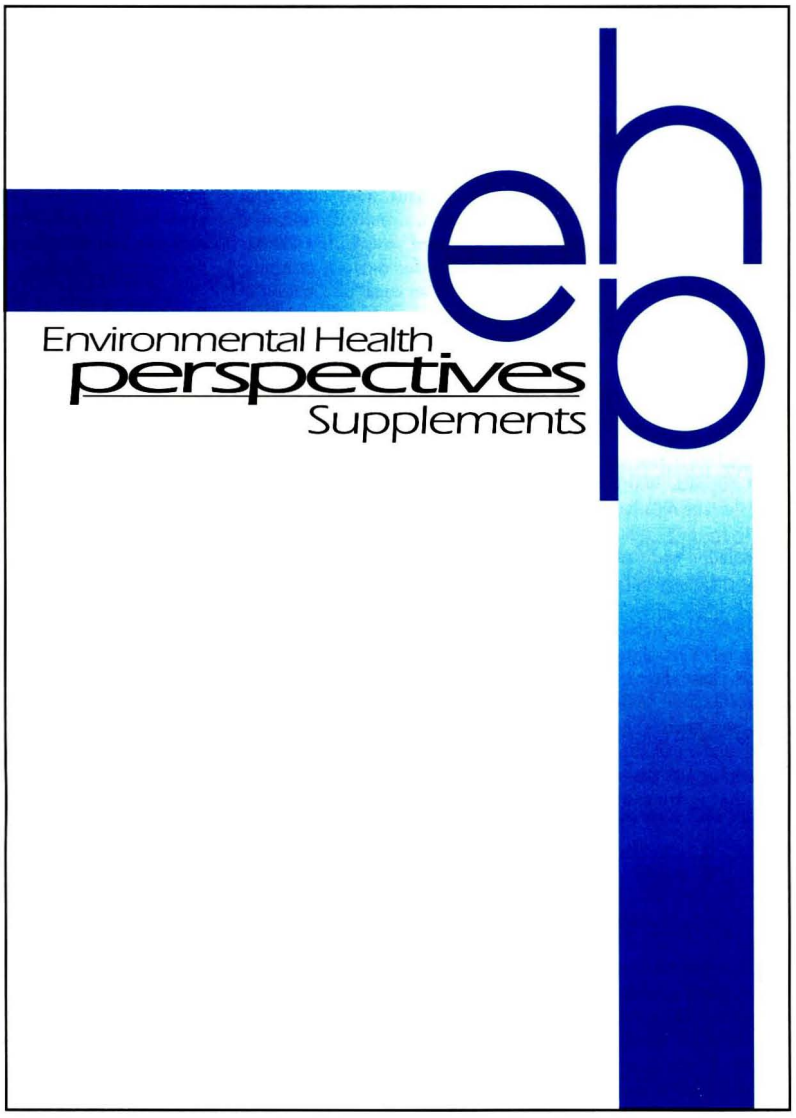

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In This Issue

Silent Killers

Prostate cancer is the number one cancer detected in men and the number two cause of cancer deaths in men. Incidence of testicular cancer has doubled in the last 50 years, making it the most common malignant disease of young men. Yet funding for research on male reproductive cancers is less than a fourth of that devoted to breast cancer, and clear answers on the causes and mechanisms of these cancers are in short supply. This month's **Focus** article (p. 802) examines the status of research on male reproductive cancers in areas including diet, genetics, natural and environmental estrogens, and occupational exposures.

Into Thin Air

Handicaps of environmental contamination, accidental exposure, and intense public criticism have set the field for a race to improve hazardous waste disposal. Waste disposal processes such as the one reported in the **Innovations** article (p. 808) offer sound alternatives to incineration and new approaches to solving this major environmental problem. The Eco-Logic process uses a closed-loop system to completely destroy contaminants such as PCBs, dioxins, and pesticides in a gas-phase thermochemical reaction that turns harmful waste into hydrogen, methane, and water. The mobile system can be used on site and then shipped to the next job, helping communities avoid the problems that accompany hazardous waste disposal sites.

Danger from Diesel Exhaust?

Epidemiological studies of cancer risk from occupational exposure to diesel exhaust fumes were prompted by reports of elevated mortality rates due to lung and bladder cancers among certain workers. Muscat and Wynder's (p. 812) review of 14 studies revealed that 4 were positive, 5 were inconclusive, and 5 were negative for risk of lung cancer from diesel exhaust exposure. They believe that short-term exposure (<20 years) to diesel engine exhaust is unrelated to increased risk of lung cancer, but that long-term exposure (> 20 years) increases the risk of lung cancer for railroad workers, but not for those in the trucking industry.

Dioxins: The Animal–Human Connection

DeVito et al. (p. 820) compare the body burdens of dioxins in experimental animals that cause toxic effects to the body burdens in humans associated with the same responses. For humans, body burdens of dibenzo-*p*-dioxins, dibenzofurans, and PCBs were summed to calculate a toxic equivalency factor for dioxins, assuming 22% body fat and an equal distribution of the dioxins. Comparative studies indicate that humans and animals respond to similar body burdens of dioxins for unambiguous responses like chloracne or induction of metabolic enzymes. Limited data on humans suggest that some individuals incur carcinogenic or noncarcinogenic effects at body burdens of dioxin within one to two orders of magnitude of those in the general population.

EMFs up Close

Levallois et al. (p. 832) attached 24-hour dosimeters to 18 adults living next to a 735-kV high power transmission line to measure exposure every minute to electric and magnetic fields. The data were compared to exposure incurred by 17 adults living far away from any transmission lines. At home the magnetic field intensity was 4.4 times higher, and the magnetic field exposure was 2 times higher among subjects living next to power lines compared to unexposed subjects. Electric field intensity was also about twice as high, clearly demonstrating that the 735-kV power line was an important contributor to residential 60-Hz electric and magnetic field exposure, and was similar to the occupational exposure incurred by electrical workers.

PAHs in Poland

Coal mines and coke oven plants located in the Silesia area of Poland are a major source of polycylic aromatic hyrocarbons (PAHs) in the air. Øvrebø et al. (p. 838) collected individual air samples in February and September using personal monitors, as well as urine samples, from workers in three different coke oven plants. Samples were also collected from people living near the coke oven plants and from those living in a non-industrialized area. There was a seasonal variation as well as an increasing concentration gradient in both PAH levels and in the urinary excretion of 1-hydroxypyrene, a metabolite indicative of the amount of PAH absorbed. 1-Hydroxypyrene levels were dependent on an individual's proximity to the source of pollution.

E-SCREENing Estrogens

Villalobos et al. (p. 844) evaluated the *in vitro* proliferative effects of estrogens using an E-SCREEN assay with four different cell stocks (BOS, ATCC, BB, and BB104) of MCF7 human breast cancer cells. The responses of the cell cultures to estrogen were characterized by their biological behavior. Among all four cell lines, the BOS cell stock was the most responsive to estrogen in terms of proliferative activity, receptor levels, and protein secretion. The authors suggest that the MCF7 BOS cell line or one with similar proliferative patterns would be the most optimal to use in the E-SCREEN assay.

Assessing Risks of Air Pollution

Herbarth (p. 852) developed a statistical method to determine threshold values and assess risk for air pollution using sulfur dioxide as an indicator component. The novel analysis was designed to eliminate temporal inhomogeneities and pseudocorrelations and to enable calculation of dose–response relationships and attributable risk associated with a relative increase in air pollution levels. He reports that the incidence of airway disease was elevated when the 24-hour average of sulfur dioxide in the air increased above 0.6 mg/m^3 for children or above 0.8 mg/m^3 for adults.

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:

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Each country has something to contribute. . . . We could create a network to share expertise and compare results obtained in different countries.

Editorial

The Advantages of International Cooperation

At the meeting on Science, Research and Development, organized by the European Commission and held April 19–24 in Noordwijkerhout, the Netherlands, 48 projects with participation from 75 laboratories in 17 European countries were presented. The multicenter approach once more stressed the idea that environmental problems are global, and we should try to solve them in cooperation with various countries. Speakers at the meeting put forth the idea that similar projects should be done on a more international basis; for example, with the United States, Japan, and Australia.

It is well known that U.S. technology aids research in other countries. One example is the Teplice program, started in Czechoslovakia in 1991. The goal of this program is to analyze the impact of air pollution on the health of the population in northern Bohemia, one of the most polluted regions in Europe. Thanks to the help of scientists from the U.S. EPA, it was possible to start an extremely fruitful collaboration. The first fruits of this project were accomplished using equipment supplied by the European Commission through the PHARE program.

In northern Bohemia, it was possible for the first time to analyze the concentrations of various pollutants in the air, including polycyclic aromatic hydrocarbons (PAHs), arsenic, lead, and mercury, and evaluate the impact of these pollutants on the health of children by analyzing functional respiratory and neurobehavioral changes. Using American technology for personal monitoring, DNA adducts were analyzed in relationship to each person's exposure to PAHs. Another technology helped analyze the effect of air pollution on sperm morphology; an increase in sperm abnormalities were related to pollution. Preliminary analyses suggest increases in low birth weight and prematurity in the pregnancy outcome project. Key to evaluating the effects of environmental pollution are new techniques to determine genetic polymorphism and sensitivity of different groups to mutagens and carcinogens.

These achievements indicate that human monitoring may enable us to determine and prevent risks to individuals. This may in turn allow us to address questions about possible adaptation to pollutants. Comparing results of the effects of air pollution on acute morbidity and mortality in Western versus Eastern European countries, it seems that people exposed to high levels of pollution for a long period of time do not exhibit increasing morbidity and mortality during air inversions as do people who have not been exposed to high levels of pollution.

Studies of genetic polymorphism may explain why different results are obtained in different populations. During recent years, paracetamol was found to be clastogenic in Czech and Norwegian studies, but not in a British study. Last year the idea was put forth that cigarette smoking induces enzymes related to biotransformation and may protect against some genotoxic insults.

In Eastern Europe, there is a different perception of the risk of environmental pollution than in Western countries. We must try to understand these societal differences. Is it possible to quantify the impact of pollution on the lifestyle of the exposed population? This impact may be indirect: it may be socially or psychologically mediated. Is it possible to measure mental changes affecting lifestyle?

Another problem is the impact of pollution on developing organisms, which is of genetic or epigenetic origin. A toxicant may act directly prenatally or postnatally, or it may be maternally mediated. We need information about the risk of each category of toxicant.

Certainly, risk assessment is important in evaluating the effect of complex mixtures, as certain factors may decrease the activity of other factors, such as genotoxicants. It is believed that in the future, the risk of pollution from traffic in metropolitan areas will increase. New efforts should be directed toward evaluating the effect of traffic on human health and determining the most sensitive biomarkers for these effects.

It would be useful to stimulate a global approach to the most important problems and to build bridges of cooperation between countries. Perhaps *Environmental Health Perspectives* could become the crystallizing point for this cooperative activity, a source for creating a picture from the various stones of the mosaic. We must try to build bridges over the oceans and tunnels through the walls of our individual scientific environments.

Each country has something to contribute: for example, Japan is well-known for mutagenicity research and Australia for studies of the effects of UV-radiation. We could create a network to share expertise and compare results obtained in different countries. It would be necessary to create a network of institutes or laboratories prepared for broad-based international research. It would be useful to start with a roundtable discussion among representatives of U.S. government agencies such as the National Institute of Environmental Health Sciences and the Environmental Protection Agency, the European Commission, and representatives from Japan and Australia. Such a network could be essential in addressing environmental problems in the next century.

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Environmental Estrogens

The estrogenic equivalent analysis that Dr. Safe presented recently in *EHP* (103:346–351) is a welcome addition to the literature, and development of this toxicologic approach is an important step in evaluating new endpoints for environmental agents (*1,2*). The hormonal potential of chemical contaminants offers many opportunities for further research, including study of the environmental etiology of breast cancer.

Complex mixtures are of great importance for human environmental exposures, but we understand neither exposure nor effect very well. Safe, as a basis for evaluating one hormonal endpoint, offers estrogenic equivalents acting in an additive fashion. In future research, we need to learn whether these combinations of chemicals, such as DDT and estrogen, may be additive or multiplicative. Examples of both exist. Thus, *in vitro* assays using MCF7 breast tumor cells found an additive effect for 10 pesticides (*3*), and *in vivo* assays of two PCB metabolites produced an apparent multiplicative effect in altering gender determination in turtles (*4*).

We also need to investigate hormonally active compounds for other relevant biological activity. For example, vinclozilin has androgenic potential (*5*); genistein may act as a free radical scavenger (*6*); and *p,p′*-DDE demonstrated potent anti-androgenic responses in the rat (*8*). Estrogenicity of genistein and other phytoestrogens may be relevant to breast cancer, but countries with high dietary intake of such compounds are generally at low risk for breast cancer. Therefore, it is possible that exogenously derived phytoestrogens act differently from steroid hormones, perhaps by altering levels of free versus bound estrogen or by increasing estrogen excretion through the biliary/fecal route (*8*).

It has become more and more apparent that toxicokinetics of chemicals is a critical component of dose–response relationships. Endogenous levels of organochlorines in women today are 10-fold higher than those of estrogen (*9*), and their effects are prolonged because of their persistence in the body. Toxicokinetics are also closely tied to PCB toxicity (*10*); thus 2,2′,5,5′-tetrachlorobiphenyl, an estrogenic compound, is short-lived in the body compared to 2,2′,4,4′,5,5′-hexachlorobiphenyl. The latter has little estrogenic behavior in rats until 20 or more days after exposure (*11*). In addition, PCBs may also have potent antiestrogenic behavior (e.g., 3,3′,4,4′-tetrachlorobiphenyl). Humans and wildlife have been exposed to mixtures of PCBs that possess a range of estrogenic activity, and the interaction of various PCB congeners has not been widely studied. PCBs may be synergistic, as in the example cited above (*4*), but their hormonal activity can also differ in different experiments, as with Aroclor 1254 (*12,13*). Indeed, the ability of chemicals in the body to act both as agonists and antagonists is well known; for example, soy products and tamoxifen can be both estrogenic and antiestrogenic. These contrasting endpoints can be attributed to different mechanism as well as to pharmacokinetics.

As Safe notes, *p,p′*-DDE is the predominant (>90%) residue of DDT in the environment. While DDT may be undetectable in some assay conditions, *p,p′*-DDT is still routinely found in women, albeit at low levels. Other isomers including *o,p′*-DDT may be currently undetectable, but they may have been present at an earlier time, so that measurement of DDE alone can serve as a surrogate for prior exposures to other isomers. Moreover, with respect to breast cancer, the effect of organochlorines may arise from mechanisms not directly involving the estrogen receptor, such as induction of P450 enzymes.

I agree with Safe that the current evidence is far from conclusive about the association of organochlorine exposure and breast cancer risk, and the many confirmatory studies now underway will improve our knowledge in this regard. The connection to male reproduction, including sperm counts, is highly speculative. However, the existing data, along with ample evidence of endocrine disruption in wildlife, provide a clear challenge for basic and epidemiologic researchers to uncover public health risks arising from environmental exposures.

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Response

In her letter, Dr. Wolff correctly points out the difficulties in assessing the potential adverse impacts of chemical mixtures containing compounds which exhibit both common (e.g., estrogenic) and diverse activities. Despite these problems, it is not unreasonable or unprecedented to determine levels and relative potencies of individual compounds in a mixture which elicit common responses and/or act through similar pathways. This method is the toxic equivalency factor (TEF) approach for hazard assessment of chemical mixtures, which by definition focuses only on specific chemical-induced responses. Regulatory agencies use a TEF approach for hazard assessment of 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds (*1*) and estrogen equivalents have previously been used for determining human exposure to

dietary and synthetic estrogenic chemicals (*2*). Ames, Gold, and co-workers (*3*) used a similar approach for developing their Human Exposure Rat Potency (HERP) index for dietary carcinogens. The major purpose of my review article (*EHP* 103:346–351) was to critically examine the hypotheses that environmental estrogens are responsible for an increased incidence of breast cancer in women and male reproductive problems (*4–6*). In my opinion, the mass-balance of human dietary exposures to "natural" and "industrial-derived" estrogens suggests that there is minimum potential of these industrial estrogens to cause an adverse endocrine-related response in humans, as I stated in the review article. The mass potency estrogen equivalents calculations, despite their limitations, would also support this conclusion. This is a narrow and focused approach and does not exclude other potential adverse effects of these compounds.

Disruption of endocrine pathways by industrial chemicals and environmental contaminants that exhibit hormone or antihormone activities have been the subject of several articles in the scientific and lay media. Environmental studies show a possible linkage between exposure to hormone mimics and possible adverse effects in fish and wildlife. It has been hypothesized (*4–6*) that these hormone mimics may be causing adverse effects in humans; unfortunately, scientific hypotheses are often treated in the press as scientific facts (*7–11*). In contrast, as scientists, we tend to be questioning and skeptical of hypotheses in the absence of data.

The normal human diet contains diverse endocrine disrupters and hormone mimics which in themselves may cause adverse effects. Therefore, consideration of environmental hormone mimics must take into account other background exposures to these same types of compounds in the human diet. My review article focused only on dietary estrogens and antiestrogens and concluded that, based on current data, adverse human impacts of industrial estrogens were unlikely. I agree with Dr. Wolff that evidence linking organochlorine exposure to breast cancer in women is "far from conclusive" and that more research is required to investigate the linkages between exposure to environmental contaminants and human disease.

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The ocean, like the air, is the common birthright of mankind.
Thomas Jefferson (1743-1826)

Forum

The Brent Spar Saga

What do you do with a floating piece of garbage that is 150 meters tall and 30 meters wide? That's the question which became the subject of a dramatic debate between the owners of the Brent Spar, a 65,000-ton decommissioned oil platform and the environmental activist group, Greenpeace.

After three years of studies, British permit procedures, and meetings with Scottish fishermen and environmental groups, Royal Dutch Shell, owner of the Brent Spar, came up with its two most feasible plans: horizontal dismantling, an on-shore disposal technique that would require the buoy to be turned onto its side, loaded onto a ship, and taken to a coastal area for decontamination and disassembly; and deep-sea disposal, a cheaper and technically simpler option of towing the buoy to a site in the North Atlantic and sinking it. Citing the expense, occupational health risks, technical difficulty, and possibility of accidental contamination of shallow estuarine waters during horizontal dismantling, Shell opted for deep-sea disposal. The chosen location was a mile-and-a-half-deep ocean trench, 150 miles northwest of the Outer Hebrides, an island group off the northwest coast of Scotland.

After more than a year of study, Greenpeace raised objections to Shell's plan, pointing out that, based on Shell's own reports, the Brent Spar still held 100 tons of sludge, possibly containing cadmium, lead, arsenic, oil, and PCBs; 30 tons of radioactive waste in the form of scaly deposits within the pumping system; and corrosion-reducing anodes made of zinc. Shell insisted that all the oil was drained in 1991, and that it had removed 100 tons of waste from the oil rig, including lubricating oil, batteries containing cadmium and lead, and light bulbs containing mercury, and made plans to go ahead with the sinking.

After a several-week occupation of the rig platform in April, Greenpeace claimed to have video footage and laboratory tests documenting the presence of oil and hydrocarbon-containing water in three of the six storage tanks, and oil in another, evidence that there might be as much as 5,000 tons of oil still aboard. Additional waste, including radioactive deposits, was not accessible for sampling. Greenpeace also criticized Shell's chemical manifest, which was based solely on estimates of quantities without recent empirical evidence.

According to Shell's reports, the sinking of the buoy would cause no significant effects on animal life in the area, and wastes would be contained in the immediate area. Greenpeace asserted that it would be difficult to estimate the environmental impact because toxicological data about the response of deep-sea organisms to environmental insults are not available. A recent report in the June 29 issue of the journal *Nature* questioned Greenpeace's reasoning, pointing out that hydrothermal vents release much larger quantities of heavy metals than the Brent Spar contains, and that some deep-ocean-floor communities rely on heavy metals as nutrients.

Aside from the environmental impact, another concern was that the sinking would set a disposal precedent. Of approximately 400 other such oil installations in the North Sea, 50 are due to be decommissioned in the next 10 years. The levels of wastes on the Brent Spar might have been used to set negligible levels of wastes, such as radioactive waste, leading to the dumping of similar levels of radioactive waste contained in items such as waste concrete rubble. In light of these considerations, Greenpeace advised that the buoy be disassembled on land.

Greenpeace

Battle on the high seas. Greenpeace activists attempt to board the Brent Spar, an oil rig slated for deep sea dumping.

When Shell refused to abandon its original plan, Greenpeace brought the battle to the sea, to the government, and to the gas stations. On June 12, just a day after European environmental ministers condemned Shell's planned action at the North Sea conference, Shell began towing the rig out to sea, followed closely by the Greenpeace vessel Moby Dick. Over the ensuing days, Greenpeace managed to place four activists on the rig, determined to go down with it if necessary.

Meanwhile, in Germany, Greenpeace appealed to strong environmentalist sentiment, securing the consensus of parties across the political spectrum against Shell. The North Sea forms part of Germany's northern border and is an important route for sea traffic and fishing. German Chancellor Helmut Kohl brought up the issue of the Brent Spar at the Group of Seven, an international meeting held in Canada in June. Kohl personally requested that British Prime Minister John Major

refuse Shell the right to dump the rig in the North Sea. Major, a strong supporter of the original plan, refused, leaving Kohl and others throughout Europe to call for the continuation and expansion of a boycott on Shell gasoline.

Economic pressure mounted as other nations, including Denmark, Sweden, the Netherlands, and some consumers in Britain, joined the boycott that began in Germany. For three weeks in June, business at Germany's 1,728 Shell stations was reportedly down by approximately 30%, representing a loss as high as 24 million dollars. In addition, German Shell stations received numerous bomb threats and 50 cases of vandalism, including one firebombing, one shooting, and an undetonated letter bomb. Although no one was injured, Shell labeled the boycotts the result of purely political actions devoid of reason and accused Greenpeace and their other opposition of instigating a terrorist attack.

Finally, amid the waning support of the European community, Shell unexpectedly dropped the North Sea disposal plan, saying that it felt it was "in an untenable position . . . without wider support from the governments participating in the Oslo-Paris Convention." The Oslo-Paris convention recently declared a ban on deep-sea disposal of such equipment.

Norway, one of the few remaining supporters of the plan, offered safe anchorage to the rig, provided that Shell meet certain requirements. The Brent Spar is floating at a location at the northern tip of the Shetland Islands as Shell waits for results of the Norwegian permitting process, which would allow them to moor the buoy for one year in Norwegian waters while they formulate a new disposal plan. Once they do, their problems may still not be over: damaged during its construction by the buildup of differential stresses on the storage tanks, the Brent Spar faces the ongoing risk of accidents during towing, upending, and disassembly. Shell estimates that on-land disposal will cost $46 million dollars plus the possible loss of a British tax incentive, versus the original estimate of $16 million for deep-sea disposal. However, while Greenpeace acknowledges that on-land disposal will be difficult, expensive, and dangerous, they insist that the dismantling is possible using present technology and is a sounder environmental alternative.

The Cost of Living

A fish caught off the coast of Spain two years ago sold for more than what most people pay for a car. The $70,000 price tag for the 715-pound bluefin tuna (in high demand in Japan) was a harbinger of financial repercussions that will result from the human race taxing its natural resources to feed and clothe a soaring population, said Lester Brown, president of the nonprofit WorldWatch Institute, speaking at the 20th annual conference of the National Association of Environmental Professionals (NAEP).

The conference, held June 10–13 in Washington, DC, focused on such complex topics as watershed management, risk-assessment methodology, and innovative methods of preventing pollution. But Brown reminded the participants that the heart of the world's environmental problems remains a glut of people. "The world cannot continue to add 90 million people a year without getting into trouble," he said. "It's been nearly three years ago that the famine started in Somalia. In 1992, 300,000 Somalis died. It took the world just 29 hours to make up that loss. That's how fast the world population is growing."

EHPnet

Recent events surrounding the successful attempt by the environmental group Greenpeace to force the Royal Dutch Shell oil company to dismantle a decommissioned oil rig rather than sink it in the North Sea focused attention on the issue of environmental assaults on the world's oceans. In a perhaps less dramatic but more detailed effort, a major new traveling exhibition at the Smithsonian Institution's National Museum of Natural History is also aimed at the issue of oceans.

Described as "the culmination of a four-year effort to study and understand environmental issues affecting the health of the world's oceans," the exhibit, Ocean Planet, opened on 22 April 1995. After eight months in Washington, DC, Ocean Planet will travel to 11 American cities through the end of the millennium. For those who may not be able to visit the exhibit, a companion exhibit is available on-line via the World Wide Web.

Upon entering the on-line exhibit, users are presented with the floor plan of the Ocean Planet Exhibition as currently presented at the Museum of Natural History. From this map users can go to any part of the exhibit hall by clicking on the name of the room they want to visit or take a special tour designed by the museum's curator. The exhibit traces the global benefits that oceans provide in terms of food and health products, recreation, and economic growth, as well as the impact of human activity on ocean ecosystems. Examples of this impact detailed in the exhibit show that polar ecosystems are no longer beyond the reach of human activity: tourism, commercial fishing, and pollution are putting pressure on populations of penguins, whales, seals, and krill; intertidal zones may support as many as two thousand species, but these interfaces between land and sea are in jeopardy from coastal development, land-based runoff, and ocean pollution; and oil pollution disasters make headlines, but hundreds of millions of gallons of oil quietly end up in the seas every year, mostly from nonaccidental sources such as road runoff.

Hyperlinks in the exhibit "rooms" such as ocean science, oceans in peril, and resources provide brief but informative descriptions of topics of environmental interest. The ocean science portion of the exhibit provides discussions ranging from recent discoveries of hydrothermal vents, previously unknown marine animals, and volcanic fields, to how ocean currents are tracked and the relationship between oceans and climate. The oceans in peril section provides overviews of marine pollution, divided into subtopics such as oil pollution, toxic contaminants, non-point-source pollution, and mining and dumping; habitat destruction, which deals with the effects of deforestation and the loss of wetlands on oceans; fishing issues such as overfishing, ecosystem changes, and pollution by bombs and poison; and global change issues including climate change, ozone depletion, and population.

Perhaps the most useful section of the exhibit for environmental researchers is the resources room. This section includes an extensive list of hyperlinks to oceanographic and environmental resources on the Internet ranging from the Scripps Institution of Oceanography, the Woods Hole Oceanographic Institute, the National Oceanic and Atmospheric Administration, and the Distributed Ocean Data System to the International Arctic Buoy Program, the Save Our Seas home page, and the Small Islands Information Network. Users interested in ocean issues should dive into this exhibit at URL:http://seawifs.gsfc.nasa.gov/ocean_planet.html.

Problems affecting availability of food may be a final wake-up call for environmental issues, Brown said. The world's oceans are being fished at their limits or beyond and are also beset by water pollution, coral reef degradation, and other environmental damage, he said. Farmers also are struggling to keep up with population growth. Land is becoming scarce, as is the water needed to grow crops. Water scarcity in China has forced farmers there to return to rain-fed farming, Brown said. And land scarcity could set back advances in more environmentally friendly energy sources, Brown noted. As food supplies dwindle and demand rises, for example, less grain will be available to make ethanol as a cleaner alternative to gasoline. The United States should adopt a national policy outlining a commitment to stabilizing world population, Brown said.

Some of the subsequent technical sessions at the conference echoed Brown's theme, specifically those dealing with sustainable development and ecosystem management. Jim Benson of the Natural Resources Conservation Service at the U.S. Department of Agriculture in Washington provided data on the impact of environmental problems on agriculture. The agency's National Resources Inventory, a study of 1.5 billion acres of nonfederal land, states that the available acreage of prime farmland fell from 339 million to 333 million acres between 1982 and 1992. Almost one out of every four cropland acres were eroding too fast to sustain soil productivity, Benson added. The NRI should help guide federal policy and provide up-to-date information on natural resource conditions, he said.

At the conference, the President's Council on Environmental Quality and NAEP awarded their third annual Federal Environmental Quality Awards, which honor federal agencies for excellence in implementing the National Environmental Policy Act, enacted 25 years ago. The Fort Worth District Army Corps of Engineers won the award for its project, a programmatic environmental impact statement of Joint Task Force Six activities along the U.S.–Mexico border. The project was organized in 1989 in response to the National Drug Control Strategy. Joint Task Force Six is a multiforce government agency charged with providing technical, logistical, operational, and engineering support to federal, state, and local law enforcement agencies throughout the southwestern United States.

The U.S. Department of Energy won the award for the continued improvement of its National Environmental Policy Act Compliance Program. Secretary of Energy Hazel O'Leary has taken bold steps to reinvent the DOE's National Environmental Policy Act program and has brought a change of culture and instilled in senior managers a commitment to openness and public participation in environmental decision-making, NAEP officials said.

NAEP, which began as a 350-member, interdisciplinary professional society in 1975, now boasts 3,300 members. It is the only overarching professional society serving the environmental professions and promotes ethical practices, technical competence, and professional standards for the environmental professions. A top goal of the group, said NAEP President Richard B. McLean, is supporting innovative and cost-effective environmental technologies.

New Rules for Medical Waste

The EPA estimates that its new proposed standards and guidelines for medical waste incinerators (MWIs) will reduce air pollution from the nation's 3,700 operating MWIs by 95% over 5 years.

EPA's proposal, *Standards of Performance for New Stationary Sources and Emission Guidelines for Existing Sources: Medical Waste Incinerators*, implements sections 111(b) and 129 of the 1990 Clean Air Act amendments. Section 129 requires the EPA administrator to establish performance standards for MWIs. Published in the February 27 *Federal Register*, the proposal targets emissions of dioxins, hydrogen chloride, lead, cadmium, mercury, and fly and bottom ash emissions from MWIs. Along with commercial medical waste incineration facilities, the new regulation mainly will affect hospitals, which produce more than 70% of the 3.4 million tons of medical waste generated in the United States each year. It will also affect nursing homes, veterinary facilities, commercial research laboratories, and bloodbanks, and the estimated 700 new MWIs installed over the next 5 years.

The EPA defines medical waste as solid waste generated when humans or animals are treated, diagnosed, or immunized, and when researchers produce or test biologicals–preparations like vaccines or cultures made from living organisms and their products.

Every year in the United States, according to the EPA, hospitals generate an estimated 2.5 million tons of solid waste, 15% of which is infectious. Laboratories, clinics, and medical offices generate even more biomedical waste, which can be anything from bandages, vials, syringes, hypodermic needles, and plastic tubing to blood, laboratory cell cultures, and human and animal tissues.

Unless the waste is treated, state and local governments usually prohibit municipal landfills from accepting it, prompting hospitals to either treat infectious waste on site or ship it to a hazardous-waste facility. Consequently, many hospitals own or share incineration facilities.

David Driesen, an attorney with the Natural Resources Defense Council (NRDC), says his organization would like to see the proposal require more pollution prevention. "A trend over the last few years has been toward good pollution alternatives and, at the state level, the closing down of small, uncontrolled incinerators at hospitals in favor of better-controlled regional facilities, often with the cooperation of hospitals," Driesen says. "The measure of success for this rulemaking is whether it will accelerate that trend."

Numerical Emission Limits

New and Existing MWIs

Particulate matter (PM):	30 milligrams per dry standard cubic meter (mg/dsm^3).
Carbon monoxide:	50 parts per million by volume (ppmv), dry basis.
Dioxins/furans:	80 ng/dsm^3 total dioxins/furans, or 1.9 ng/dsm^3 toxic equivalency (TEQ), determined by measuring the total dioxins/furans congener concentration and adjusting the results to account for each congener's toxicity.
Hydrogen chloride:	42 ppmv, dry basis, or 97% reduction.
Sulfur dioxide:	45 ppmv, dry basis.
Nitrogen oxides:	210 ppmv, dry basis.
Lead:	0.10 mg/dsm^3.
Cadmium:	0.05 mg/dsm^3.
Mercury:	0.47 mg/dsm^3, or 85% reduction.
Fly ash/bottom ash:	0% opacity from any fly ash or bottom ash storage or handling area on facility property.

In an April 28 statement to the EPA, the Chicago-based American Hospital Association (AHA) said it supports "the enactment of reasonable regulations that are necessary to protect the environment and public health. However, the proposed regulations appear to be overly restrictive, unnecessarily costly, and burdensome," with no significant environmental improvements or reduced risks, the statement said. AHA has more than 5,000 member hospitals and health systems and 50,000 personal members.

At the EPA Emission Standards Division in Research Triangle Park, North Carolina, Rick Copland says examiners will take a close look at all comments, including those from AHA "to see how they developed their cost estimation."

Medical waste incinerators are subject to widely varying state and local regulations. An April 1990 EPA survey showed that 38 states had MWI-specific regulations or permit guidelines in place or on the drawing board. The other states regulate MWIs under less stringent general incinerator requirements.

Subpart Cc of the new regulation proposes emission guidelines and compliance schedules for states to use in developing regulations to control existing MWI emissions. The proposed guidelines establish emission limits for specific pollutants (see table) and set out additional requirements. Facilities must train and qualify MWI operators and develop and annually update site-specific training manuals for each MWI. Annual testing and monitoring must be performed to show compliance with emission limits for dioxins, particulate matter, cadmium, lead, mercury, carbon monoxide, and hydrogen chloride. "With respect to dioxins," NRDC's Driesen emphasizes, "there is evidence it is already at potentially unsafe levels in human bodies. Mercury similarly is a pollutant that accumulates, causes water pollution, and moves up the food chain. Some pollutants tend to dissipate. This group has serious health consequences. We need to reduce their amounts *and* avoid adding them to the environment." Most dioxin sources have not yet been identified, Driesen adds. "But the data we do have say medical waste incinerators are right up there in terms of generating dioxins."

A continuous emissions monitoring system (CEMS) would track opacity and carbon monoxide emissions. The guidelines require monthly opacity testing to determine compliance with fly and bottom ash emissions. An annual stack test would monitor emissions of other pollutants. If an MWI passed all three annual compliance tests in a three-year period, the MWI could forego testing for that pollutant for the next two years. MWI facilities must either comply with a state plan within one year after the EPA approves the plan or comply with the state plan within three years after the EPA approves the state plan if the owner/operator documents and submits measurable, enforceable steps it will take to comply with the state plan. Finally, facilities must comply with operator training and qualification and inspection requirements within one year after the EPA approves a state plan.

In addition to the provisions for existing MWIs, the proposed regulation would also cover emissions from new MWIs. The rule would regulate site-selection for MWIs built after the final rule effective date, including a requirement of comprehensive air quality analysis and analysis of the potential effect of air, ground, and water pollution on visibility, soils, and vegetation. Facilities would submit results to the EPA and state and local officials, make results available to the public, and provide for a public meeting and prepare a comment and response document.

Driesen says his organization looks to the proposed rule for pollution-prevention incentives that do more than set standards. "The proposal was weak in that regard," he adds. "We're looking for requirements that encourage recycling, incineration alternatives, and pollution prevention efforts. The proposed regulations mention alternatives but don't require that anyone do anything with regard to pollution prevention. Emission standards seem to be aimed at accommodating waste streams that could be cleaner in the first place."

General standards would probably improve things, Driesen concludes, "but they fall short of state requirements for maximum achievable emission reductions. A well-controlled facility can do a lot better than what EPA is proposing."

"Individual incinerators emitted less than our proposed standards," EPA's Copland says, "but when we set a standard it has to be achievable by all incinerators. We feel the proposed standards are as stringent as our data would support." Copland adds, "This was a proposal. No one is required to do anything yet. We are taking comments and reassessing virtually everything. The final rule could look very different from the proposal." The final rule will take effect in April 1996.

Wayne Thomann, director of Occupational and Environmental Safety at Duke University Medical Center in Durham, North Carolina, says the newest MWI proposal won't affect his facility, which began in 1992 to use "administrative controls to assure compliance with EPA's evolving regulations on chemical emissions that affect public health and the environment." Thomann says his facility no longer burns the plastics and heavy metals that release priority pollutants. In response to the EPA's 1990 Clean Air Act Amendments, he says, "We decided to change our waste mix. We didn't need to burn plastics and heavy metals. Now we won't burn anything but pathological waste, animal carcasses, and bedding." Duke now uses commercial contractors to dispose of hazardous waste. "Since we took ourselves out of the loop [for these hazardous emissions]," Thomann says, "we're doing as well or better than the emissions standards being proposed."

If the MWI standards and guidelines were to take effect as proposed, the EPA estimates the nationwide annual cost of waste incineration per unit of medical waste treated for new MWIs would increase from $136 a ton to $161 a ton. For existing MWIs, the cost of waste incineration per unit of waste treated would rise to $222 a ton from the regulatory baseline cost of $185 a ton.

In terms of costs to the hospital industry and, ultimately, to hospital patients, the EPA estimates that for new MWIs the industry would have to raise prices to cover higher waste disposal costs by an average 0.03% over current revenues of $224 billion a year. For existing MWIs, this would mean an average price increase of 0.1% over current revenues. Put another way, AHA spokesperson Alicia Mitchell estimates total capital cost requirements for compliance could be as high as $1.6 billion, and annual owning and operating costs could be as high as $344 million per year.

The EPA believes medical waste generators now operating medical waste incinerators have three choices: continue to operate their on-site incinerator and comply with the proposed emission limits, install an alternative medical waste treatment technology on site, such as autoclave, microwave or chemical treatment, or contract with a commercial medical waste disposal service for off-site treatment and disposal of medical waste. For existing MWIs, the EPA estimates 80% of facilities now burning waste on site will switch to another treatment and disposal method to avoid the cost of installing air pollution control equipment.

Selenium Secrets

An ounce of prevention is said to be worth a pound of cure, but little is understood about the mechanisms of cancer that might be targeted as prevention strategies.

Researchers are looking at several naturally occurring elements and vitamins that may potentially protect against cancer. Despite a somewhat checkered past, selenium, an element obtained through the diet, may have a promising future in cancer prevention.

Selenium is widely distributed in inorganic form in soil and in organic form in certain foods. An excess or a deficiency in selenium intake can cause a variety of clinical symptoms and toxicities in humans. Most people in developed countries receive adequate amounts of selenium in its organic form (selenomethionine) found in cereals, grains, fish, and certain vegetables. Historically, selenium has been classified as a nutrient, but it is classified also as a toxin and a carcinogen. Recently, selenium has been labeled a "chemoprotective agent."

Research in the 1970s showed that selenium binds to and detoxifies poisonous levels of mercury. Mercury and other heavy metals oxidize low-density lipoproteins in the blood, a process that promotes arteriosclerosis and eventually leads to heart disease. Studies have shown that people in Japan with high levels of serum selenium who also eat mercury-tainted fish have a much lower rate of heart disease than the U.S. population.

Research on Canadian Eskimos provides another example of the protective properties of selenium. Éric Dewailly, director of the Environmental Health Service at Quebec's Public Health Center, has studied the Inuit from northern Quebec over the past decade. The Inuit have higher than normal blood levels of lead, mercury, and polychlorinated biphenyls (PCBs), which can be especially toxic to children and fetuses (see Dewailly et al., *EHP* vol. 101, no. 7, p. 618 and a related article, Chan et al., *EHP* vol. 103, no. 7-8, p. 740). Also, the smoking rate among Inuits is approximately 65%, about twice that of southern Canadians. Surprisingly, however, the Inuit have low levels of cancer and heart disease.

Researchers believe the Inuit may be protected by their intake of such protective substances as selenium and omega-3 oil from their diet of muktuk (skin of beluga whales) and other marine animals. Dewailly explains that "Inuit whole-blood selenium levels are about 10 to 15 times the levels found in the U.S. population." Although this evidence is intriguing, some researchers question whether confounding factors such as a shorter life span may account for the difference in disease rates. Because the average life expectancy for Inuits (62 years) is slightly lower than for the U.S. population, one theory is that Inuits do not live long enough to develop certain cancers. However, because cancer incidences for a population are typically age-adjusted, this may not be a real factor.

Results of epidemiological studies are inconsistent on the protective role of selenium against chemically and virally induced cancers. In a 1993 study published in the *Journal of the National Cancer Institute*, selenium, in combination with vitamin E and beta-carotene, successfully protected against spread of esophageal and stomach cancer in a clinical intervention trial conducted in Linxian, China.

Studies in laboratory animals also support the protective role of selenium in cancer. Rat, mouse, and hamster models have been used to study liver, breast, colon, skin, and pancreatic cancers. A review titled "The Chemoprotective Role of Selenium in Carcinogenesis," published in the *Journal of the American College of Toxicology* in 1986, stated: "Of 35 studies published since 1949, 31 have shown that selenium produced an inhibitory response, whereas only 3 reports have found that there was no effect." The review continued, however, "In 1 case, selenium . . . increased pancreatic ductular carcinoma yields," indicating there is still no clear-cut case for selenium's protective role.

Laurie Chan/CINE

Fish tales. Traditional seafood diets may protect against environmentally related diseases.

Most of these animal studies have used the inorganic form of selenium (selenite, selenate, or selenium dioxide). The primary concern with these compounds is that they may be toxic at doses required to achieve chemoprotection. To address this problem, Karam El-Bayoumy, associate division chief at the American Health Foundation, and his colleagues are attempting to develop novel synthetic organoselenium compounds that are chemoprotective and have low toxicity.

Results of several studies published in a review article in the November 1994 issue of *Carcinogenesis* show that one promising organoselenium compound from El-Bayoumy's laboratory has reduced tumors in breast, lung, and colon cancer in animals caused by several different carcinogenic agents, including one present in tobacco smoke. Requests to conduct detailed toxicology testing are now being accepted by the National Cancer Institute. Following this testing, the compound may enter phase I clinical trials, although no timetable can yet be estimated.

Computer Recycling Takes Hold

Old computers never die, they just go to the attic . . . or the basement, or the local elementary school. At least, that's where they've gone until now. But the likelihood that outdated computers will start ending up in landfills is increasingly high. According to scientists at Carnegie-Mellon University, the average computer now becomes obsolete within 12 months of production. They predict that about 150 million personal computers will be in landfills around the world by the year 2001—enough to fill an acre-wide hole three-and-a-half miles deep.

"Today, two computers become obsolete for every three purchased," D. Navin-Chandra, an assistant professor at Carnegie-Mellon, recently told *Fortune* magazine. "By 2005, the ratio will be 1 to 1, which means we should be able to recycle computers as fast as we make them."

The lack of a recycling infrastructure for computers poses a threat not only to municipalities faced with collecting and disposing of the computers in their landfills, but also for the manufacturers, who might be held responsible for any leakage of toxic materials from these computers. Some computers have leachable quantities of lead and other toxic materials, although they are present only in small quantities.

Computers have at least some components that are economical to recycle. Specialty recyclers like the Handy and Harman company have been involved in

the disassembly and recovery of materials from computers, printers, and other electronic hardware since the early 1960s. Their business is growing at the rate of 15–20% a year.

"The principal economics with a computer is in the recovery and refurbishment of the subsystems—the hard drive, the keyboard, and occasionally the monitor," says Steven Foulk, marketing executive with Handy and Harman. "The next level involves recovery of the integrated circuits and various components including the processor and memory. The final level is the recovery of precious metals including gold and copper."

Once a computer is more than 4–5 years old, Foulk says, the subsystems are generally not worth refurbishing. At that point, the value comes in "mining" the computer for its raw materials. The typical desktop computer contains about $50 worth of usable material. This includes aluminum valued at $9.37, gold at $6.45, and copper at $5.56. Unfortunately, the costs of collection, dismantling, purification, and smelting can run $45–47 per unit, making it little more than a break-even operation. Plastics, which at $12.07 per unit have the highest value of all components, present a particular problem.

"There are too many different kinds of plastics being used in most computers, which makes them difficult to separate," says Foulk. "Maybe 20 percent is recyclable, and the rest is either incinerated or landfilled."

Responding to pleas from recyclers and environmentalists, computer manufacturers are beginning to design their products with end-of-life management in mind. Two schools of design are emerging: design for the environment (DFE) and design for disassembly (DFD). IBM's Engineering Center for Environmentally Conscious Products is pioneering advances in both these fields.

"Starting in 1991, we emphasized design that used fewer materials and less energy," says J. Ray Kirby, director of the IBM center. "Within the second year, we focused on how to design to assist in recovery and recycling. Our PS/2E qualifies for the government's Energy Star Logo. It tak7es less energy to run than most PCs and the plastic cover has 25 percent recycled content."

IBM has only a limited take-back program. The company operates a number of collection centers in Europe, which has a more aggressive stance on take-back. Computers are sent to Scotland where the keyboards are melted down and remolded. In the United States, IBM is piloting some programs to evaluate the costs of take-back, but these are not yet available to all customers.

Joseph Tart

Will not compute. Rather than shelving the problem of what to do with outdated computer equipment, several companies are pushing for recycling and reuse.

Hewlett-Packard operates product recovery centers in Roseville, California, and Grenoble, France, which together retrieve nearly 800,000 pounds of computer and related equipment each month. Products are disassembled and sorted into types of components. Reusable and resalable parts, such as computer chips, are recovered and refurbished. The remaining parts are recycled to the maximum extent possible.

Apple sponsors periodic trade-in programs for its personal-use computers through colleges and universities. Students can receive a $250 discount on a new Apple computer or printer when trading in an older model. Computers are sent to Fox Electronics in San Jose, California, which recovers the integrated circuits and sends other components to other recycling firms. Apple's most recent buy-back program netted over 15,000 computers.

Recyclers identify four major problems that must be addressed if computer recycling is to make significant inroads. First, computers need to be designed for easier disassembly, which currently runs about $20 per desktop computer in labor costs alone. Second, separation systems for mixed plastics need to be developed. Third, recycling efficiency for metals needs to be improved. And finally, recycling processes need to be developed for elements, particularly exotic elements such as rhodium and terbium, which are not currently being recycled, but which may become critical because rare elements are used in advanced computing systems.

"For recycling to be effective, the infrastructure to take computers apart has to be as big as the manufacture," says Foulk. "The infrastructure for recovery of the metals is in place. The infrastructure for dismantling and recovering subsystems that still have value is developing. Collection and transportation is in its infancy."

Measuring UV's Effects

Long-term exposure to UV-B rays, the spectrum of sunlight with a wavelength shorter than 320 nanometers, is known to contribute to a variety of human ailments including premature aging of the skin, nonmelanoma skin cancer, and cataracts. It is also suspected to play a role in melanoma skin cancer and suppression of the immune system. Stratospheric ozone is the most important factor determining the amount of UV-B radiation reaching the earth's surface. Concern about human exposure to UV-B has been increasing since decreases in atmospheric ozone were discovered over Antarctica in 1985. Unfortunately, there is no worldwide network for measuring changes in UV radiation, so there is no clear understanding of how much UV radiation is increasing in different locales, or whether such increases might be responsible for observed changes in biota. But that situation is about to change.

In coordination with the U.S. Global Change Research Program, the EPA has

installed spectroradiometers—devices that will provide long-term data on the UV-B flux reaching the earth's surface—in five cities in the United States: Atlanta, Georgia; Research Triangle Park, North Carolina; Gaithersburg, Maryland; Boston, Massachusetts; and Bozeman, Montana. Data from these devices will be useful in studies ranging from the effects of UV-B flux on the incidence of cataracts to the failure of striped bass eggs to reach maturity.

The device, called a Brewer spectroradiometer, is capable of measuring radiation across the UV range in half-nanometer wavelengths. Spectroradiometers provide continuous measurements, feeding data automatically into computers inside the buildings on which the instruments are installed. Continuous measurement is crucial since most biologic effects from UV-B are sensitive to accumulated doses rather than a threshold dose. The main advantage of spectroradiometers over other UV-monitoring instruments is that the biologic effectiveness for any spectrum can be calculated. Additionally, the details of the spectrum bear vital information about the composition of the atmosphere, such as total ozone column, total oxygen saturation column, and particle scattering, all of which affect the amount of UV-B reaching the earth's surface. The relationship between these various phenomena are complex and not well understood. Ground-based UV measurements, coupled with other meteorological data such as cloud cover, are necessary to explore atmospheric changes and the resultant effects on the biosphere.

"Until now, the data has not been good enough to resolve the many issues with respect to changes in the UV-B flux," says Larry Cupett, acting director of the EPA's Atmospheric Processes Research Division. "We know that changes in the total ozone column can influence the amount of UV-B reaching the earth's surface. But is there a long-term trend? What is the influence of cloud cover, particulate scattering, and ozone in the planetary boundary layer? Until you understand all the parameters, you can't identify which ones will have long-term effects. And until we get a technique that can accurately measure UV-B, the correlations with various biological effects will be suggestive."

Project scientists estimate they will need at least 5–7 years' worth of data to begin to detect long-term changes. Data gathered from each site will be posted on the World Wide Web under the EPA's home page beginning later this year. Additional cities will be included if funding becomes available.

Menace in the Mix

New research underway at Duke University could yield clues to how chemicals used by U.S. soldiers during the Persian Gulf War may have intermingled and caused neurotoxic effects in some veterans. The combined, or synergistic, effects from three chemicals: pyridostigmine bromide, DEET, and permethrin, may have caused some of the symptoms reported by Gulf War veterans, including chronic fatigue, rashes, headaches, weight loss, and joint pain, according to Mohamed Abou-Donia, a professor of pharmacology at Duke University who is spearheading the research.

"It's a plausible hypothesis that synergism occurred," says Ernest Hodgson, head of the toxicology department at North Carolina State University, who has devoted much of his work to studying the synergism of chemicals. "That's not to say [the hypothesis] is an appropriate lead for further investigation," Hodgson cautions. "Dramatic cases of synergism are really not that common."

Abou-Donia's preliminary findings are arresting, however: they show that, when introduced alone, the chemicals caused no harmful effects on laboratory animals. However, when the chemicals were administered two or more at a time, the animals underwent significant neurological damage. Abou-Donia and his colleagues, toxicologist Ken Wilmarth and biochemist John Locklear, tested the chemicals on chickens because they are more sensitive than rats to chemicals that harm the central nervous system and because federal agencies call for the use of chickens when screening chemicals for possible neurological effects.

Findings from Abou-Donia's research, due to be published soon, may supply at least one missing link in the chain that may one day conclusively tie a number of symptoms reported by Gulf War veterans to environmental exposures they suffered during the war. Soldiers there endured environmental hardships ranging from oil well fires and infectious parasites to pesticides, insecticides, and anti-nerve gas pills originally intended to protect them. Abou-Donia's research is being funded by a grant from former presidential candidate and veterans' advocate H. Ross Perot.

Ironically, the three chemicals being evaluated at Duke were issued by the Department of Defense to protect soldiers. At the outset of the conflict, U.S. and British troops were given a 21-count package of 30-mg pyridostigmine bromide, anti-nerve gas pills that would counter the effects of potential Iraqi chemical warfare. "It shields an enzyme present in the brain and peripheral nervous system in a reversible manner, for a short period of time," says Abou-Donia, explaining how the chemical functions.

Though the DOD and a Defense Science Board Task Force on Gulf War Effects concluded in their 1994 report that the Iraqis did not use chemical or biological weapons against coalition forces, rumors of chemical warfare apparently circulated widely among soldiers throughout the conflict. According to Abou-Donia, fear prompted many soldiers to take more than the recommended dosage of pyridostigmine bromide pills. "During the war, over 50 percent of U.S. service personnel seen in the health service complained of symptoms relating to pyridostigmine bromide," Abou-Donia says.

The other two chemicals Abou-Donia and his colleagues are studying are *N,N*-diethyl-*m*-toluamide (DEET), an insect repellent, and permethrin, a liquid insecticide. According to Abou-Donia, DEET (used in a 90% concentration) was used due to concern about insect-borne tropical illnesses. Soldiers' uniforms were impregnated with permethrin, says Abou-Donia. Some veterans have reported that combat uniforms were doused with the liquid, then distributed in plastic bags. Because soldiers would presumably wear the uniforms for an extended time—a period of days, perhaps—extensive dermal contact with the permethrin would have occurred. Both DEET and permethrin have low acute toxicity, Abou-Donia says. "If the two chemicals had been given alone, they would not have caused harm," he quickly points out. In 1992, New York state banned the use of insect repellents containing more than 30% concentrations of DEET because of concerns over health effects (see *EHP*, vol. 102, no. 11, p. 910).

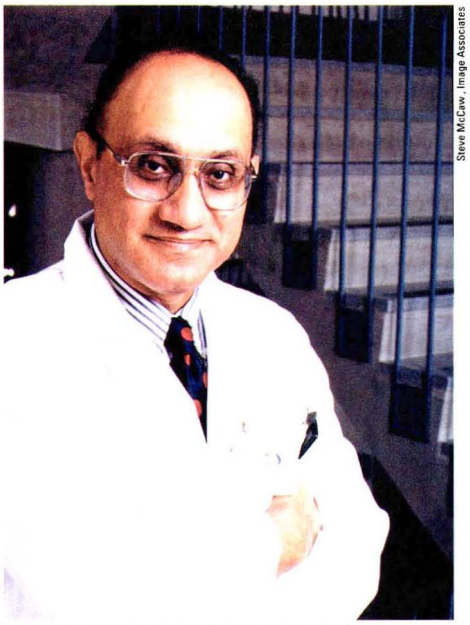
To test the combination of chemical exposures in the lab, the researchers administered pyridostigmine bromide orally and both the DEET and permethrin dermally via subcutaneous injection. Though soldiers in the battlefield would have absorbed the latter two chemicals dermally, Abou-Donia and his colleagues had to inject the chickens subcutaneously to deliver precisely measured and statistically viable quantities.

Abou-Donia's team is also investigating the hypothesis that the chemicals the Gulf War soldiers were exposed to generated a delayed toxic impact known as organophosphate-induced delayed neurotoxicity (OPIDN). OPIDN assaults both the central and peripheral nervous systems, producing symptoms such as weak-

ness, lack of coordination, and even paralysis. After analyzing brain tissue samples from laboratory chickens, the researchers found that nerve damage was linked to the decreased activity of an enzyme present in tissues, neurotoxic esterase. The team is searching for biomarkers present in the animals' blood that will show nervous system damage, and are comparing animal blood samples with those from affected veterans. "If we could find a biomarker, then we could find a treatment for existing populations," Abou-Donia said.

While Abou-Donia's research sheds light on the connection between environmental exposures and some medical symptoms associated with what is unofficially referred to as Gulf War Syndrome, it does not explain why some individuals appear to be more sensitive to the chemical effects.

"We would like to try to see if, in fact, we could identify a population segment that is naturally predisposed to chemical sensitivity," says Abou-Donia describing MCS, a medical phenomenon that is widely acknowledged, yet little understood, even by scientists and physicians closest to the issue. "Maybe there is a genetic variant we need to identify," Abou-Donia suggests. A more complete understanding of genetically dependent chemical sensitivity could be useful should the DOD deploy soldiers in future military operations.



Steve McCaw, Image Associates

Researching synergism. Duke's Mohammed Abou-Donia is investigating whether the illnesses plaguing Gulf War soldiers are the result of exposure to chemical mixtures.

One scientist interested in studying Persian Gulf veterans with symptoms resembling multiple chemical sensitivity is physician Claudia Miller, an expert on MCS and an assistant professor of environmental and occupational medicine at the University of Texas Health Science Center at San Antonio. As a staff physician at the Houston VA's Persian Gulf Regional Referral Center, Miller evaluates the health of Gulf War veterans.

According to Miller, certain people may have genetically determined metabolic differences that make them susceptible to chemical sensitivity—a phenomenon that falls into a new area of study called ecogenetics. "We rely heavily on epidemiological studies, which are based on crude estimates of past exposure," Miller says of her work with Gulf War veterans and other MCS patients. "We're trying to correlate [chemical exposures] with health problems," a process that is error-prone because it relies on anecdotal information from patients, but which Miller calls "invaluable" for the information it supplies.

Of the veterans she has evaluated who have unexplained illnesses following their service in the Persian Gulf, a large portion have reported the onset of new chemical intolerances which commonly include diesel exhaust, solvents, gasoline, tobacco smoke, hairspray, and fragrances. Miller refers to this as a toxin-induced loss of tolerance (TILT), a term she prefers to chemical sensitivity "because available data on MCS patients and Gulf War veterans point away from MCS as a syndrome, but perhaps toward what may be an emerging new mechanism or theory of disease." Describing TILT, Miller says, "Once their tolerance level is exceeded, they don't respond normally to low-level exposures." These veterans exhibit the typical two-phase response observed in MCS patients: during the induction phase, loss of tolerance occurs following an acute chemical event or a less-acute series of events. This loss of tolerance can involve any of a wide range of chemicals such as medications, caffeine, foods, and other chemically unrelated substances. In the triggering phase, patients experience symptoms when they are exposed to tiny amounts of such common substances, but these responses overlap in timing, thereby masking any individual reaction to a single chemical. What is relevant to the Gulf veterans' illnesses about TILT, says Miller, is the fact that a wide range of environmental agents (solvents, pesticides, combustion products, etc.) appear to be capable of initiating this process.

According to Miller, further research on chemically sensitive Gulf War veterans will require studies conducted in a controlled environmental medical unit—a hospital-like environment built and furnished with materials that don't emit chemical vapors and equipped with an efficient air filtration system. Only in such an environment, Miller says, could subjects be observed to see whether they improve, and if so, then be re-exposed to very low levels of chemicals, one at a time, under double-blind, controlled conditions and then evaluated for symptoms.

Studies on Gulf War health effects are as politically significant as they are scientifically important. Confirmation of a link between chemical exposures and negative health effects would allow affected Gulf War veterans to receive compensation under the veterans' disability compensation program.

The U. S. Department of Veterans Affairs established three research centers—in Boston; East Orange, New Jersey; and Portland, Oregon—in October 1994 to study how environmental and toxic hazards may affect health. Among six research projects underway at the Boston center is a study to examine the relationship between war-time exposure and chronic fatigue, chemical hypersenstitivity, and post-traumatic stress disorder. At the New Jersey center, scientists are gathering information on illnesses suffered by Gulf War veterans, hoping to examine a characteristic symptom profile and connect certain risk factors with the development and progression of unexplained illnesses. Researchers at the Portland center are screening veterans for medical, chemical, or biological markers that may confirm exposure and disease, as well as studying how chemical agents, including pyridostigmine and pesticides, may affect the nervous system.

NIEHS Employees Honored by NIH Director and Public Health Service

NIEHS employees were honored recently at the National Institutes of Health for outstanding research in areas such as the gene for inherited breast cancer, reproductive toxicants, and slippery DNA, and for innovative management of a federal facility.

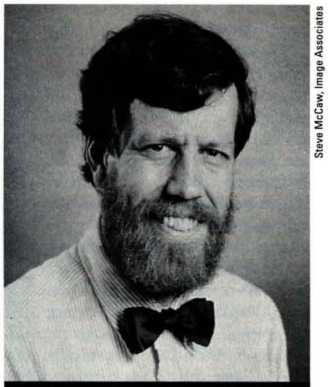
The NIH Director's Group Award was presented to the Comparative Carcinogenesis Group of the Laboratory of Molecular Carcinogenesis for its role in the isolation and characterization of the breast and ovarian cancer susceptibility gene, *BRCA1*. Identification of the *BRCA1* gene has major implications for public health, including the potential for early tumor detection, improved prognosis, and new therapeutic strategies. Members of the group include J. Carl Barrett, Michelle Bennett, Heather Brownlee, Charles Cochran, Andrew Futreal, Astrid Haugen-Strano, Lori Terry, and Roger W. Wiseman.

Robert Chapin, a toxicologist in the Reproductive and Developmental Toxicology Group of the Experimental Toxicology Program, received the NIH Director's Award in recognition of his work in the detection of human reproductive toxicants and in assessing human reproductive risks associated with chemical exposures. Chapin has devised new methods for screening large numbers of chemicals for reproductive toxicity in rodents and has worked to develop specific protocols in this area.

Mutations arise when DNA bases are copied out of sequence, generating base additions or deletions. The human genome contains many slippage-prone sequences that may be increased in certain tumors. Thomas Kunkel, a research geneticist in the Laboratory of Molecular Genetics, has discovered that a frequent cause of this human mutator condition is a defect in the repair of the newly produced mutations.

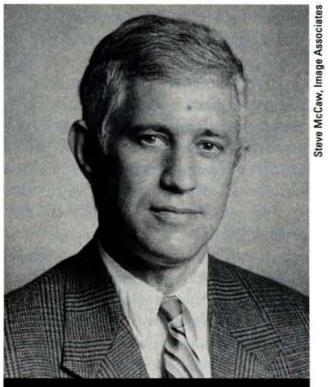
Kunkel received the NIH Director's Award for his studies of the mechanisms of DNA-slippage and its role in carcinogenesis and HIV variation.

Thomas M. Bedick, chief of the NIEHS Facilities Engineering Branch, received the Public Health Service Meritorious Service Medal for his role in the renovation of 50 office spaces for use as scientific laboratories. The award commended Bedick for exemplary leadership and innovative management in building an effective professional team.



Steve McCaw, Image Associates

Robert E. Chapin



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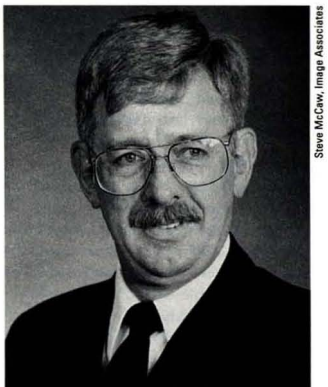


Steve McCaw, Image Associates

Thomas M. Bedick

Steve McCaw, Image Associates

(Front row, left to right) **Lori Tarry, Andrew Futreal, Roger Wiseman, J. Carl Barrett, Astrid Haugen-Strano;** (back row, left to right) **Michelle Bennett, Charles Cochran, Heather Brownlee.**

Environmental Justice Leaders Meet

At a recent meeting hosted by NIEHS Director Kenneth Olden, environmental justice leaders reviewed the Department of Health and Human Services' Environmental Justice Strategy (see Special Report on next page). At the meeting in Research Triangle Park, North Carolina, leaders emphasized the need for a coordinated approach to ethnic and regional networks focused on health and justice in low-income and minority populations. Senior staff from the NIEHS and the National Toxicology Program identified opportunities for improved partnerships with the networks through the hazardous waste and minority worker training program, environmental justice partnership grants, and other core professional training and research activities. Olden stressed the importance of expanding collaborative efforts with the networks over the next year, as the NIEHS and DHHS implement the environmental justice strategy.

Leaders who attended the meeting include: Richard Moore, director of the Southwest Network for Economic and Environmental Justice and chair of the National Environmental Justice Advisory Committee, Mary Crowe of the Indigenous Environmental Network, Charles Lee of the United Church of Christ's Commission on Racial Justice, Connie Tucker of the Southern Organizing Committee, Robert Bullard, director of Clark Atlanta University's Environmental Justice Resource Center, and Yin Ling Leung of the Asian Pacific Environmental Network.

NIEHS

Justice team. (left to right) Sharon Beard, Allen Dearry, Mary Crowe, Charles Lee, Kenneth Olden, Richard Moore, Connie Tucker, Robert Bullard, Yin Ling Leung, and Jerry Poje.

Special Report

Strategic Elements for Environmental Justice*

Goal:

To ensure that disproportionately high and adverse environmental and health effects experienced by low-income and minority populations are addressed, as appropriate, in the programs of the Department of Health and Human Services (HHS), and that these programs encourage the full involvement of affected parties.

Introduction

On February 11, 1994, President Clinton signed executive order 12898, *Federal Actions To Address Environmental Justice in Minority Populations and Low-Income Populations.* This Executive Order requires each federal agency to make achieving environmental justice part of its mission by identifying and addressing, as appropriate, disproportionately high and adverse human health or environmental effects of its programs, policies, and activities on minority populations and low-income populations in the United States and its territories and possessions. Under the terms of the executive order, each federal agency is required to develop an agency-wide environmental justice strategy.

"Environmental Justice" seeks to ensure that no population is forced to shoulder a disproportionate burden of the negative human health and environmental impacts of pollution or other environmental hazards. HHS's strategic plan is one component of the overall federal effort to address such disproportionate burdens in low-income communities and minority communities.

This outline of the HHS strategy for addressing disproportionate environmental and human health burdens in low-income and minority communities is divided into six sections. The first and most important section describes the Department's objectives for creating partnerships with the public in the activities described in the strategy. The next four sections cover the activity areas addressed in the strategy: Public Education and Training, Services, Data Collection and Analysis, and Health Research; the last section describes strategies for interagency coordination of these programs. Policy oversight mechanisms within HHS will be maintained to assure ongoing attention of HHS agencies and programs to advancing the agenda of environmental justice and to safeguarding against unintended environmental consequences of their activities.

For this document, "public" means any affected or interested party, with special emphasis on minority and low-income communities and workers, and including, but not limited to, state and local governments and agencies, tribal nations, Congress, other federal agencies, review bodies, community groups, environmental and other interest groups, business and industry, labor, religious and social organizations, the media, academia, professional and technical organizations, educational organizations, employees and contractors, and members of the general, unaffiliated public.

Oversight and coordination for implementation of this strategy will be the responsibility of the Assistant Secretary for Health working with the Public Health Service (PHS) agencies. The Environmental Health Policy Committee (EHPC) is an HHS committee established to coordinate policy development for environmental health activities related to the mission of the PHS and its agencies. It serves as the primary focal point within the Department of Health and Human Services for promoting the exchange of environmental health information and for providing review, advice, and consensus facilitation where necessary on environmental health research, exposure assessments, risk assessments, and risk management procedures. The EHPC is chaired by the Assistant Secretary for Health and includes agency heads and other leaders from PHS agencies with environmental health missions. HHS agencies will be required to prepare evaluation plans to measure progress on their objectives related to reducing the human health burdens of disproportionately high and adverse environmental exposures in low-income and minority communities. Agencies will engage public participation in the development of their implementation plans.

The objectives and strategies outlined in this plan are based on current HHS activities and programs in place. The strategic plan organizes these programs so as to address key environmental justice goals within existing resources and authorities. The key to the success of this strategy will lie in fostering partnerships with members of the public, especially residents of low-income and minority communities, and in promoting collaboration and coordination between HHS agencies, so that HHS's programs can be as effective as possible in addressing the health impacts in low-income and minority communities of disproportionately high and adverse environmental exposures.

I. Public Partnerships

Objective 1: In the context of HHS programs and available resources, ensure that members of the public are meaningful partners in all appropriate departmental activities to address the health impacts of disproportionately high environmental hazards in low-income and minority communities, including education, training, provision of services, data collection, and research.

Strategies:

- Work with affected communities and potentially affected communities to enhance their capacity to participate in the partnership.
- Engage the public's involvement in identifying a full range of alternative approaches to developing a broad-based consensus on what the objectives should be to address disproportionate environmental hazards in low-income and minority communities, and on how to achieve those objectives.
- Ensure that each HHS agency provides opportunities for meaningful participation by interested members of the public, including residents of minority and low-income communities, before making decisions that will affect the public.

Objective 2: In the context of HHS programs and available resources, provide mechanisms by which each relevant HHS agency can develop public participation plans.

Strategies:

- Improve the working relationships within and among HHS agencies and between HHS agencies and the public in order to facilitate and maintain credible and open decision-making processes and to coordinate and integrate public participation activities.
- Encourage cooperation between HHS and contractors in identifying and resolving major issues that are relevant to the objectives of the executive order.

*This report was prepared by the Subcommittee on Environmental Justice, Environmental Health Policy Committee, DHHS.

- Educate HHS grantees pursuing environmental justice-related activities funded by HHS agencies about HHS public participation objectives, and encourage them to meet those objectives.

II. Public Education and Training

Objective 1: In the context of HHS programs and available resources, educate residents and workers in affected communities through effective outreach, education, and risk communication.

Strategies:

- Develop a plan of action in collaboration with target communities that includes scientific investigation as well as appropriate educational campaigns to educate minority and low-income populations about environmental and occupational hazards; make sure that educational materials are appropriate, understandable, and efficacious (including preparation in languages other than English where needed).
- Conduct training workshops in health-risk communication and education for community members and workers; teach workers and members of low-income and minority communities about the relationship between pollution and adverse health effects and about the importance of various disease-prevention approaches, including pollution prevention and hazard abatement.
- Engage low-income and minority children and youth and their families in activities to address health impacts of disproportionately high and adverse environmental exposures through schools and the Head Start program.

Objective 2: In the context of HHS programs and available resources, establish strong ties with community-based organizations, workers' groups, public health agencies, and educational and religious institutions that may be able to help increase awareness of environmental hazards among those at risk.

Strategies:

- Identify barriers that may inhibit agencies from developing positive working relationships with these organizations.
- Educate HHS staff about the disproportionate environmental and health burdens in low-income and minority populations; send agency staff to visit affected communities whenever practicable and appropriate.
- Identify a mechanism for conducting ongoing relations with community-based organizations and leaders, public health agencies, and educational institutions within affected communities and with those interacting with these communities, especially historically black colleges and universities and other minority institutions (HBCUs/MIs).
- Encourage health professionals serving low-income and minority populations to participate in environmental and occupational health education workshops, scientific meetings, seminars, and other forums designed to enhance their knowledge of possible adverse health outcomes associated with exposure to environmental and occupational hazards.
- At the request of community organizations, conduct environmental health education seminars on the possible health effects of exposure to environmental and occupational hazards.

Objective 3: In the context of HHS programs and available resources, make environmental and occupational health data more available to the public and inform the public of how to gain access to this data.

Strategies:

- Use community-based directories of organizations and individuals that promote environmental and occupational health awareness among underserved and low-income and minority populations to identify partners for collaborative educational and information-sharing activities.
- Establish repositories of environmental and occupational health data in public schools, public libraries, community colleges and universities, community organizations, and State Offices of Minority Health.
- Continue HHS efforts to expand the amount and scope of health information that is made available to the public; for example, make data from several surveys and the vital statistics program available on CD-ROM and in computer microdata tape format and on Internet.
- Collaborate and consult with members of at-risk communities, workers, and national minority organizations to determine the most effective methods of translating and disseminating occupational safety and health information.

Objective 4: In the context of HHS programs and available resources, focus training efforts to enhance the availability of specific skills and services needed by low-income and minority populations affected by disproportionately high and adverse environmental exposures.

Strategies:

- Protect low-income and minority workers disproportionately represented in hazardous occupations with programs to promote safe workplaces and work practices, including efforts such as the HHS program to train hazardous waste workers in the proper procedures to use when cleaning hazardous waste sites and disposing of hazardous materials.
- Expand on existing occupational and environmental medicine training opportunities, including professional training, continuing medical education, and curriculum development, for health care providers and public health personnel who serve a significant number of the minority and low-income populations at high risk for occupational exposure, and work with the professional organizations and societies of these providers continue to build an adequate workforce of environmental medical expertise that can help address the environmental health needs of low-income and minority populations disproportionately affected by high and adverse environmental exposures.
- Train residents of minority and low-income communities for certification in cleanup and remediation of environmental hazards.

III. Services

Objective 1: In the context of HHS programs and available resources, identify specific disproportionately high and adverse environmental hazards affecting workers and people in minority and low-income communities, identify the health problems associated with these hazards, and identify the needs and concerns of the people affected.

Strategies:

- Actively solicit information on specific environmental and occupational hazards and on people's health needs.
- Develop a method by which to assess local problems; use the assessment method as a basis for community education and involvement. Where appropriate, work through existing local groups and networks.
- Target existing HHS programs to train and equip residents of minority and low-income communities to carry out community and residential audits of environmental hazards.
- Design interventions to address the problems identified.

Objective 2: In the context of HHS programs and available resources, assess the capacity of low-income and minority communities affected by disproportionately high and adverse environmental exposures to diagnose, treat, and prevent environmentally sensitive medical problems, and as appropriate, seek to remedy any deficiencies.

Strategies:
- Provide technical assistance to low-income and minority communities impacted by disproportionately high and adverse environmental exposures to develop primary and preventive health programs aimed at specific environmental and occupational hazards.
- Provide as necessary for the medical testing of communities and workers with disproportionately high and adverse environmental exposures to determine the extent of exposure to hazardous substances.
- Identify community resources and barriers to care in order to promote access to primary care services in disproportionately affected communities; consider translation and outreach services, transportation, evening hours, and types of health services available.
- Work with state primary care associations and other state and local agencies to assure access to quality environmental and occupational medical care in affected communities.

Objective 3: In the context of HHS programs and available resources, take advantage of existing HHS programs that promote the economic potential of individuals and communities and provide opportunities for meaningful career development; use these programs as appropriate to advance the goals of the executive order by promoting the development of necessary environmental remediation and related services within disproportionately affected low-income communities and minority communities so as to provide them with an economic return.

IV. Data Collection and Analysis

Objective 1: In the context of HHS programs and available resources, improve the collection of monitoring and surveillance data on disproportionately high and adverse environmental hazards in minority and low-income communities and on the health status of residents.

Strategies:
- Continue HHS efforts to collect, maintain, and analyze data on disproportionately high and adverse exposures to environmental hazards and on indicator conditions (health outcomes associated with important environmental factors) in minority and low-income communities.
- Wherever possible and appropriate, ensure that the data collected is sufficient to permit analysis of any linkages between exposures and health outcomes.
- Work with state, local, and tribal health officials, environmental health officials, regional health officials, and other federal agencies to improve health and environmental surveillance and monitoring activities in minority and low-income populations disproportionately impacted by high and adverse environmental exposures.

Objective 2: In disproportionately and adversely affected minority and low-income communities, and in the context of HHS programs and available resources, focus studies so as to provide low-income and minority residents with effective surveillance, monitoring, treatment, and prevention of adverse health effects.

Strategies:
- Use existing and new data to identify and target communities with disproportionately high rates of adverse effects from hazardous environmental conditions.
- Develop a coordinated, comprehensive program capable of addressing multiple environmental health and social problems in low-income and minority communities with disproportionately high and adverse exposures.
- Foster active partnerships and collaborations across HHS agencies, with state, local, and tribal governments, with private and voluntary sector groups, and with affected low-income populations and minority populations with disproportionately high and adverse environmental exposures.
- Involve members of the public in activities to collect data in affected communities wherever feasible and appropriate.

Objective 3: In the context of HHS programs and available resources, use information from State birth and disease registries to investigate the health effects of disproportionately high and adverse environmental exposures in low-income populations and minority populations.

Strategies:
- Evaluate existing registries and make recommendations regarding the methodologies they use.
- Consult with and assist states as appropriate to improve the capacity of their birth and disease registries.

V. Health Research

Objective 1: Design environmental and occupational health research programs within HHS in partnership with minority and low-income communities.

Strategies:
- Identify mechanisms such as regional meetings, register notices, and advisory and review bodies that can be used to engage the participation of low-income and minority communities and workers in the assessment, design, and conduct of environmental and occupational health research.
- Promote and institutionalize public participation in all phases of research through focus groups and peer review procedures.
- Incorporate information from low-income and minority communities and workers on their diseases and exposures when devising any environmental and occupational health research agenda.
- Collaborate and coordinate with community-based organizations, business and industry, academia, labor, and health professionals concerned about disproportionate environmental and health burdens in low-income and minority populations to develop new and relevant models for health research.

Objective 2: In the context of HHS programs and available resources, identify and characterize environmental and occupational factors that have the greatest disproportionate adverse impact on the health status of low-income and minority communities.

Strategies:
- Compile and document the extent of the problem by analyzing available data; document gaps in critical information.
- Identify high-risk populations, communities, industries, and occupations and document the environmental and occupational factors that have the greatest adverse impact on human health.
- Conduct epidemiologic research and surveillance on illnesses and injuries that disproportionately affect minority and low-income workers.
- Where appropriate, have studies take into account additional exposures due to such factors such as subsistence consumption of fish and wildlife and that study indicators are appropriate to the group, population, or community under study.
- As appropriate in the analysis of disproportionate adverse environmental and health impacts on low-income and minority communities, use and develop new models for occupational and environmental science research that can be used in population-, community-, and industry-based studies of 1) exposures and diseases among small numbers of people, 2) human exposures to low levels of a known environmental or occupational hazard (especially chronic, low-level exposures), 3) human exposures to combinations and mixtures of hazards at low levels for extended periods and at acute levels for short periods, and 4) new biological markers that can be used in identifying risk factors.
- Keep community members informed of the results of studies.

Objective 3: In the context of HHS programs and available resources, establish a coordinated program of environmental and occupational health research among the HHS agencies that is consistent with an overall departmental strategy for addressing disproportionate environmental and health burdens in low-income and minority populations.

Strategies:

- Maintain a structure within the HHS Subcommittee on Environmental Justice that will provide ongoing monitoring and evaluation of health research activities relevant to addressing the disproportionate environmental and health burdens in low-income and minority populations.
- Establish and document specific coordination processes that address emerging issues in environmental and occupational health research.
- Establish HHS health research priorities, including issues of disproportionately high and adverse environmental health impacts in low-income and minority populations, through a regular Science Managers' Conference.
- Design and support collaborative interagency environmental and occupational health research projects to address adverse health impacts that fall disproportionately on low-income communities and minority communities.

VI. Interagency Coordination

Objective 1: Foster interagency coordination (both within HHS and between HHS and other agencies) in all activities related to addressing disproportionately high and adverse environmental health impacts in low-income and minority populations, including public education, training, the provision of services, regulatory activities, data collection, and research.

Strategies:

- Ensure that all HHS environmental justice-related activities and agency plans for implementation are reviewed by the Environmental Health Policy Committee (EHPC), chaired by the Assistant Secretary for Health, comprising agency heads, senior management representatives from PHS components with environmental health responsibilities, and liaisons from other key Federal agencies.
- Foster more in-depth coordination across agencies through the EHPC Subcommittee on Environmental Justice. This subcommittee includes representatives from the EHPC member agencies as well as from HHS components such as the Office for Civil Rights, the Administration for Children and Families, and the Health Care Financing Administration.
- Identify ways of better using the Healthy People 2000 program to address needs and goals relevant to disproportionate environmental health burdens in low-income and minority populations. Many of the existing Healthy People priority areas and objectives (such as Environmental Health, Cancer, and Occupational Safety and Health) have relevance to these disproportionate environmental health burdens and can be used as focal points for interagency activity.
- Coordinate plans and activities between federal, state, tribal and local agencies and community organizations through regional health officials and offices of minority health.
- Foster collaboration by conducting a regular conference of high-level scientists from federal environmental health research agencies. Such collaboration is essential in order to generate data needed for key activities (regulation, risk assessment and avoidance, public education, pollution prevention and mitigation) and to help minority and low-income communities improve their environmental health and ensure environmental justice. (See the Research section.)
- Promote multiagency representation on working groups, steering committees, and other bodies addressing issues germane to environmental justice.

Objective 2: In the context of HHS programs and available resources, develop, identify, and implement interagency projects aimed at reducing adverse environmental health effects in low-income and minority populations that can both exemplify and test interagency coordination processes. (See examples.)

Examples of Projects:

THE MISSISSIPPI DELTA PROJECT

Project Description: The overall goal of the Delta Project is to demonstrate that partnerships between government, academia, private sector organizations and community residents can identify key environmental hazards (and barriers to this identification), promote environmental quality, reduce and, where possible, prevent these hazards from impacting on health and the environment, with emphasis on persons in underserved communities. This goal will be pursued jointly by federal agencies (the Environmental Protection Agency, the National Institute of Environmental Health Sciences, the National Library of Medicine, the National Institute for Occupational Safety and Health/CDC, the National Center for Environmental Health/CDC, and the Agency for Toxic Substances and Disease Registry), state and local health departments, local community groups, and institutions of higher education, particularly those that serve large minority populations. By joining the interests, authorities, and resources of the relevant federal and state agencies, a more comprehensive and effective effort can be implemented to reduce and, where possible, prevent the health and environmental impacts of environmental hazards.

Because of the demographics and economic profiles inherent to the Mississippi Delta Region, this project will give special emphasis to identifying and reducing the disparities of environmental hazards experienced by disadvantaged communities and minority communities. Working closely with communities and historically black colleges and universities (HBCUs) in the region will be an essential component of this project.

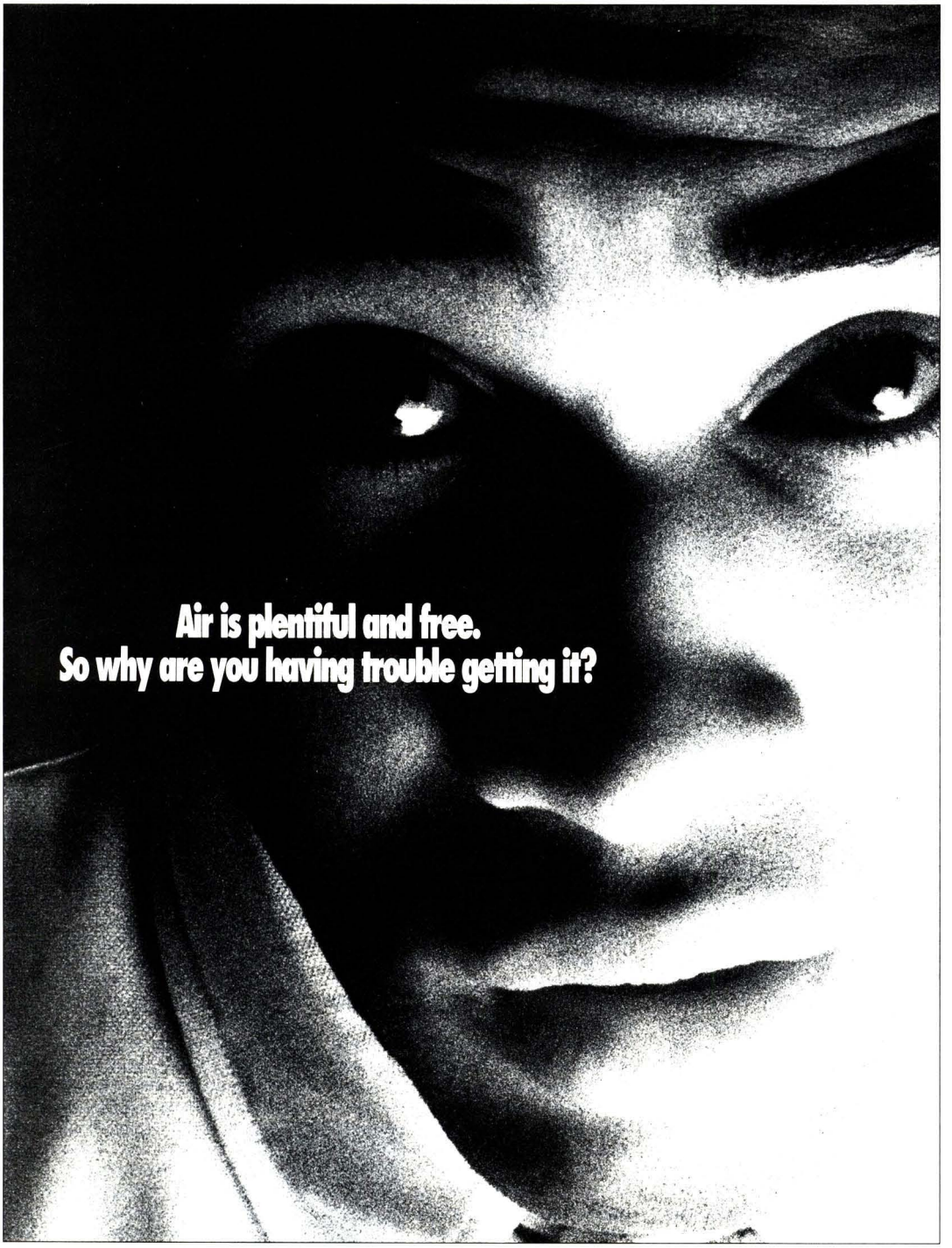
Overall objectives of the Delta Project are these:

- Identify key environmental hazards and barriers to recognizing hazards that may affect the health and quality of life of people who live in communities believed to be at risk. Where necessary, based on assessments of hazards and exposure, conduct biologic testing of individuals believed to be exposed. This effort may result in the development and implementation of appropriate public health actions, based on demonstrated need, including actions recommended to prevent or reduce current exposures to toxic substances.
- Assess the potentially harmful impact on high-risk populations of exposure to key environmental hazards.
- Empower and educate the community about environmental hazards. Evaluate impact of educational efforts to ensure that health care providers familiar with the recognition and treatment of illness associated with exposure to environmental hazards.
- Enhance capacity building in state and local health departments, environmental departments, academic institutions, and community non-profit groups to address environmental public health issues associated with minority health.
- Through collaborative efforts with state regulatory agencies and other federal agencies, increase the awareness of the importance of environmental public health among students at Head Start Centers, other preschools, and primary through college-level institutions in the Delta region.

- Provide pollution prevention and health promotion education regarding exposure to environmental hazards
- Ensure that efforts occur that lead to enhanced community empowerment and involvement in addressing environmental public health issues.
- Identify and coordinate state and federal actions to address environmental health issues in Delta Region.
- Evaluate and disseminate the effectiveness of strategies to prevent health and environmental impacts of key environmental hazards.

Federal Science Managers' Conference on Environmental Justice Research

Project Description: This interagency project is proposed as a model participatory research planning conference involving senior federal research managers, key stakeholders from at-risk communities, workers, and representatives from industry, academia, and state research agencies. Building upon the process and content of the *Symposium on Health Research and Needs to Ensure Environmental Justice*, this conference for FY 1995 will emphasize developing a sustainable environmental justice research agenda focused on central topics in Executive Order 12898: multiple and cumulative exposures and impacts; subsistence consumption of wildlife; involvement of people of color and low-income populations in epidemiological and clinical research; demographic and exposure-related research at federal facilities; more effective public involvement in research and the development of research strategies; and building a sustainable federal infrastructure to support environmental justice research.

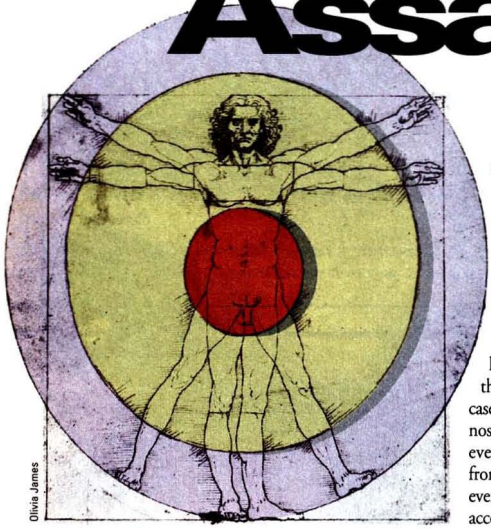


If you cough a lot, wheeze, are short of breath or feel tightness in your chest, you just might have asthma. If you do, you need treatment to control it. So find out for sure. Catch your breath and see a doctor.

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

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

Assault on the Male


Olivia James

According to Tom Bruckman, men and women are not created equal—when it comes to researching the sex-specific cancers that strike and kill them. In 1994, the National Cancer Institute spent five times more ($267 million) studying breast cancer than prostate cancer ($57 million), points out Bruckman, the executive director of the American Foundation for Urologic Disease. But the fact that prostate cancer is now the number one cancer detected in men, and the number two cause of cancer deaths among men, has begun to mobilize its victims. "Support groups have exploded into action," says Bruckman. "Four years ago, there were about 10 groups across the country. Now there are almost 500. We are learning from the experience women have had in lobbying for research and in helping themselves out."

The facts are indeed scary. Not only is the rate of prostate cancer growing in elderly men (it accounts for 92% of male cancers), but its brother, testicular cancer, is also increasing (its incidence has doubled in the last 50 years), and is now the most common malignant disease in young men. And then there is the ongoing, ominous debate about falling sperm levels.

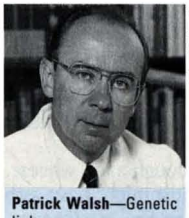
But the sex-selective cancers do share some depressing similarities. This year, more than 244,000 men will be diagnosed with prostate cancer, and about 40,000 will succumb. About 183,000 women will develop breast cancer, and 46,240 will die. Other sex-selective cancers such as cancer of the cervix, ovary, and uterus, will account for 26,400 deaths, while testicular and other male reproductive cancers will kill 580 men annually.

Breast cancer can affect one in eight women, if they live long enough, and 13% of men develop clinically significant prostate cancer at some time in their lives, usually late in life. The NCI says that every 3 minutes, a new case of prostate cancer is diagnosed in the United States, and every 15 minutes a man dies from it. One-third of them will eventually die from the disease, according to the NCI.

The statistics are bad enough, says Bruckman, but what really scares men is the knowledge that after age 45, the prostate starts changing. No one can predict if that transformation will result in a painful, but cancer-free, enlargement or a slow-growing carcinoma that, when it finally produces symptoms, is often too advanced to cure. Metastasized prostate cancer is fatal because chemotherapy can't target the slow-growing carcinoma. Prostate cancer increases faster with age than any other major cancer; researchers have found that by age 80, approximately 60–70% of men have evidence of incidental carcinoma at autopsy. By the year 2000, the incidence of prostate cancer is expected to increase by 90%, and deaths will rise by 37%.

What prostate cancer shares with breast cancer, according to Stanford epidemiologist Alice Whittemore, a leading prostate cancer researcher, is that "there are no neat models to tie all the risk factors together: those things you eat, are exposed to, and how your genetic disposition handles everything. We know so little about what causes these diseases."

This is also true of testicular cancer. Although cancer of the testis is a disease of young men and is highly treatable, researchers don't know what causes it, or even if it is related in some fashion to other male genital cancers. The glands share hormonal influences. Testosterone, the male hormone, is made in the testicles and is the substance that makes the 1.5-inch walnut-shaped prostate develop and stimulates it to manufacture secretions for sperm. The question for researchers is which men are at risk for cancer of their reproductive system and why.


Johns Hopkins U.

Patrick Walsh—Genetic linkages may soon provide insights into prostate cancer.

A Fat Risk?

Like any good mystery, there are many clues leading researchers down disparate avenues, often to contradictory evidence. "I don't know if any one theory holds the truth or is flawed," said Johns Hopkins Urologist-in-Chief Patrick Walsh. "All we can say is that there are a number of intriguing leads, so nothing should be dismissed."

The first clue to teasing apart influences on prostate cancers is population studies that revealed up to one-third of all men examined at autopsy are found to have microscopic, incidental prostate cancer. And this 30% incidence is found in men around the world, so it is likely caused by some universal factor such as age. In some men, this latent cancer never becomes a problem, but in others it kills them. Death rates vary dramatically from country to country. A 1982 study by the International Agency for Research on Cancer, in Lyon, France, looked at prostate cancer incidence in five continents and found a 25-fold difference between incidence rates in black American men living in San Francisco and Japanese men. In 1990, a team from Johns Hopkins, led by urologist John Isaacs, further refined the comparison, concluding that the initiation rate of prostate cancer was the same in Japanese and American men, but that there appeared to be differences in the rate of promotion or progression to clinically evident prostate cancer. Their observations were supported by other studies that found that immigrants who move from low-risk areas to the United States assume Americans' higher risks.

So what are the risk factors that increase the chance of the cancer progressing in American men, but cause very few Japanese men to die of prostate cancer? One obvious environmental culprit is the fatty Western diet, which is said to contribute to a number of cancers, including breast and colon,

colon, but not testicular. As early as 1975, researchers found that prostate cancer death in 32 countries was highly associated with total fat consumption, a finding similar to that for breast cancer. Follow-up studies have also made the case for diet. A 1993 Harvard study of 48,000 men found that those men who consumed high amounts of saturated and unsaturated fats had the highest risk of ending up with advanced or fatal cases of prostate cancer, but there was no association between fat intake and early stages of prostate cancer. Taking these findings a step further, the research team, led by Edward Giovannuci and Walter Willett, discovered a link between the disease and a type of fat called alpha-linolenic acid, which is found in meat as well as in dairy products and other foods.

Stanford U.

Alice Whittemore—There are no models to tie all the risk factors together.

This May, results of a four-year case–control study of prostate cancer among men of different ethnic and racial backgrounds living in five locations confirmed that fat is a risk factor for advanced cancer, but this time it was saturated fat. The study led Stanford's Whittemore to conclude that there is a "causal role in prostate cancer for saturated fat intake, but the data suggest that other factors are largely responsible for interethnic differences in risk. Fat is definitely a factor, but there are others," she says.

Researchers are also studying ways that fat could promote cancer. Some say it may dramatically affect prostate cell membranes, altering them in such a way that cells are more likely to turn cancerous. Another theory is that a diet high in animal fat raises a man's level of testosterone, which in turn bolsters the likelihood that cancerous cells will multiply and form a tumor.

The answer is not simple. For example, a high-fat diet is believed to play a role in other diseases, such as colon and breast cancer. The death and incidence rates of prostate cancer are higher among American blacks than American whites, yet the incidence of breast and colon cancer is about the same in the two groups. If fat is the culprit, why isn't the rate of prostate cancer as high in whites?

Other researchers point to different aspects of the diet that could influence cancer. An experiment reported in the March 1 issue of the *Journal of the National Cancer Institute* found modified citrus pectin, given orally to rats, inhibited spontaneous metastasis in prostate cancer. The researchers, from the Michigan Cancer Foundation and University of Michigan School of Medicine, found that modified citrus pectin, a complex polysaccharide, inhibited the adhesion of prostate cancer cells to rat endothelial cells. In other labs, scientists have linked deficiencies in vitamin A, a fat-soluble vitamin found in yellow vegetables, to development of different kinds of tumors. But the type of vitamin A seems to matter: supplements of one kind of vitamin A, beta-carotene, found in certain vegetables, appear to lower risk of prostate cancer, while other forms of vitamin A, found in animal fat, raise it.

Another vitamin seems to be related to geographic incidence of prostate cancer. A 1992 study in the journal *Cancer* demonstrated a global north–south graduated pattern of prostate cancer, with the highest rates in the north. The theory explored by the authors, Carol Hanchette and Gary Schwartz of the University of North Carolina at Chapel Hill, is that insufficient levels of vitamin D from UV radiation may increase the chance of the cancer. It could explain a number of observations, say the authors, such as why more men in the Scandinavian countries, Canada, and the United States have prostate cancer than men in Asia, and why black men are susceptible—people with dark skin absorb less sunlight and thus have lower levels of vitamin D.

Genetic Mechanisms

There are some clues about the inheritability of prostate cancer. A 1992 study by Johns Hopkins researchers Bob Carter and Patrick Walsh, published in the 15 April 1992 issue of the *Proceedings of the National Academy of Science,* showed for the first time the close association between a family history of prostate cancer and a man's likelihood of developing the disease early in life. Says Walsh, "Your risk of the disease is twice as high if your father or brother has it. But the risk could grow to 90 percent depending on the number of affected relatives you have and the age at which they develop the disease." But researchers say this familial form of prostate cancer probably accounts for only about 10% of the cases, similar to the inheritability of breast cancer. And they suspect it is autosomal dominant—a single gene from the mother or father can lead to development of disease. But the "prostate gene" has not been mapped yet. "I think genetic linkages are bound to give us paydirt soon, but there are a number of interesting leads on environmental factors, as well," says Walsh. "Nothing should be dismissed."

Late last year, researchers in another Johns Hopkins laboratory identified a genetic alteration linked to prostate cancer that they believe is the most frequently occurring genetic error associated with the disease. In almost all of the human prostate cancer samples he studied, oncologist and urologist William Nelson found deactivation of the gene that codes for glutathione *S*-transferase (*GSTP1*), an enzyme that detoxifies environmental carcinogens. He also found that some men with noncancerous prostate tumors produce the enzyme while others don't, indicating that alteration of the gene may cause the tumors to become cancerous. Nelson noted that diet and genes may form an "alluring" nexus in this situation: "Vegetables like broccoli and brussel sprouts trigger high production of these enzymes." Inherited gene mutations likely speed up initiation of the cancer, and environmental

Facts

Every three minutes a man is diagnosed with prostate cancer in the United States. Every 15 minutes a man dies from it.

Prostate cancer is the number one cancer detected in men and the number two cause of cancer deaths among men. Testicular cancer is the most common malignant disease in young men.

This year, 244,000 men will be diagnosed with prostate cancer and 40,000 will die from it. Comparatively, 183,000 women will be diagnosed with breast cancer and 46,240 will die.

events may promote it or protect against it, Nelson says.

In May, a major gene discovery offered the hope of a test to predict the course of prostate cancer in individuals and hope of corrective gene therapy. Johns Hopkins researcher John Isaacs and Carl Barrett, head of the NIEHS's molecular carcinogenesis laboratory, identified a gene, called *KAI1*, that prevents prostate cancer cells from metastasizing, or spreading out from the gland. It is only the second human gene known to block deadly metastasis. The gene's action had been shown in mice injected with human prostate cancer. According to Barrett, the research team first proved that metastatic prostate cancer cells lacked the *KAI1* gene, but that it was present in prostate cancers that didn't spread. The gene was isolated, injected into cultures of human prostate cancer cells, and then transferred to mice. These mice had much less cancer spread than mice without the gene. "We don't know how this gene affects the invasive ability of cells, but it is quite unique. Most genes found to date in cancer regulate the growth of cells," says Barrett. "We want to identify what factor, potentially environmental, that causes the gene to turn off." Currently, Barrett is trying to determine whether *KAI1*'s effects are limited to prostate cancer, and the Johns Hopkins team is looking to see if the *KAI1* gene was absent in cancer that spread in 200 prostate cancer patients.

There have been few leads on the genetic influences of testicular cancer, which is expected to be diagnosed in 7,100 American men this year, killing 370. No clear abnormalities in known oncogenes or tumor-suppressor genes have been linked to testicular cancer, according to University of Chicago urologist Robert Smith. Smith reported in May in the *Journal of Urology* that evidence from his laboratory points to the possibility that one or more tumor-suppressor genes are inactivated on chromosome 11, near where a tumor-suppressor gene for Wilms' tumor, a cancer of the kidney that particularly affects children, and for gonadoblastomas have been found. Smith said it appears that these genes may be important, although "it is likely that testicular cancer results from a multistep process involving alterations in many genes."

Many scientists point to abnormalities in the development of the testes as a risk factor. Research at Oxford University found 32% of patients with the cancer had atrophy of the testis, and 75% may have reduced sperm count. These results point to another pre-

Michael Reese Hospital

Gail Prins—There is a compelling case for the effects of estrogen on the prostate.

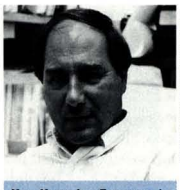
dominant risk category for both testicular and prostate cancer: natural and synthetic estrogens.

Hormonal Balance

The first hint that the level of sex hormones circulating in men had an impact on male reproductive cancer was the observation that prostate cancer is rarely found in men with undeveloped testes and in castrated men. The evidence has since been stacking up to form an alarming picture that paints estrogen and testosterone as cancer culprits.

The prostate and the testes are regulated by sex hormones called androgens, which are made in the testes. Foremost among these hormones is testosterone, produced in the testes but controlled by a hormone from the pituitary gland, called luteinizing hormone (LH). Testosterone circulates in the blood and enters the prostate gland by diffusion, where it is transformed by an enzyme into the hormone dihydrotestosterone (DHT), which is more than twice as potent as testosterone. Both hormones can bind to the same receptors on the prostate cell, which activates genes. During this process, the hypothalamus monitors the amount of testosterone in the blood and boosts or cuts back on production of LH. Estrogens can promote or retard the process naturally, but the precise mechanisms of how a hormonal imbalance leads to cancer is not yet understood. Environmental estrogens mimic the action of natural estrogens; they have the same chemical key that can turn hormone production on or off.

Conversely, estrogen receptors can be blocked by weak estrogen impostors that block sex hormone production. The result could be falling sperm counts, as found in 1992 by Danish researchers who performed a meta-analysis of 61 studies worldwide, and concluded that mean sperm concentrations have been cut almost in half between 1940 and 1990. The world's attention was riveted to the finding, especially when University of Florida researcher Louis Guillette, who reported similar findings in alligators, told a congressional committee, "every man in this room is half the man his grandfather was." But the Danish findings are controversial; some scientists analyzed the same data and found increasing sperm counts, while others say sperm production can be affected by venereal disease, and the difference in rates of venereal disease may account for the findings.



Steve McCaw, Image Associates

Ken Korach—Estrogen is a possible player in prostate and testicular cancers.

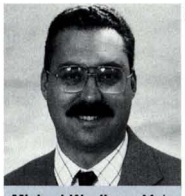
Another theory is that prenatal exposures to estrogens are responsible for rising numbers of testicular cancers. That link was explored by John McLachlan, director of the Center for Bioenvironmental Research at Tulane University, who found several decades ago that the synthetic estrogen diethylstilbestrol disrupted fetal testicular development, producing undescended testes and lower sperm counts. Since then, researchers have found dozens of chemicals, from pesticides to the plastic resin that lines food cans, that may disrupt the endocrine system via the estrogen receptor, and further investigation has found that estrogen mimics may act in different ways, producing various responses. The pollutant DDT has been found to produce testicular cancer in male rodents, while exposure to PCBs produced enlarged testes and increased sperm production in laboratory rats. Rex Hess, the University of Illinois researcher who reported growth of the enlarged testes at this year's meeting of the Society of Toxicology, says he thinks there is a critical window in male development where even a small extra amount of estrogen will produce delayed, harmful effects. "If you expose the organ to estrogen before it is ready, the organ may be impaired so that it can't function normally in adults. The toxic effect would be delayed."

In that context, some research findings make sense. For example, one study found that young black men had serum testosterone levels that are 15% higher than their white counterparts, accounting for their increased risk of developing the cancer. Similarly, other research determined that pregnant black women consistently had higher levels of estrogen than white pregnant women.

Gail Prins, a reproductive physiologist at the University of Illinois, has shown how excess estrogens can affect the prostate gland. Prins administered a single high dose of estrogen to rats on days one, three, and five after their birth, a critical time during development of the rat prostate gland (analogous to the second and third trimester in humans). She found a majority of the rats later developed prostate cancer. "This makes a compelling case, as much as you can make an analogy between rodent and human uterine exposure of estrogen," says Prins. She is now trying to uncover the actual mechanism—perhaps growth factors or increased DNA transcription—by which early estrogen exposure leads to prostate damage.

On the other hand, the dangers of no estrogen exposure have been revealed in an estrogen knock-out mouse produced in the NIEHS lab of Ken Korach, chief of the Receptor Biology Section. The consequences for both genders of the knock-out mouse was a tendency to infertility, Korach found. "It had a dramatic effect on the testes, reducing them to one-half their normal size in adult males. This means that estrogen has a real role in male reproduction." Korach has found that animals exposed to too much estrogen during a critical period of genital development have reduced sperm counts: "It's possible that the estrogen feedback system is disrupted, and sperm production is suppressed," he says. But Korach cannot yet say that estrogen balance is emerging as a primary actor in prostate or testicular cancer. "Right now, it should be considered a possible player."

Several highly publicized studies in the late 1980s reported that men who had vasectomies were more likely to develop prostate cancer because of high levels of testosterone circulating in the blood. But more recent data have discounted that argument, attributing the rates to better reporting as these men were more likely to see physicians about urologic complaints.



NCI

Michael Waalkes—Male cancers are multifactorial diseases.

The hormonal surge brought on by frequent sexual activity is also a risk factor, physicians at the Harvard School of Public Health reported in the May 20 issue of the *British Medical Journal*. Frequent sex triggers excess testosterone and DHT production, which promotes cell division in normal prostate functioning. Harvard physician Christos Mantzoros says in the report that high DHT levels might spur cancerous cell proliferation. Based on the fact that men who cannot produce DHT never develop prostate cancer, the NCI is now sponsoring an 18,000-patient trial of the drug finasteride, which controls noncancerous prostate enlargement by controlling DHT levels.

Hormone-modulating chemicals, such as dioxin, a by-product of wood-pulp bleaching, seem to work in other sinister ways. Dioxin appears to affect both the prostate and the testes because it decreases, by up to 80%, "the amount of sperm available to ejaculate," says University of Wisconsin toxicologist Richard Peterson. "Sperm production is slightly lessened, but sperm storage is greatly hampered. And the most robust effects occur at the lowest levels. Dioxin is in a class of chemicals that merit more research because there is already a certain background level in utero and through lactate exposure." According to Korach, "Dioxin also alters liver steroid metabolism, resulting in a possible hormonal imbalance."

Risky Business

Some researchers are questioning how a man's occupation might affect his reproductive health. Research this year from the Memorial Sloan-Kettering Cancer Center suggested extreme high (above 80°F) and low temperatures (less than 60°F) over a number of years during work may be a risk factor for testicular cancer, since the testis is a temperature-sensitive organ. The study, presented in the *Archives of Environmental Health*, found a significant risk factor for men exposed to high temperatures in their work environment for more than 10 years. And a study presented in 1994 from Kansas State University said that farmers in that state were at significantly higher risk for some cancers, including prostate cancer. The researcher, R. Scott Frey, told participants at the American Public Health Association meeting that data on almost 70,000 farmers indicate that there may be something in the farm environment, such as pesticides, that increases their cancer risk. But he added the results are preliminary, and more research is needed to assess the full scope of underlying causes of the cancer risks that Kansas farmers face.

Finally, several studies have looked at whether the trace mineral cadmium, found in welding shops, increases the risk of prostate cancer by reacting with zinc; the prostate has the highest concentration of zinc of any organ in the body. Michael Waalkes, a researcher at NCI's Frederick Cancer Research and Development Center, has found that both a rat's prostate and testes are sensitive to the chemical, which can cause cancer. But, Waalkes says he can't yet make the leap to saying that human glands are damaged. "It's difficult to say what portion of the rat prostate is analogous to the human prostate, so I don't think there is a good rat model there. And there is no good animal model for testicular cancer."

"All in all, I think that both of these male cancers are multifactorial diseases," says Waalkes. Like all researchers who seek to solve these complex biological riddles, he says the real question is which risk factor is the most important. Figuring this out will require continuing basic research into both male and female reproductive biology and toxicology to understand how environmental chemicals may exert their influence.

Renee Twombly

Factors

The gene that codes for an enzyme that detoxifies environmental carcinogens is deactivated in almost all human prostate cancers. A gene that prevents the spread of prostate cancer has been found to be absent in animals with metastatic prostate cancer.

Exposure to environmental estrogens, particularly *in utero*, from pesticides, plastic resins, and other sources may disrupt the endocrine system, causing prostate and testicular cancers.

Certain occupations may carry higher risks for male reproductive cancers: extreme high and low temperatures may play a role in testicular cancers, and exposure to cadmium and zinc in welding shops may increase risk for prostate cancer.

Where a man lives may have a lot to do with his risk for prostate cancer: ethnic groups with high-fat diets show higher rates of incidence, and men who live in northern climates and receive insufficient levels of vitamin D from UV radiation have higher rates.

Biennial Report on Carcinogens

A Work in Progress

You can't please all of the people all of the time. Never does this adage hold more true, perhaps, than in the case of determining which agents in our environment are probable carcinogens. In the ongoing process of reviewing and revising the criteria for listing such chemicals in the *Biennial Report on Carcinogens*, however, the NIEHS has followed a policy of open, public meetings and solicitation of scientific opinions from all the stakeholders in the hope of capturing the majority opinion. According to George Lucier, director of the Environmental Toxicology Program at the NIEHS, the latest version of the criteria may have accomplished that goal.

The *Biennial Report on Carcinogens* is mandated by the Public Health Service Act, which states that the secretary of the Department of Health and Human Services shall publish a report containing a list of all substances "which are either known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and to which a significant number of persons residing in the United States are exposed." The process of preparing the report, which is carried out by the National Toxicology Program, has in recent years been the subject of controversy between government, industry, public interest groups, and others who disagreed about both the process itself and the outcome of listing for particular chemicals. In 1994, one particular controversy, surrounding whether glass wool should be listed as a substance reasonably anticipated to be a human carcinogen, refocused attention on the listing issue and prompted officials at the NIEHS and DHHS to direct a review of the listing process and possible revision of the criteria.

Process

In an atmosphere of criticism of government agencies for making behind-the-scenes scientific and regulatory decisions, officials at the NIEHS began the review process with the intention of fostering public discourse on the subject open to all organizations and individuals with an interest or stake in the outcome. To this end, an ad hoc working group of the NTP Board of Scientific Counselors, a primarily nongovernment group that reviews the scientific activities of the NTP, was formed to receive public comments on the criteria and review and make recommendations for revising the listing process. Members of this working group were made up of representatives from academia, industry, labor, public interest groups, state and local health departments, international experts in carcinogenesis, members of the NTP Executive Committee, and NIEHS staff. On 24–25 April 1995, at a public meeting in Washington, DC, the group set about its task of examining the existing process and criteria and determining if changes were needed, and, if so what should be done.

Kenneth Olden, director of the NIEHS and the NTP, charged the ad hoc working group in the first plenary session of the meeting with addressing the adequacy of existing criteria for listing substances and with deciding whether to incorporate mechanistic data into these criteria. The criteria may include the consideration of sensitive subpopulations or procedures to evaluate the results of animal bioassays or epidemiology studies. The second plenary session was devoted to presentation of public comments concerning the criteria. Comments were presented by representatives from such varied groups as the Chlorobenzene Producers

Association, the United Auto Workers, and the Center for Science in the Public Interest. Following the public comments, participants met in breakout sessions and then reconvened in the third plenary session to report on their deliberations and recommendations.

There was a consensus among the working group that the current criteria for listing substances in the *Biennial Report on Carcinogens* should be revised, although proposals ranged from slight revisions to more substantive changes. Although many recommendations were made, most members of the working group felt that mechanistic data should be used in the selection process. It was also decided that formal guidelines for de-listing chemicals should be incorporated into the biennial report.

Revisions

Based on the recommendations of the ad hoc working group, Lucier and William Jameson at the NIEHS developed revised criteria for review by the NTP's Board of Scientific Counselors. Upon review of the proposed revisions at a meeting June 29, the board passed several resolutions regarding the *Biennial Report on Carcinogens*: mechanistic information should be used in the selection process; the current criteria should be revised; the number of categories should remain at two; revised criteria should include a change in the wording of the categories; an explanatory paragraph regarding the basis of the categories should precede the criteria; and a formal mechanism for de-listing substances should be instituted.

The results of these resolutions are included in the proposed revised criteria as follows: Conclusions regarding carcinogenicity in humans or experimental animals will be based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to, dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive subpopulations, genetic effects or other data relating to mechanism of action, and/or factors that may be unique to a given substance. For the purpose of the *Biennial Report on Carcinogens*, the degrees of evidence are as follows:

1. Known to be Human Carcinogens: There is sufficient evidence of carcinogenicity from studies in humans that indicates a causal relationship between the agent, substance, or mixture and human cancer.

2. Reasonably Anticipated to be Human Carcinogens:

•There is limited evidence of carcinogenicity from studies in humans which indicate that causal interpretation is credible but that alternative explanations such as chance, bias, or confounding could not adequately be excluded, or

•There is sufficient evidence of carcinogenicity from studies in experimental animals that indicates there is an increased incidence of malignant and/or combined benign and malignant tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor or age at onset.

These recommendations must be reviewed by the NTP Executive Committee, which is made up of heads of agencies or their designates with an interest in NTP activities such as the CDC, NCI, FDA, and other DHHS officials. Upon approval by this committee, final proposed revisions will be submitted to DHHS Secretary Donna Shalala for approval. Submission to Shalala is expected by the end of 1995.

Commentary

Though not everyone may be completely satisfied with the proposed revisions, certainly everyone would agree that the revision with the most impact is the consideration of mechanistic data in the scientific review process preceding formal listing of a chemical. Lucier says that the addition of such information is important because "it allows us to better compare rodent and human responses, which will enable better and more accurate listings of chemicals reasonably anticipated to be carcinogens. Mechanistic data will allow us to strengthen the scientific basis for listings, and in some cases, chemicals could be listed primarily on the presence of convincing mechanistic data that the chemical is likely to cause cancer in humans."

Addition of such information may also address the concerns of groups who question the validity of using animal bioassays as the primary basis for extrapolating human risk, although Lucier stresses, "the Biennial Report on Carcinogens is only one part of hazard identification, the first step in the process of risk assessment which spurs regulatory action for chemicals or classes of chemicals." According to Lucier, some chemicals may be listed as a result of using mechanistic data and some may be de-listed. In the long run, the numbers may work out to be much the same. Still, the process of using mechanistic data in addition to existing human and animal data on toxicity is not just a scientific exercise. Bringing the weight of scientific evidence to bear on the problems of protecting human health from exposure to carcinogens hits at the heart of Congress's intent in creating the *Biennial Report on Carcinogens*: "to disseminate prudent information which will prevent human cancer through helping people to take prudent steps to reduce exposure."

Kimberly G. Thigpen

An ECO-LOGICal Way to Dispose of Waste

Eco-Logic

The "Destructor." Organic compounds such as PCBs, dioxins, furans, pesticides, and solvents are destroyed by this process.

Managing toxic waste is one of the country's biggest industrial and environmental headaches, but technology is right around the corner that may make the process safer, cleaner, and eventually cheaper than incineration.

Eco-Logic International, Inc., based in Michigan and Canada, has developed a process that can completely destroy organochlorines and organic matter, including compounds such as PCBs, dioxins, furans, and chlorinated pesticides. The company's patented gas-phase thermochemical process uses a reduction reaction of hydrogen with organic and chlorinated organic compounds at elevated temperatures. Hazardous contaminants are transformed into excess hydrogen, methane, and a small amount of water vapor. The gases produced in the reaction either recirculate into the process or provide supplementary fuel for the system. And because it is a closed-loop system, no contaminants escape.

Currently, incineration is the method of choice to dispose of municipal, medical, and hazardous waste, but evidence indicates that this technology is flawed and may create more problems than most communities are equipped to handle. Under high-temperature combustion, organic compounds containing carbon, hydrogen, and often chlorine are oxidized. Other substances potentially incinerated include sulfur, arsenic, and metals such as chromium, mercury, and lead. Both the toxic emissions produced during the burning process (sometimes from accidents) and toxic ash left over after combustion are often more hazardous than the original materials. These emissions consist of products of incomplete combustion, escaping heavy metals, and new combinations of materials as a result of the burning process. The fly ash—the particulate matter emissions which may include dioxins and furans—goes up the smokestack, releasing toxins including trace organics and PCBs directly into the air.

The bottom ash—the part which falls to the bottom of the incinerator after a burn—is created because municipal waste contains approximately 25% noncombustibles. The bottom ash may be more hazardous than the original waste because it concentrates heavy metals that cannot be destroyed by combustion. A 1995 study sponsored by the Center for the Biology of Natural Systems found that 70% of the airborne dioxin deposited in the Great Lakes comes from the incineration of municipal and medical waste. Another 20% comes from certain steel mill operations and the burning of hazardous waste from within 300 miles of the lakes to as far away as 1,250 miles.

Toxic releases are insufficiently tested and can pose health hazards. The EPA Dioxin Reassessment states current exposures to dioxin carry a cancer risk of 1 in 1,000 to 1 in 10,000. A Harvard University School of Public Health study indicates that air pollution, especially fine particles from combustion of fossil fuels, including incinerators, may result in roughly 60,000 deaths each year, and that tens of thousands, especially children, are made sick.

Eco-Logic

On the road again. A mobile system means communities can avoid the divisions and dangers that come with incineration siting.

Grinding Heat

At 850°C or higher, hydrogen combines with organic

contaminated compounds in a reaction known as reduction, producing lighter, smaller products. Water, which can serve as a reducing agent and a source of hydrogen, enhances the reaction. And because hydrogen can produce an atmosphere devoid of free oxygen, the possibility of dioxin or furan production is eliminated. Wastes suitable for this kind of destruction include contaminated soils and landfill leachates, lagoon and harbor sediments, wood treatment and pulp mill wastes, contaminated electrical equipment, pesticide wastes, chemical warfare agents, and organic wastes mixed with low-level radioactive wastes.

When treating high-concentration organic wastes, the process produces excess gas, which can be compressed and stored for later use, either as boiler fuel or as a fuel product for resale. Although the technology does not treat metals such as mercury, copper, iron, and cadmium or nuclear waste, it may be used to separate these various materials from organochlorines.

The Eco-Logic technology is the brainchild of Douglas Hallett. Hallett spent 16 years with Environment Canada, part of the time as senior scientific advisor, where he worked on both the Great Lakes and Love Canal. In 1987, Hallett founded Eco-Logic International to develop and commercialize the initial concept he conceived in 1985. Wayland Swain, former EPA director of the Large Lakes Laboratory in Grosse Ile, Michigan, heads the U.S. operations.

A laboratory-scale process unit was constructed in 1988 and tested extensively. Because the results were so favorable, a mobile demonstration-scale unit was constructed and commissioned in 1991. The unit was taken to Hamilton Harbour, Ontario, where coal tar-contaminated harbor sediment and PCBs were successfully processed. The results were audited by the Waste Water Technology Center, formerly part of Environment Canada. The Ontario Ministry of the Environment also participated in the audit. The results confirmed consistent destruction efficiencies of 99.9999% or better on coal tar-contaminated sediments dredged from the harbor.

During October and November 1992, the same unit was taken through a second round of tests for 28 days as part of the U.S. EPA's Superfund Innovative Technology Evaluation (SITE) program in Bay City, Michigan. The agency conducted extensive tests, treating wastewater containing an average 4,600 parts per million PCBs and waste oil containing an average 24.5% PCBs. Both wastes were tested in triplicate. A specific quantity of perchloroethylene (PCE), a reliable surrogate measure for PCBs, was added to the waste stream as a control, and the unit was put through a 72-hour engineering performance run.

According to an EPA report on the test, the technology was quite effective: "A 99.9999% destruction and removal efficiency for PCBs during all the test runs; a 99.99% destruction efficiency for perchloroethylene; net destruction of trace feedstock dioxin and furan compounds during all test runs; and successful completion of a 72-hour engineering performance test."

The Bay City test was not *completely* successful, however: the thermal desorption unit, which separates the contaminants from the soil, did not perform to design specifications. The EPA applications analysis report concluded: "The combination of material handling problems and inadequate organics desorption showed a need for further development."

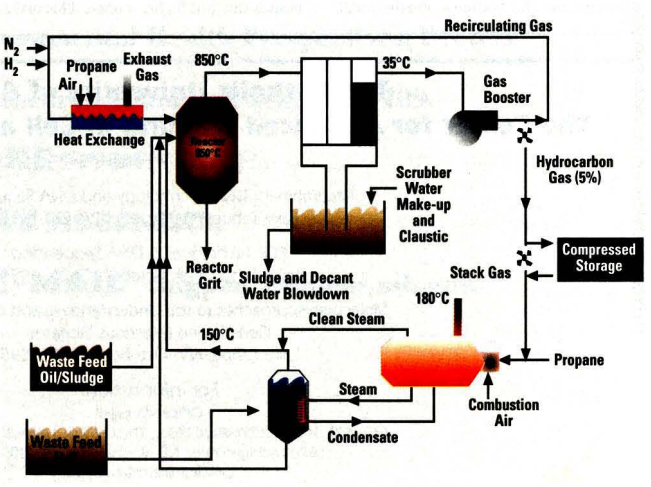
Martin Hassenbach, a spokesperson for the company's marketing department, describes how Eco-Logic has addressed the problem. After the 1992 tests, Hassenbach explains, the company went back to the drawing board to design a new device to replace the thermal desorption unit. The device, called a thermal desorption mill (TDM), uses rotating steel balls to break up the soil at the time it is being heated. "We had these big clumps of soil that were not breaking down properly," he said. "We could get at the contamination outside, on the surface of the soil, but we could not desorb the contamination inside the lumps." With the new design, the soil or sediment is ground into a powder during the heating process. The soil is passed through without oxygen into an enclosed device that is heated and supplied with hydrogen-rich methane gas from the reactor. Hydrogen strips the contaminants from the soil. Contaminants are then carried to a reactor where they are broken down into methane gas. The remaining methane gas is recirculated through the TDM to heat the unit. With the new TDM, the amount of toxic materials that remains in the soil is reduced to the low parts per billion range. Says Swain, "Since the Toxic Substances Control Act demands 1 part per million, we are now 1,000 times better than that."

On 15 July 1994, the EPA released a detailed "Technology Evaluation Report" of the Superfund trial program which independently verified previous reports on the success of the Bay City project. Based on the operating experience and test results at Bay City, commercial-scale process units were constructed and are now being used.

Gordon Evans, economist and EPA project manager for Eco-Logic's testing program in Bay City, stated, "The bottom line is that the machine does what it's advertised to do."

The Costs of Progress

Evans says, however, that the costs of the process are currently higher than incineration. "According to our studies, if we use Eco-Logic to dispose of 30,000 gallons of oil [contaminated with PCBs], it will cost $1,800 a ton. . . . At this point, it costs between $1,200 to $1,300 a ton for incineration."



Eco-Logic

Keeping to itself. A closed-loop destruction process means no uncontrolled emissions.

Responding to this analysis, Swain suggests that it does not factor in the additional expenses of waste transportation to and from the incinerator: "Those costs are eliminated with our technology," says Swain. "Residue ash requires proper disposal, usually in a landfill, a procedure that adds to the cost and may be limited because available landfills are rapidly filling up."

Swain adds, "Operating economies to treat water-bearing waste are eventually expected to be three to five times cheaper than incineration technologies of comparable capacities. Incineration technologies consume large amounts of energy to heat up the water component to combustion temperature. Additionally, because incineration technologies utilize air for combustion and must destroy all the organic matter, they often require 10 times the volume of the Eco-Logic process for the same residence time of reaction."

William A. Suk, chief of the Chemical Exposures and Molecular Biology Branch of the NIEHS's Division of Extramural Research, calls the Eco-Logic process, "an interesting technology and one that probably has merit." However, Suk adds, "Hazardous waste is a mixture. In order to eliminate PCBs and hydrocarbons, you don't get rid of metals and visa versa. No one process does it all."

Out of Site, Out of Mind

Eco-Logic has already begun to be used around the world. The company has signed with General Motors of Ontario, Canada, to dispose of that company's wastes; Dofasco Steel will use the process to clean up a PCB-laden mine in northern Ontario; and the National Procurement Development Program of the Australian Department of Environmental Protection is planning to use the process to destroy 200 tons of hazardous pesticides currently stored in Western Australia.

Incinerators run better—environmentally and economically—when they run continuously, which requires a steady stream of waste. Thus, in order to keep running, an incinerator may be forced to take in waste from a number of different communities. This raises the issue of fairness. Should one community become the disposal site for others? Or, should one community accept the risks of an incinerator malfunctioning? Local citizens fear that a design on paper, however promising, may not translate into adequate functioning once an incinerator is on-line. An incinerator site also leads to other irritants such as noise, truck traffic, and unpleasant odors. And communities that house incinerators usually see their property values go down.

One advantage of the Eco-Logic apparatus is that it is highly mobile. This makes it attractive to communities that are troubled by the presence of incinerators. Once the job is done, the machine is hoisted on top of a large trailer and moved on to the next site. The apparatus is contained on two 45-foot drop-deck flatbed trailers. An additional trailer, housing the on-line mass spectrometer, the process control unit, and other analytical units completes the equipment. Setup takes only a few days, and the minimum run may be less than a single unit's daily capacity.

Swain believes it is just a matter of time before the process is fully accepted. Current demand is greater than the company's supply; sales are now projected well into 1996. "For the kind of thing we do—dispose of any organic contaminant in any matrix in any concentration—I know of no other technology presently that can do it," Swain says. "Eventually people will see this as part of the future."

Liane Clorfene Casten

Suggested Reading

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Roy Kimberly A. Thermo-chemical reduction process destroys complex compounds. Hazmat World Dec: 77–79 (1992).

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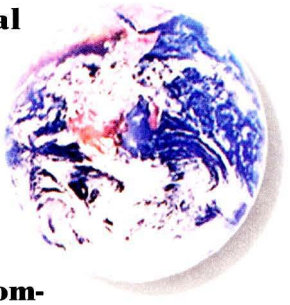

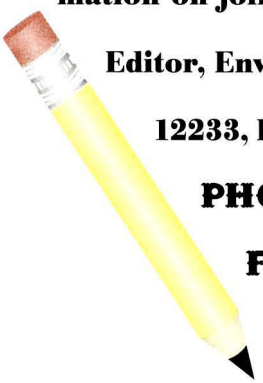

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Diesel Engine Exhaust and Lung Cancer: An Unproven Association

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The risk of lung cancer associated with diesel exhaust has been calculated from 14 case–control or cohort studies. We evaluated the findings from these studies to determine whether there is sufficient evidence to implicate diesel exhaust as a human lung carcinogen. Four studies found increased risks associated with long-term exposure, although two of the four studies were based on the same cohort of railroad workers. Six studies were inconclusive due to missing information on smoking habits, internal inconsistencies, or inadequate characterization of diesel exposure. Four studies found no statistically significant associations. It can be concluded that short-term exposure to diesel engine exhaust (<20 years) does not have a causative role in human lung cancer. There is statistical but not causal evidence that long-term exposure to diesel exhaust (>20 years) increases the risk of lung cancer for locomotive engineers, brakemen, and diesel engine mechanics. There is inconsistent evidence on the effects of long-term exposure to diesel exhaust in the trucking industry. There is no evidence for a joint effect of diesel exhaust and cigarette smoking on lung cancer risk. Using common criteria for determining causal associations, the epidemiologic evidence is insufficient to establish diesel engine exhaust as a human lung carcinogen. *Key words*: diesel exhaust, diet, lung cancer, occupation, smoking. *Environ Health Perspect* 103:812–818 (1995)

Diesel engine exhaust contains respirable carbonaceous particulates that adsorb organic chemicals, including the polycyclic aromatic hydrocarbons benzo[*a*]pyrene and 1-nitropyrene. These organic extracts are carcinogenic to rodents when administered topically or by implantation. Inhalation of high concentrations of whole diesel exhaust causes destruction of defensive pulmonary mechanisms and promotes the development of primitive lung adenocarcinoma in animal models (*1*). At lower levels of exposure that do not reduce pulmonary clearance, diesel exhaust is not carcinogenic. This suggests that the mechanism of carcinogenic action for diesel inhalation is particle overloading and subsequent inflammation of the lung, and not the mutagenic effects of the organic fraction of diesel exhaust (*2,3*). The results from recent experiments support this concept. Many rats were chronically exposed to high levels of diesel exhaust or carbon black (*4*). Carbon black has a similar carbonaceous core as diesel exhaust minus the mutagenic organic fraction. Both diesel exhaust and carbon black produced the same incidence of lung tumors in experimental rats.

The relevance of these laboratory findings has possible implications for interpreting studies of diesel exhaust and human lung cancer. Cigarette smoke particulates cannot induce damage to human bronchial epithelium when the lung's ciliated mucus-producing epithelium is intact (*5*). It is therefore uncertain whether the much lower concentrations of diesel exhaust in traffic or industrial settings, compared with animal inhalation studies, can cause bronchial damage and subsequent cancer in humans. Furthermore, unlike cigarette smoke inhaled through the mouth, diesel exhaust is inhaled through the nose and encounters upper respiratory defense mechanisms.

Elevated mortality ratios of lung cancer have been documented in industries that use diesel engines. These observations have implicated diesel exhaust as a possible human lung carcinogen. In recent years, several case–control and cohort studies have been conducted to assess the independent role of diesel emissions in lung cancer. This paper reviews the epidemiologic findings and evaluates the evidence for causality according to standard criteria.

Prior Reviews

Diesel engines have been used increasingly in various industries since the 1930s, although they were not in widespread use until about 1950. Diesel engines are the power source of railroad locomotives, heavy equipment vehicles, and some buses and trucks. Diesel engines are also used in mining and dock operations. The health effects of diesel exhaust have been critically addressed by several groups. In 1981, the National Research Council (NRC) of the National Academy of Sciences found no evidence for a carcinogenic effect of diesel exhaust in epidemiologic studies, although the lack of high-quality research in this area was acknowledged (*6*). I.T.T. Higgins, a member of the NRC committee, stated in a separate position paper that the cancer risk from diesel exhaust emissions was "uncertain" (*7*). Concerns were raised about inadequate allowance for asbestos exposure and cigarette smoking in occupational cohort studies. A similar conclusion was reached by Wynder and Higgins in 1986 (*8*). Reviews by Schenker (*9*) and Steenland (*10*) concluded that the evidence was suggestive but inconclusive. The International Agency for Research on Cancer concluded in 1989 that based on the evidence from animal studies, "diesel engine exhaust is probably carcinogenic to humans" (*11*). The U.S. Environmental Protection Agency has proposed classifying diesel exhaust as a probable human carcinogen (*12*). The National Institute for Occupational Safety and Health concluded that diesel exhaust is a potential human carcinogen (*13*).

These evaluations were based primarily on results of experimental animal studies and elevated standardized mortality ratio statistics of lung cancer in some diesel-exposed workers. The epidemiologic studies were done on truckers and other motor vehicle drivers (*14–28*), traffic controllers (*29*), coal miners (*30*), construction workers (*18,25,31,32*), railroad union members (*16,33*) and dock workers (*34*). These investigations lacked accurate exposure information on diesel exhaust and individual smoking habits or had insufficient follow-up times to account for the potential latent effects of diesel exposure.

More recent case–control and cohort studies have provided additional information on the health effects of diesel exhaust (Table 1). These studies are reviewed here.

Case–Control Studies of Lung Cancer

In a French case–control study conducted by Benhamou et al. (*35*), the smoking-adjusted odds ratio (OR) was 1.42 (95% CI, 1.07–1.89) for motor vehicle drivers. The types of motor vehicles were not specified. There was no trend in the odds ratio with duration of employment. Information on exposure to diesel exhaust was not obtained.

Damber et al. (*36*) interviewed surro-

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Table 1. Studies of diesel exhaust and lung cancer

Reference	Design, sample size	Exposure measurements	Bias	Relative risks	Probability/ confidence units	Adjusted for smoking	Evaluation
Benhamou et al. (*35*)	Hospital-based case–control study, 1,625 cases	Motor vehicle drivers	Low	1.42	$p<0.05$	Yes	Inconclusive
Damber and Larsson (*36*)	Case–control study in Sweden, 600 lung cancer cases	Nonsmoking truckers	Moderate	5.4 (duration not evaluated)	0.8–26.6	Yes	Inconclusive
Hall and Wynder (*37*)	Hospital case–control, 502 male case–control pairs: diesel occupation	1) Occupation 2) Self-report	Low	1.4 (duration not evaluated)	0.8–2.4	Yes	Negative
Siemiatycki et al. (*38*)	Population-based study, 3,726 male cancer patients	Job title	Minimal	1.2 (for "substantial" exposure)	0.6–2.4	Yes	Negative
Boffetta et al. (*39*)	Hospital case–control, 2,584 cases; 5,099 controls	1) Occupation 2) Self–report	Low	1) 0.95 2) 1.21	0.78–1.16 0.78–2.02	Yes	Negative
Boffetta et al. (*40*)	Cohort of 463,000 men	1) All occupations 2) Truck drivers 3) Railroad workers	Minimal	1) 1.18 2) 1.24 3) 1.59 Trend	0.99–1.44 0.93–1.66 0.97–2.89 NS	Yes	Negative
Howe et al. (*43*)	43,286 railroad workers, 175 deaths	1) Possible exposure 2) Probable exposure	Low	1) 1.03 2) 1.35	$p<0.01$	No	Inconclusive
Garshick et al. (*44*)	Case–control study, 1,256 lung cancer deaths among railroad workers	Air samples	Minimal	1.41	1.05–1.88	Yes	Positive for long-term exposure
Garshick et al. (*48*)	Retrospective cohort, 55,407 railroad workers. 1,694 lung cancer cases identified.	Air samples	Minimal	1.45 for 40-to 44-year olds	1.11–1.89	No	Positive for long-term exposure/ inconclusive
Gustavsson et al. (*49*)	SMR study of 695 Swedish bus garage workers; 20 lung cancer cases Weighted regression Low exposure High exposure	Exposure scale based on industrial data	Minimal	1.22 1.34 low 2.43 high	 $p<0.05$ $p<0.05$	No	Inconclusive
Steenland et al. (*50*)	Case–control study, 1,288 male lung cancer deaths	Job title	Low/ moderate	1.89 for ≥ 35 year exposure	1.04–3.42	Yes	Positive for long-term exposure
Hayes et al. (*51*)	Pooled case–control studies, 2,291 male cases	Truck drivers	Low	1.5	1.1–1.9	Yes	Inconclusive
Swanson et al. (*53*)	Population-based study, 3,792 male cases	1) Heavy-truck drivers 2) Light-truck drivers	Moderate	1) Trend w/ duration 2) Trend w/ duration	$p<0.05$ $p<0.05$	Yes	Positive for long-term exposure
Emmelin et al. (*54*)	Case–control study of 50 male Swedish dock workers with lung cancer	Exposure scale based on industrial data	High	2.9–6.8 for estimates of high exposure	Not significant	Yes	Inconclusive

SMR, standardized mortality ratio.

gates of 600 lung cancer case–control pairs in Northern Sweden. Of 92 truck drivers, 83 were smokers. Among the nonsmoking truck drivers (three cases and six controls), the OR was 5.4 (95% CI, 0.8–26.6). The types of truck engines (gasoline or diesel) were not specified.

Hall and Wynder (*37*) interviewed 502 male case–control pairs in hospitals. Using industrial hygiene criteria to define diesel exposure, the crude OR was 2.0 (95% CI, 1.2–3.2). After adjustment for cigarette smoking, the OR was 1.4 (95% CI, 0.8–2.4). Using National Institute of Occupational Safety and Health criteria to define exposure, the unadjusted risk was 1.7 (95% CI, 0.6–4.6) for a high probability of exposure, and 0.7 (95% CI, 0.4–1.3) for a moderate degree of exposure. There were no specific occupational groups that had an elevated risk of lung cancer. The statistical analyses were based on the usual occupation. Information on duration of employment was not analyzed.

Siemiatycki et al. (*38*) conducted a population-based study of multiple cancer sites. They Interviewed 3726 male cancer patients in Montreal hospitals. An industrial hygienist classified each job according to the potential for exposure to diesel exhaust and other emissions or air pollutants. The risk of squamous cell lung cancer for "nonsubstantial" diesel exhaust was 1.9 (90% CI, 1.0–3.5) and "substantial" (defined as exposure levels above the median cumulative exposure index) diesel exhaust was 1.2 (90% CI, 0.6–2.4) compared with unexposed subjects. Compared to subjects exposed to "nonsubstantial" gasoline exhaust, the respective risk estimates were 2.3 (90% CI, 1.0–5.2) and 1.2 (90% CI, 0.4–3.8). The statistically nonsignificant findings and the low ORs associated with high levels of diesel do not implicate diesel exposure as a risk factor.

Boffetta et al. (*39*) examined occupational histories for 2584 cases and 5099 controls. The crude and smoking–adjusted

ORs for occupations with probable diesel exposure was 1.31 (95% CI, 1.09–1.57) and 0.95 (95% CI, 0.78–1.16), respectively. The OR for possible diesel exposure was 1.0. No increased risk was observed for truck drivers. The unadjusted and adjusted odds ratio for subjects who self-reported exposure to diesel exhaust was 1.45 (95% CI, 0.93–2.27), and 1.21 (95% CI, 0.78–2.02), respectively.

Cohort Study

In the American Cancer Society's (ACS) Cancer Prevention Study II, Boffetta et al. (*40*) calculated the mortality rates for 461,981 men aged 40–79 after 2 years of follow-up. The relative risk (RR) associated with self-reported exposure to diesel exhaust was 1.18 (95% CI, 0.97–1.44). The chi-square trend test for duration of exposure was $0.05 < p < 0.10$. The RR was 1.59 (95% CI, 0.94–2.69) for railroad workers and 1.24 (95% CI, 0.93–1.66) for truck drivers, after adjusting for smoking and other risk factors.

Among truck drivers, the relative risk was 1.22 (95% CI, 0.77–1.95) for those who reported exposure to diesel exhaust and 1.19 (95% CI, 0.74–1.89) for drivers who reported no exposure to diesel. Among truck drivers who worked for at least 16 years and reported diesel exposure, the risk was 1.33 (95% CI, 0.64–2.75). The risk for diesel-exposed truck drivers who worked 1–15 years compared to truck drivers who were unexposed to diesel was 0.87 (95% CI, 0.33–2.25).

The RR for heavy equipment operators was 2.6 (95% C, 1.12–6.06), although this was based on only five lung cancer deaths. The RR for miners was 2.67 (95% CI, 1.63–4.37). The increased risk associated with mining should be interpreted with caution because it was based on 15 lung cancer deaths, and some mining operations have never used diesel-fueled motors in underground pits (*41*). In addition, high levels of radon daughters in some mines increase the risk of lung cancer (*42*). The evidence from this cohort study does not implicate diesel exhaust as a lung cancer risk factor.

Railroad Workers

Howe et al. (*43*) examined the lung cancer mortality experience of 43,826 retired Canadian railway workers employed from 1965 to 1977. The probability of exposure to diesel fumes was evaluated by the Department of Industrial Relations. The relative risk was 1.20 ($p<0.013$) for possible exposure, and 1.35 ($p<0.001$) for probable exposure. There were no data on years of employment and smoking habits. The mortality rates of other tobacco-related cancers (except bladder) and emphysema among diesel-exposed employees were slightly elevated, suggesting that the smoking prevalence was higher than for other workers. The results from this well-done study require careful interpretation due to the lack of information on cigarette smoking, asbestos exposure, and duration of diesel exposure.

Garshick et al. (*44*) compared 1,256 lung cancer deaths to two age-matched controls in a retrospective cohort study of 650,000 active and retired railroad workers. The baseline study year was 1959, when diesel engines had nearly replaced all steam engines in the railroad industry (*45*). Consequently, few workers were exposed to asbestos. Information on cigarette smoking habits was obtained from the next of kin. An industrial hygienist conducted sampling tests to detect the levels of diesel exhaust in selected jobs. The extent of diesel exposure in other job categories was determined by job activities and degree of contact with diesel equipment. This exposure classification system was verified against a survey sent to each worker in these jobs. Asbestos exposure was based on surveys and on medical and industrial literature, although exposure to asbestos occurred primarily during the steam-engine era. An increased risk of lung cancer was found for younger employees (<65 years of age) who worked in a diesel-related job for 20 or more years (OR = 1.41, 95% CI, 1.05–1.88). This risk was adjusted for pack-years of smoking and asbestos exposure.

The methodologic advantages of this study include a more precise exposure assessment of diesel exhaust and statistical adjustment for cigarette smoking (pack-years) and asbestos. A sufficient latent period was allowed for, and data on long-term exposure were available. A possible bias was imprecise smoking histories obtained from next of kin. This study provides evidence of a risk associated with long-term diesel exhaust exposure. However, surrogate information on cigarette smoking is often inaccurate (*46,47*), and no association with lung cancer was found for older workers. The authors state that older workers were exposed to diesel exhaust for only a short period, although this needs verification.

The same group conducted a retrospective cohort study of 55,407 white, male railroad workers who were exposed to little or no asbestos (*48*). Members of the cohort had worked for 10–20 years in the railroads after 1959. Jobs with possible exposure to diesel were identified by job titles and job descriptions. An industrial hygiene survey was subsequently conducted to determine the probability of exposure in these jobs. Most railroad workers kept the same jobs during their tenure. Death certificates were obtained on 88% of the cohort. There were 1694 deaths attributed to lung cancer. For older employees who were exposed to diesel for less than 20 years as of 1980, there was no increased rate of lung cancer. The relative risk associated with 20 years of diesel exposure was 1.45 (95% CI, 1.11–1.89) for 40–44 year olds and 1.33 (95% CI, 1.03–1.73) for 45–49 year olds. These younger groups also had the lowest exposure to asbestos. There was no trend with increasing years of diesel exhaust, but when the most recent years of exposure were excluded from the analysis (the four years preceding death), a trend in cumulative exposure was found. Some concerns in this study include the ambiguous data on trend tests and the lack of information on cigarette smoking. However, it is likely that there was little variability in socioeconomic class and therefore the workers may have had similar smoking habits. It should be noted that the two studies of lung cancer by Garshick et al. (*44,48*) were conducted in the same occupational setting.

Motor Vehicle Drivers and Mechanics

Gustavsson et al. (*49*) examined the cancer incidence of 695 bus garage workers in Sweden between 1945 and 1970. These workers were employed as mechanics, servicemen, or hostlers for at least 6 months. All motorized buses in Sweden have been diesel-powered since the end of World War II. Twenty cases of lung cancer occurred in this group. The intensity of the exposure to occupational diesel exhaust and asbestos was assessed by industrial hygienists. The authors fitted a weighted regression model using a cumulative exposure measure to subjects with lung cancer and to subjects who died of lung cancer. The statistically significant relative risks were 1.34 for low-level exposure to diesel exhaust and 2.43 for high-level exposure to diesel exhaust. There were no data on smoking habits.

Steenland et al. (*50*) conducted a case–control study of Teamsters Union members. Death certificates were obtained for more than 10,000 members who filed claims for pension benefits. At least 20 years of tenure in the union was required to claim benefits. Of these, 1,288 men died of lung cancer. The Teamsters records did not have information on the types of truck engines used by members. Data were obtained on the number of years employed in each job. Additional information on occupation, smoking history, and asbestos exposure was obtained from next-of-kin.

Members were classified into jobs with potential diesel exposure based on job category (e.g., diesel truck driver, gasoline truck driver, etc.).

Using the Teamster employment data, elevated but nonsignificant odds ratios between 1.27 and 1.69 were found for long-haul drivers, short-haul drivers, truck mechanics, and other jobs with possible diesel exposure, after adjusting for smoking. The risk of lung cancer by duration of employment after 1959 (the approximate year when most trucks were diesel-powered in the United States) for long-haul truck drivers was, for 1–11 years, 1.08 (95% CI, 0.68–1.70), for 12–17 years, 1.41 (95% CI, 0.9–2.21), for >18 years, 1.55 (95% CI, 0.97–2.47) (linear trend test, $p<0.05$). No trend in duration was found for short-haul truck drivers or for truck mechanics.

From the next-of-kin interviews, nonsignificant odds ratios between 1.25 and 1.54 were found for truck drivers and other jobs with potential diesel exposure. Diesel truck drivers who were employed for 1–24 years had no significantly increased risk of lung cancer. The odds ratio for 56 drivers who drove diesel trucks for 35 years or more was 1.89 (95% CI, 1.04–3.42) after adjusting for smoking. However, for 102 drivers who drove both gasoline-powered and diesel trucks for 35 years or more, the OR was 1.34 (95% CI, 0.81–2.20). There was no increased risk associated with driving both gasoline-powered and diesel vehicles for less than 35 years. Some possible limitations in this study include the validity of smoking information obtained from the next of kin, and a low response rate for questions on employment history (68%). The study results show a statistical association between >35 years of diesel exposure and lung cancer risk.

Hayes et al. (*51*) pooled data from three case–control studies in the United States. More than 1400 lung cancer case–control pairs were interviewed directly. Although detailed occupational data were not available, the risk of lung cancer among truck drivers employed for 10 or more years was 1.5 (95% CI, 1.1–1.9) after adjusting for daily cigarette smoking. The risk associated with other motor-vehicle-related occupations was 1.4 (95% CI, 1.1–2.0). There was no information on the types of engines in this latter group.

Burns and Swanson (*52*) and Swanson et al. (*53*) conducted a population-based study of 3992 males with lung cancer. Cases were diagnosed between 1984 and 1987 and were identified through the Detroit cancer registry. Patients or surrogates were interviewed. Information was collected on smoking habits and occupation. Over 90% of subjects who were approached responded, although only 44% of the case interviews were completed by the subjects themselves, compared to 70% of control interviews. For white, male subjects employed as drivers of heavy trucks, the smoking-adjusted risk estimate was 1.4 (95% CI, 0.8–2.4) for 1–9 years, 1.6 (95% CI, 0.8–3.5) for 10–19 years, and 2.5 (95% CI, 1.4–4.4) for 20+ years. For drivers of light trucks, the odds ratio was 1.7 (95% CI, 0.9–3.3) for 1–9 years and 2.1 (95% CI, 0.9–4.6) for 10+ years. A significant linear trend was found with increasing years of driving heavy and light trucks. No information was available on engine type. There was no increased risk for industrial equipment operators.

Dock Workers

In a study of Swedish dock workers covering the years 1950–1974, 50 lung cancer cases and 154 matched controls were compared (*54*). Company records on annual fuel consumption and annual machine hours were used to calculate indirect measures of diesel exposure. The response rate was 67% and many interviews were conducted among next-of-kin. Some ex-smokers were combined with nonsmokers in the analysis. The odds ratios associated with diesel exhaust among reported nonsmokers was 1.6 for medium exposure and 2.8 for high exposure. Among smokers, the odds ratio associated with diesel exhaust was 10.7 for medium exposure and 28.9 for high exposure. However, the odds ratios for smokers and nonsmokers at each level of diesel exposure had overlapping confidence intervals.

Unmeasured Confounders

The effects of several possible confounders have not been assessed in the studies discussed.

Smoking. Because cigarette smoking is the predominant cause of lung cancer, studies of diesel exhaust and lung cancer require precise statistical adjustment for cigarette smoking. This is especially important in studies showing weak associations. However, the statistical measures of smoking do not reflect with precision the actual exposure of the respiratory tract to cigarette carcinogens. A traditional measure of smoking history is pack-years, which is the product of the duration (years) and intensity of smoking (average number of cigarettes per day). Only some studies used pack-years as a statistical covariate. This measure is only an approximate method of estimating exposure to cigarette tar and particulates. Information obtained from next-of-kin adds further uncertainty in accurately classifying smoking habits. A more refined measure of smoking is total lifetime tar intake. Zang and Wynder (*55*) calculated that men who smoked >20 pack-years have an odds ratio of lung cancer that varies from 26.9 to 48.4 depending on their lifetime tar intake.

Even lifetime tar indices are an inexact measure of cigarette carcinogen intake. The tar values are determined by the Federal Trade Commission (*56*) using outdated methods. The tar values for different cigarette brands are determined by smoking machines under standard laboratory conditions taking one puff per minute of 2 sec duration and a 35-ml volume. These standard conditions were established in 1936 to reflect the smoking habits of nonfiltered cigarettes. Today, most smokers use filtered cigarettes. The inhalation patterns of cigarettes differ between smokers of nonfiltered cigarettes and smokers of filtered cigarettes.

Apparently, the low relatively elevated odds ratios in studies of diesel engine exhaust and lung cancer may be confounded by incomplete statistical adjustment for smoking.

Asbestos. Truck drivers may be exposed to airborne asbestos in the driver's cab. After a clinical report of asbestosis in a truck driver (*57*), dust samples were taken from the cabs of 10 trucks (*58*). Three cabs contained airborne asbestos fibers, and seven cabs contained synthetic fibrous minerals. The airborne concentration of fibers was not determined. These fibers likely originated from the insulation materials in the cab. The joint effect of asbestos and cigarette smoking on the risk of lung cancer in truckers (*59*) needs to be considered.

Dietary Fat. Saturated fat is a lung tumor promoter in animal models (*60–62*) and has been related to the development of lung cancer in epidemiologic studies (*63–67*). International comparisons of lung cancer rates also implicate dietary fat as a lung tumor promoter. Although the prevalence of smoking has been higher in Japan than in the United States since 1955, lung cancer rates in Japan have been substantially lower (*68*). This paradox may reflect the lower per capita intake of dietary fat in Japan.

There is little information on the dietary habits of diesel-exposed workers. In one survey of 206 long-distance truck drivers, Wynder and Miller (*69*) found a high consumption of dairy products and fatty foods. Sixty percent of truckers reported eating two or more eggs per day. Their consumption of butter, margarine, and cheese was also higher than that reported in national surveys, reflecting frequent meal consumption at roadside restaurants. Other

studies suggest that blue-collar workers such as skilled technicians or laborers eat more meat and fewer vegetables compared to professionals and other white-collar workers (*70*).

Body weight. Leanness is an independent risk factor for adenocarcinoma of the lung in epidemiologic studies (*71,72*). The mechanisms for these observations are unknown but could reflect increased metabolic rate. The possible confounding effects of diet and body weight have not been accounted for in epidemiologic studies of diesel exhaust and lung cancer.

Conclusion

Determining whether diesel engine exhaust is a human lung carcinogen is clearly a complex undertaking (*73*). The results from earlier experimental, mutagenesis, and epidemiologic studies may be less relevant than more recent studies, as changes in emission control technology have altered the chemical composition of diesel exhaust. Diesel particulate extracts are mutagenic in some bioassays, although urinary mutagenic activity was not associated with levels of diesel exhaust exposure in a population of railroad workers (*74*). Toxicologic data suggest that short-term metabolic "overload" from diesel exposure induces lung tumors in rats. It is unknown whether this is an appropriate model for human exposure, although cigarette smoke reduces pulmonary clearance in causing human lung cancer. If the results from rat studies are predictive of human health effects, it is unclear whether cumulative, long-term exposure to diesel emissions can overload defensive pulmonary systems. The epidemiologic studies do not provide evidence for a short-term (<20 years) carcinogenic effect of diesel exhaust.

The results from epidemiologic studies are not consistent in different study populations, although some studies were done with greater precision than others. Few studies have shown a dose-dependent relationship independent of cigarette smoking. The lack of a dose effect with cumulative diesel exposure indices must also be interpreted with caution. In general, epidemiology is too imprecise a science to detect trends in weak associations.

There is statistical evidence that long-term employment (>20 years) for locomotive engineers, diesel mechanics, trainmen, and other railroad workers is associated with an increased risk of lung cancer (*44*). Two other studies in the railroad industry (*43,48*) found statistical evidence linking long-term diesel exposure to a small increase in lung cancer rates, although the lack of adjustment for smoking habits requires a cautious interpretation. There is little evidence of a dose–response relationship because only men who had the longest duration of exposure (>20 years) in the two Garshick studies had increased risks of lung cancer. The study by Howe et al. (*43*) had no data on duration of employment. There was no association between employment in the railroad industry and lung cancer risk in the American Cancer Society study, although only 14 deaths occurred in this group (*40*). Case–control studies by Burns et al. (*52*) and Hall and Wynder (*37*) found no association between railroad employment and the risk of lung cancer, although these studies lacked information on diesel exposure. In summary, there is statistical information linking long-term exposure (>20 years) to a small increased risk for locomotive engineers, brakemen, and diesel mechanics.

A limitation in the studies of truck drivers is the inadequate characterization and statistical control for cigarette smoking. This does not necessarily imply a deficiency in the data collection instruments. It is important to recognize that when cigarette smoking is a strong confounder in studies of weak associations, traditional measures of cigarette smoking may not be precise enough to allow complete control for confounding in statistical models. The various studies of truck drivers found no increased lung cancer risk with short-term employment. Steenland et al. (*50*) observed a statistical increase of lung cancer among diesel truck drivers who were employed for 35 or more years. In contrast, Boffetta et al. (*40*) found no significant increased risk with employment as (primarily) diesel truck driving in the American Cancer Society cohort study. Boffetta et al. (*39*) also found no increased risk of lung cancer in truckers who reported exposure to diesel exhaust in a case–control study. Hall and Wynder (*37*) found no increased risk for truck drivers in a separate case–control study, although the types of trucks were unspecified. Burns et al. (*52*) found a significant increased risk for drivers in a case–control study, although no information was available on diesel exposure.

Swanson et al. (*53*) found a trend with years of employment for drivers of heavy trucks, but also found a trend with drivers of light trucks. Similarly, Hayes et al. (*51*) found a significant increased risk for truck drivers in a case–control study, but also found a significant risk for other motor-vehicle occupations besides truck driving. Doll has pointed out that internal study inconsistencies are more likely to reflect chance findings than identification of occupational risk factors (*74*). In this case, there may be other factors associated with driving trucks besides engine type related to lung cancer. Benhamou et al. (*35*) reported a significant increase for motor vehicle drivers but did not specify the type of vehicle. In summary, the findings for motor-vehicle drivers are inconsistent. Among those studies with positive findings, it is unclear whether the associations reflect an effect of diesel exposure. There is little evidence of a dose–response trend in these data. The study of Swedish dock workers is inconclusive. It was not possible to separate the independent effects of cigarette smoking from diesel exposure.

Elemental carbon has been used as a marker of exposure to diesel exhaust in industrial hygiene surveys. The average concentration of elemental carbon in the cabins of diesel and gasoline trucks is higher than in residential environments, but not elevated above highway background levels (*75,76*). Because drivers of diesel trucks and drivers of gasoline trucks are exposed to similar concentrations of diesel, this could explain in part the similar risk estimates of lung cancer for these two groups in some epidemiologic studies. Average respirable concentrations of particulates are 1–7 times higher for railroad workers (17–134 $\mu g/m^3$) (*77*) than average truck driver exposures (20 $\mu g/m^3$) (*75*), after adjustment for smoking. These differences suggest that the low odds ratios in studies of railroad studies cannot be generalized to other diesel-exposed occupational groups. These hygiene measurements do not necessarily reflect historic levels in these industries, although the much higher exposure of railroad workers to diesel raises questions concerning the validity of the elevated risks associated with trucking.

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Disinfection By-products in Drinking Water: *Critical Issues in Health Effects Research*

October 23–25, 1995

The Carolina Inn
Chapel Hill, NC

Organized by: ILSI Health and Environmental Sciences Institute (HESI)
Sponsored by: HESI Water Quality Technical Committee and
U.S. Environmental Protection Agency
Cosponsored by: National Institute of Environmental Health Sciences

Workshop Objectives

- Provide a public forum for the presentation and discussion of recent research findings on potential health effects of disinfection by-products from chlorine, chlorine dioxide, chloramines, and ozone in drinking water
- Provide a comparative evaluation of available information on by-products resulting from various disinfection methods
- Discuss case studies for application of biologically based risk assessment models; examine data requirements for use of these models in risk assessment
- Evaluate data needs and risk assessment approaches for non-cancer effects of disinfection by-products
- Review health effects and risk assessment issues associated with exposure to disinfection by-product mixtures
- Explore opportunities for interaction between the disciplines of toxicology and epidemiology in design and prioritization of future research
- Review the directions of current research on disinfection by-products and outline future research needs and priorities

A report summarizing the workshop sessions and recommendations will be prepared and distributed to all participants

Audience

This three-day workshop will provide a forum for scientists from the international scientific, regulatory, and public health communities to describe their current research findings related to critical issues in health effects research of disinfection by-products in drinking water. The presentations and panel discussions are intended to assist scientific investigators, regulators, water treatment professionals, and risk managers concerned with making decisions regarding the interpretation of data from safety assessment studies and their application to the risk assessment of disinfection by-products.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Comparisons of Estimated Human Body Burdens of Dioxinlike Chemicals and TCDD Body Burdens in Experimentally Exposed Animals

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Humans are exposed to mixtures of polyhalogenated aromatic hydrocarbons, and the potential health effects of these exposures are uncertain. A subset of this class of compounds produce similar spectra of toxicity in experimental animals as does 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and these chemicals have been classified as "dioxins." In this study, we compared the body burdens of dioxins that produce effects in experimental animals to body burdens associated with these effects in humans. Human body burdens were estimated from lipid-adjusted serum concentrations of dioxins, assuming dioxins are equally distributed in body fat and an adult has 22% body fat. The toxic equivalency factor (TEF) method was used to calculate body burdens of dioxins in humans. These calculations included dibenzo-*p*-dioxins, dibenzofurans, and polychlorinated biphenyls. In the general population, average background concentrations were estimated at 58 ng TCDD equivalents (TEQ)/kg serum lipid, corresponding to a body burden of 13 ng TEQ/kg body weight. Populations with known exposure to dioxins have body burdens of 96–7,000 ng TEQ/kg body weight. For effects that have been clearly associated with dioxins, such as chloracne and induction of CYP1A1, humans and animals respond at similar body burdens. Induction of cancer in animals occurs at body burdens of 944–137,000 ng TCDD/kg body weight, while noncancer effects in animals occur at body burdens of 10–12,500 ng/kg. Available human data suggest that some individuals may respond to dioxin exposures with cancer and noncancer effects at body burdens within one to two orders of magnitude of those in the general population. *Key words*: dioxins, polychlorinated biphenyls, risk assessment, toxic equivalency factors. *Environ Health Perspect* 103:820–831 (1995)

Over the last 30 years, an abundance of studies have clearly demonstrated that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is extremely toxic to experimental animals (*1–3*). Fish and wildlife are also sensitive to the toxic effects of this chemical (*4*). TCDD is carcinogenic in male and female rats and mice, male hamsters, and male and female fish (*5,6*). Reproductive and developmental toxicity has been observed in all experimental animals tested. Immunotoxic effects occur in mice, rats, and nonhuman primates exposed to low doses of TCDD (*7*). Evidence to date indicates that the actions of TCDD are mediated by the Ah receptor (*8,9*) which functions as a signal transducer and transcription factor. In many ways the actions of the Ah receptor are similar to those of the steroid hormone receptors (*10,11*), although the Ah receptor is not a member of this superfamily of proteins (*12,13*). Other halogenated dibenzo-*p*-dioxins and dibenzofurans substituted in all four lateral positions also have high binding affinity to the Ah receptor and induce the same spectrum of toxicity as TCDD (*14*). In addition, certain polyhalogenated biphenyls, naphthalenes, and diphenyl ethers are Ah receptor agonists. Humans are exposed to complex mixtures of these chemicals; estimates of daily exposure to TCDD or "dioxinlike" (all 2,3,7,8-halogenated dibenzo-*p*-dioxins and dibenzofurans as well as the dioxinlike polychlorinated biphenyls) chemicals is 3–6 pg TCDD equivalents/kg/day in the United States (*15,16*). The subclass of the polyhalogenated aromatic hydrocarbons with dioxinlike activity are referred to as dioxins in this article.

Although the toxic effects of dioxins in experimental animals are unequivocal, their toxic effects in humans are less certain. Chloracne is the only toxic effect induced by dioxins for which there is unequivocal evidence linking exposure to effect in humans (*17*). The uncertainty of other toxic effects of dioxins in humans is due to the scarcity of human populations with high dose exposures, limited data on the body burdens of dioxins present in these populations, the difficulty in assessing sensitive toxic endpoints in humans, and the lack of knowledge about likely, but unknown, genetic factors that may influence the relative susceptibility of individuals. Dioxins produce some of the same biochemical alterations in humans and experimental animals (*18*). Several recent epidemiological studies suggest an association between dioxin exposure and increased incidence of cancer (*19–23*) and increased incidence of altered glucose tolerance in exposed populations (*24,25*). One way to determine the strength of an association between dioxin exposure and a toxic effect in humans would be to compare the dose of dioxin that is required to produce an effect in animals to the dose of dioxin in humans that is associated with a similar toxic effect. While it is clear that for some toxic effects, such as lethality and body weight loss, there are marked species differences in susceptibility to dioxins, many recent studies have also noted that for other endpoints, such as reproductive and developmental effects, most animal species respond at similar doses (*9,26*). Thus, the dose of dioxin that produces a particular effect in experimental animals might be expected to be similar to the dose of dioxin associated with that same effect in humans.

Although the hypothesis that toxic doses of dioxins in animals and humans are similar for most responses is theoretically testable using data from accidentally exposed human populations, there are some difficulties. In particular, it is often difficult to determine the human dosage at the time of exposure. In experimental studies, animals are administered a known amount of dioxin and evaluated at a specific time after the treatment. In humans the actual exposure is unknown and often difficult to estimate. Several epidemiological studies determined serum concentration of dioxins in exposed and control populations (*19–25*). Although the dose to the individuals in these studies is uncertain, the body burdens of dioxins in these populations can be estimated at a specific point in time. In addition, serum and tissue dioxin concentrations from populations in the United States with-

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out any unusually high exposures have been reported from several different laboratories (*27–30*). All humans in industrialized countries are presumed to carry a body burden of dioxins based primarily on consumption of minute quantities of dioxin in the food supply. Here we compare the body burdens of dioxins that produce effects in experimental animals to the body burdens associated with effects in humans, based on the clinical findings observed during epidemiological studies. A comparison of the *in vitro* effects of dioxins on human and animal tissues and cell cultures is also presented. This analysis suggests that some of the effects observed in experimental animals also occur in humans and that the body burdens of dioxins associated with these effects (adaptive and/or toxic) are similar between animals and humans.

Methods

Comparisons of animal and human tissues or cell lines studied under *in vitro* conditions are shown in Table 1. This list is not meant to be exhaustive. The data presented are from peer-reviewed literature and include only those papers that compared animal and human tissues in the same study or laboratory.

We estimated human body burdens based on analyses of dioxin in serum or tissue in the cited literature. Several assumptions were used to derive body burdens from these values. Dioxins are assumed to be equally distributed in the body lipid with all tissues having the same concentration of TCDD when expressed on a lipid-adjusted basis (*31–33*). Thus, serum levels presented as lipid-adjusted are assumed to be equivalent to adipose tissue levels expressed as lipid-adjusted values. In addition, we assumed that for the average person, 22% of the body weight is lipid or fat (*34*). To estimate body burdens in humans, lipid-adjusted serum or adipose tissue concentrations (expressed as ng TCDD/kg or TEQ/kg) were multiplied by 0.22 (*34*), the fraction of body weight that is fat.

Some of the body burden estimates in humans presented here are based on tissue concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin alone. In all cases, humans were likely to have been exposed to many dioxinlike chemicals that bind to the Ah receptor and produce the same spectrum of toxic effects in experimental animals as TCDD (*2,14,26*). To account for exposure to additional dioxins, the toxic equivalency factor method (TEF) was used (*14,35–38*). TEFs are relative potency factors used to convert the amount of dioxins in a sample to TCDD equivalents or TEQs (*14,35–38*). TEFs were assigned only to 2,3,7,8-chlorine substituted dibenzo-*p*-dioxins and dibenzofurans, the coplanar polychlorinated biphenyl(s) (PCBs) (IUPAC nos. 77, 81, 126, and 169) and the mono-*ortho*-substituted PCBs (IUPAC nos. 105, 114, 118, 156, 157, 167, and 189). The TEF values used for the dibenzo-*p*-dioxins and dibenzofurans were the U.S. EPA interim TEF values, which represent an internationally accepted convention for assessment of dioxins (*37,38*). The TEF values used for the dioxinlike PCBs were the World Health Organization values, which resulted from a recent international meeting of dioxin and PCB experts (*38*). Hence, body burdens for this complex mixture of related chemicals are expressed in terms of TEQs.

Body burden estimates in populations exposed to background levels of dioxins were based on published studies that measured serum concentrations of 2,3,7,8-chlorine substituted dibenzo-*p*-dioxins (CDDs) and dibenzofurans (CDFs) and dioxinlike PCBs in populations with no unusually high exposure to dioxins (*27–30,39*). Serum concentrations of CDDs and CDFs have been measured in a number of different populations from several studies. Schecter (*27*) presented data indicating that the average whole-blood CDD/CDF concentration in U.S. (n = 100) and German (n = 85) populations were similar when presented on a TEQ basis (41 and 42 ng TEQ/kg whole blood, lipid adjusted). More extensive studies of U.S. populations indicate that the national average for serum CDD/CDF concentrations is 28 ng TEQ/kg serum lipid (*39*). Much smaller studies of congener-specific PCB serum or adipose tissue concentrations have been published that indicate that average dioxinlike PCB concentrations range from 8 to 17 ng TEQ/kg tissue lipid in U.S. populations (*28,30*). The range of average tissue TEQ concentrations for CDDs/CDFs is 28–41 ng TEQ/kg lipid and for the PCBs the range is 8–17 ng TEQ/kg lipid. Based on these studies, average background dioxin tissue concentrations range from 36–58 ng TEQ/kg lipid. In these populations, TCDD contributes approximately 15% of the total TEQ.

Body burden estimates in exposed populations were based on the published literature. These populations were assumed to have background exposures, in addition to the specific exposures determined in the study. The level of dioxins in exposed populations were often determined years after the initial exposure. Body burdens were estimated at the time of maximal exposure assuming the rate of total body elimination of dioxins is linear with respect to time and dose and a assuming 7.1-year half-life (*40*).

Determination of maximum body burdens in experimental animals was based on the administered dose and the rate of elimination of dioxin from the animal. Total body half-life of TCDD in experimental animals was assumed to be first order with respect to time and dose. In several cases, body burdens in animals were based on tissue levels determined in the study.

Effects seen in epidemiological studies have been divided into two categories. The first category (Table 2) is for effects that have been causally associated with exposure to dioxins. These are effects for which there is strong evidence that the responses observed are due to exposure to dioxins and/or related compounds. Typically, adverse effects with demonstrated causality

Table 1. Comparison of the effects of TCDD exposure on human and animal tissue *in vitro*

Effect	Species/tissue	Concentration (nM)	Reference	Appendix note[a]
TCDD binding to Ah receptor (K_d)	Mouse (C57Bl/6)	0.27	(*42*)	a
	Human	1.6	(*42*)	a
Induction of CYP1A1 (EC_{50})	Lymphocytes			
	Mouse	1.3	(*46*)	b
	Human	1.8	(*46*)	b
Cytotoxicity (LOEL)	Embryonic palate			
	Mouse	0.1	(*47*)	c
	Rat	100	(*47*)	c
	Human	100	(*47*)	c
Inhibition of proliferation (LOEL)	Thymocytes			
	Mouse	0.1	(*48*)	d
	Human	0.1	(*49*)	d
	Lymphocytes			
	Mouse	3.0	(*50*)	e
	Human	0.3	(*50*)	e
Inhibition of IgM secretion (LOEL)	Lymphocytes		(50)	e
	Mouse	3.0		
	Human	0.3	(*50*)	e

LOEL, lowest observed effect level.
[a]The data and methodology used to determine each value are presented in the appendix under the letter indicated.

Table 2. Responses in humans causally associated with exposure to dioxins and comparable effects in experimental animals

Effect	Species	Experimental dose	Body burden (ng/kg)	Reference	Appendix note[a]
Chloracne	Human		95–3,000	(*51,52*)	f
	Monkey	1,000 ng/kg	1,000	(*53*)	g
	Rabbit	4 ng/rabbit, 5 days/week/4 weeks	23	(*54*)	h
	Mouse	4,000 ng/kg, 3 days/week/2 weeks	13,900	(*55*)	i
Downregulation of EGFR (maximal effect)	Human (placenta)		2,130	(*18,56*)	j
	Rat (liver)	125 ng/kg/day, 30 weeks	2,582	(*57*)	k
	Mouse (liver)	10,000 ng/kg	10,000	(*58*)	l
Induction of CYP1A1 (maximal effect)	Human (placenta)		2,130	(*18,56*)	j
	Rat (liver)	125 ng/kg/day, 30 weeks	2,582	(*59*)	k
Induction of liver CYP1A1 (LOEL)	Rat	1 ng/kg	1	(*60*)	m
	Mouse	1.5 ng/kg/day, 5 days/week/13 weeks	23	(*61*)	n
Hepatic sequestration	Human		300	(*62*)	o
	Rat		100	(*62*)	o
Background	Human	TCDD	1.1		p
		PCDD/PCDF	6–9		p
		PCDD/PCDF/PCB	8–13		p
	Rat		1	(*67*)	q
	Mouse		4	(*61*)	r

Abbreviations: EGFR, epidermal growth factor receptor; LOEL, lowest observed effect level; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PCDD, polychlorinated dibenzodioxin; PCDF, polychlorinated dibenzofuran; PCB, polychlorinated biphenyl.

[a]The data and methodology used to determine each value are presented in the appendix under the letter indicated.

are associated with high-level exposure and are severe enough to clearly indicate a relationship with such exposure. Chloracne is an example of such an adverse effect. In addition, biochemical changes such as induction of CYP1A1 and decreases in EGF receptor autophosphorylation are included in this category because there is significant experimental evidence that these effects occur through activation of the Ah receptor and are therefore causally related to exposure to dioxinlike chemicals.

A second category (Table 3) was assigned for effects associated with dioxin exposure for which a causal link has not been definitively proven. Effects included in this category are decreased birth weight, decreased growth, delayed developmental milestones, cancer, decreased testosterone levels, and increased risk of diabetes. In both Tables 2 and 3, body burdens in experimental animals are presented for comparable toxic effects to those seen in the epidemiological studies.

Table 4 presents body burdens in experimental animals that produce an effect for which no comparable human epidemiological data are yet available. The current epidemiological database consists primarily of studies on adult male populations; few studies of women or children are available. Only effects seen at low doses or body burdens in experimental animals were chosen for this table to estimate the low end of the animal effect range; effects such as thymic atrophy, the wasting syndrome, or death are not included. The specific assumptions and data used to derive each value presented in Tables 1–4 are presented in the appendix.

Results

Comparisons of the *in vitro* effects of TCDD on animal and human tissues or cell lines are shown in Table 1. A number of investigations have found the Ah receptor present in humans to have a similar but slightly lower binding affinity for TCDD than the Ah of many other species (*42–45*). The concentration of TCDD required to produce equivalent effects in animal and human tissues is not significantly different for responses as varied as induction of CYP1A1 in lymphocytes and thymocyte proliferation (Table 1). For several responses, the effective concentration of TCDD differs in animal and human tissue by an order of magnitude or greater. Cytotoxic effects induced by TCDD in organ cultures of developing palate occur at concentrations 1000 times lower in mouse tissue than in either human or rat tissue (*48*). Cultures of embryonic human and rat palatal shelves respond at the same concentrations (*48*). Inhibition of lymphocyte proliferation and secretion of IgM in mouse splenic lymphocytes requires 10 times the concentration of TCDD compared to human tonsilar lymphocytes (*50*).

Comparisons of body burdens associated with *in vivo* effects demonstrate similar correlations between animals and humans. Body burden estimates in individuals with chloracne vary by almost two orders of magnitude (Table 2). In subjects with chloracne, exposures resulted from either industrial or accidental poisonings. In experimental animals, species differences in body burdens of TCDD that induce chloracne vary by almost three orders of magnitude, with the rabbit the most sensitive and the hairless mouse the least sensitive. The range of body burdens that result in chloracne in humans (96–3,000 ng TEQ/kg body weight) and animals (23–13,900 ng TCDD/kg body weight) are similar. It should be noted that the first of these ranges represents interindividual variation while the second includes interspecies variation.

Body burdens in the general population were determined based on TCDD alone, total PCDDs/PCDFs, and total PCDDs/PCDFs/PCBs (Table 2). The average body burden of TCDD in the general population is approximately 1.1 ng TCDD/kg body weight. The average body burden in the general population for total PCDDs/PCDFs is 9 ng TEQ/kg body weight and for total PCDDs/PCDFs/PCBs is 13 ng TEQ/kg body weight.

Rice oil contaminated with PCDFs and PCBs, among other contaminants, was ingested by men and women from Taiwan (Yu-Cheng incident); these individuals have been carefully studied since the poisoning incident (*18,56,63–65*). Biochemical changes in placentas from the women exposed during the Yu-Cheng incident are similar to the biochemical changes in rodent liver from animals exposed to TCDD. Near maximal downregulation of human placental epidermal growth factor receptor autophosphorylation occurs at similar body burdens, as do comparable decreases in hepatic epidermal growth factor receptor in rats and mice (Table 2). Maximal induction of hepatic cytochrome P-450 1A1 (CYP1A1) in rats and mice by TCDD occurs at body burdens similar to those that elicit maximal increases of CYP1A1 in human placenta from the individuals exposed during the Yu-Cheng incident. The lowest observable effect level (LOEL) for enzyme induction in animals is 1 and 23 ng TCDD/kg body weight in rats (*60*) and

Table 3. Responses in humans associated with dioxin exposure and comparable effects in experimental animals

Effect	Species	Experimental dose	Body burden (ng/kg)	Reference	Appendix note[a]
Cancer	Human		109–7,000	(19,23)	s
	Hamster	100 µg/kg/month/ 6 months	137,000	(68)	t
	Rat	100 ng/kg/day, 2 years	2,976	(69)	u
	Mouse	71 ng/kg/day, 2 years	944	(70)	v
Tumor promotion	Rat (liver)	125 ng/kg/day, 30 weeks	2,582	(71)	k
	Mouse (skin)	7.5 ng/week, 20 weeks	830	(72)	v
Decreased birth weight	Human	Maternal body burden	2,130	(18,56)	j
	Rat	400 ng/kg, maternal dose	400	(73)	w
	Hamster	2,000 ng/kg, maternal dose	2,000	(8)	w
Decreased growth	Human	Maternal body burden	2,130	(63)	j
	Rat	1,000 ng/kg, maternal dose	1,000	(75)	w
Delayed developmental milestones	Human	Maternal body burden	2,130	(64,65)	j
Object learning	Monkey	0.151 ng/kg/day, Maternal body burden	42 ng/kg	(66)	x
Decreased testosterone	Human		44–122	(41)	y
	Rat	12,500 ng/kg	12,500	(76)	u
Altered glucose homeostasis	Human		99–140	(24,25)	z, aa
	Guinea pig	30 ng/kg	30	(78)	bb
	Rat	100 ng/kg/day, 30 days	2,000	(79)	u

[a]The data and methodology used to determine each value are presented in the appendix under the letter indicated.

Table 4. Low dose effects in animals exposed to dioxins

Effect	Species	Experimental dose	Body burden (ng/kg)	Reference	Appendix note[a]
Decreased offspring viability	Rhesus monkey	0.76 ng/kg/day, 4 years	345	(80)	x
	Rat	1,000 ng/kg	1,000	(74)	w
	Hamster	18,000 ng/kg	18,000	(81)	w
Altered lymphocyte subsets	Marmoset	0.3 ng/kg/week, 24 weeks and 1.5 ng/kg/week, 12 weeks	10	(82)	cc
		10 ng/kg	10	(83)	cc
Enhanced viral susceptibility	Mouse	10 ng/kg	10	(84)	dd
Endometriosis	Monkey	0.151 ng/kg/ day/4 years	69	(85)	x
Decreased sperm count	Rat	64 ng/kg, maternal dose	64	(86)	w
Testis abnormalities	Rat	12,500 ng/kg	12,500	(76)	u
	Mouse	100 µg/kg	100,000	(77)	ee

[a]The data and methodology used to determine each value are presented in the appendix under the letter indicated.

mice (*61*), respectively, which is within the range of background human body burdens of 13 ng TEQ/kg body weight.

Disposition of dioxins is dose dependent in animals and humans (*62*). The body burden necessary for hepatic sequestration is similar for rats and humans (*62*). In animals, the body burden of TCDD that produces a carcinogenic effect ranges from 944 ng TCDD/kg body weight in mice (*70*) to 137,000 ng TCDD/kg in hamsters (*68*) (Table 3). Body burdens in animals exposed to carcinogenic doses of TCDD are 73- to 10,500-fold greater than background human TEQ body burdens. In epidemiological studies that indicate an association between TCDD exposure and increased incidence of cancer, body burdens were estimated between 109 and 7,000 ng TCDD/kg at the time of highest human exposure. Background human TEQ body burdens are approximately 8–540 times less than human TEQ body burdens estimated from the studies that associated dioxin exposure with increased cancer incidence.

Decreased birth weights were reported in children born to women exposed during the Yu-Cheng incident (*18,56*). These women were highly exposed and had an average body burden of approximately 2,130 ng TEQ/kg body weight. Body burdens of dioxins in experimental animals that decrease birth weight range from 400 to 2,000 ng TCDD/kg body weight in rats and hamsters (*73,74*) (Table 3).

Children of the Yu-Cheng mothers are not only smaller at birth but remain smaller throughout childhood compared to children of unexposed women (*63*). In rats, pups of dams exposed to 1,000 ng TCDD/kg body weight not only have decreased birth weights but consistently weigh less than controls up to 63 days of age, though they do recover upon reaching sexual maturity (*75*).

The Yu-Cheng children also exhibit delayed developmental milestones (*64,65*). Behavioral effects after perinatal TCDD exposure have been observed in rhesus monkeys born to mothers exposed to approximately 5 ppt TCDD in the diet (*66*). Body burdens in the rhesus mothers were 42 ng TCDD/kg body weight, which is approximately 51 times less than the TEQ body burden in the Yu-Cheng women, but only 3.2 times higher than average TEQ body burden in the general population.

Although some of the responses seen in experimental animals appear to occur in humans at similar body burdens, there are significant differences in the body burden estimates for decreased testosterone levels (*41*) and between human and animals. Based on these limited data, if decreased testosterone in humans is due to dioxin toxicity, then some humans may be approximately 280 times more sensitive than are rats for dioxin-induced decreases in testosterone.

Increased incidence of diabetes in populations exposed to dioxins has been reported in two studies with body burdens ranging from 99 to 140 ng TEQ/kg. While TCDD-induced diabetes has not been studied in experimental animals, there are reports of altered glucose homeostasis. Alterations in glucose uptake in adipocytes isolated from guinea pigs treated with TCDD occurs at body burdens 3–4 times lower than human populations with increased incidence of diabetes and altered glucose tolerance (*24,25*). Decreased serum glucose in rats occurs at body burdens 14–20 times higher than the increased incidence of diabetes and altered glucose tolerance in humans.

Table 4 presents estimated body burdens of TCDD in experimental animals from studies that report low-dose effects for which no comparable human studies are available. LOELs for decreased offspring viability/fetal viability vary from 345 ng/kg in monkeys to 18,000 ng/kg in hamsters. Alterations in lymphocyte subsets in juvenile marmosets is 10 ng TCDD/kg body weight (*82,83*). Enhanced viral susceptibility, as measured by increased mortality, occurs in mice at body burdens of approximately 10 ng TCDD/kg (*84*), which is equivalent to the body burden seen in unexposed humans and approximately twice the level in untreated mice. Effects such as increased incidence of endometriosis in rhesus monkeys (*85*) and decreased sperm count in offspring of rats treated with TCDD (*74,86*) occur at body burdens approximately five times that of unexposed human populations.

Discussion

A number of investigators have found the Ah receptor present in human tissues to have a similar, but slightly lower, affinity for TCDD than those receptors present in many other species (*42–45*). For example, a recent study determined that the apparent binding affinity of TCDD to the Ah receptor ranged from 0.4 to 15 nM in 115 human placentas and from 1 nM in the TCDD responsive C57Bl/6J mouse to 16 nM in the TCDD nonresponsive DBA/2 mouse. The binding affinity of TCDD to the Ah receptor is similar in mice, rats, hamsters, guinea pigs, and monkeys (*87*), and there is no obvious correlation between TCDD binding affinity to the Ah receptor and species sensitivity to the lethal or toxic effects of TCDD (*87*). Thus, our knowledge of the quantitative relationship between binding affinity and interspecies responsiveness does not provide adequate information to determine whether humans are more or less responsive than other species based solely on the binding affinity of TCDD to the Ah receptor.

Comparisons of human tissues or cell lines with similar animal tissues or cell lines demonstrate that from relatively simple responses, such as enzyme induction to more complex phenomena, such as cytotoxicity and proliferation, human tissue responds in the same manner as animal tissue and at similar concentrations (Table 1). These *in vitro* studies suggest that humans will respond to dioxin and that some of these responses may be adverse.

The doses of dioxins that produce lethality in experimental animals can vary by more than three orders of magnitude; guinea pigs are the most sensitive and hamsters are the least sensitive (*1–3*). Because of this large variability in lethal effects, there has been an expectation that large species differences exist for all other effects. The data presented in the tables indicate that for a particular effect, some species may be extremely sensitive and some may be resistant, but many species respond at similar doses (i.e., within an order of magnitude). All experimental mammalian species examined respond to most of the adverse effects of dioxins at some dose. It is possible that humans may be resistant to some of the toxic effects of dioxins, but it seems highly unlikely, given the data currently available, that humans are refractory to all of the toxic effects of these chemicals.

Dioxins are unequivocally potent toxicants in experimental animals, yet the human health effects of exposure to these chemicals remain controversial. Comparisons of human and animal body burdens alone cannot prove a cause-and-effect relationship between toxicity and exposure in humans observed in an epidemiological study. However, this information can be used to increase or decrease our confidence that a particular adverse health effect observed in an epidemiological study was associated with the exposure to dioxins.

In addition, the present analysis required several assumptions in estimating both animal and human body burdens. These assumptions were required due to the lack of complete data on pharmacokinetics, toxic equivalency factors, species extrapolation, and, for humans, lack of information on daily dose or exposures. Hence, the information presented here can be used to direct research efforts to provide more accurate information on these topics.

There are some uncertainties associated with the assumptions used to estimate body burdens of dioxins in animals and humans. Unlike the experimental animal toxicology studies examined, humans are exposed to multiple chemicals. However, in the epidemiological studies, many of these chemicals interact with the Ah receptor as either agonists, partial agonists, or possibly antagonists. Assumptions of the relative potency of the chemicals and their distribution in the humans will result in uncertainties that are difficult to quantify given the present database. However, these uncertainties are likely well within an order of magnitude because body burdens of TCDD alone represent 10% of the total TEQ body burden due to all the PCDDs, PCDFs, and PCBs (Table 2).

Human body burdens are estimated using the TEF methodology. The TEF values derived by the U.S. EPA and the World Health Organization were based on scientific judgment as well as experimental data (*37,38*). In setting a TEF value, more weight was given to long-term, *in vivo* studies than to *in vitro* or acute *in vivo* studies (*14,36–38*). In fact, although wide ranges of TEF values have been reported for specific congeners, the variability is within a factor of 10 when the *in vivo* data are used to set the TEF value (*14,37,38*).

The TEF methodology assumes additivity of toxic potential. The use of the TEF methodology has been validated for complex mixtures of chlorinated dibenzo-*p*-dioxins for effects such as enzyme induction and tumor promotion (*88*). The interaction of mixtures containing both dioxin-like and non-dioxinlike chemicals has not been studied as thoroughly. There are reports of antagonistic (*89–91*) and synergistic (*92,93*) interactions of dioxins and non-dioxinlike PCBs. The demonstration of nonadditive interactions increases the uncertainty of these values. Finally, the TEF scheme includes only full agonists of the Ah receptor. The use of TEFs and the assumption of additivity have been approved by both the World Health Organization and the U.S. EPA as a default, but interim, approach given the enormity of the task to test for all possible interactions of complex mixtures and in the relative absence of consistent data to the contrary (*94*). Clearly, the TEF values and assumptions regarding additivity need to be updated as more data become available.

Estimates of body burdens in animals and humans assume that the half-life of elimination of dioxins is a first-order process which is independent of the body burden or dose. There is significant evidence that disposition of TCDD is dose dependent (*95–97*). Induction of a binding protein in the liver has been proposed by Andersen et al. (*98*) to explain the dose-dependent disposition of TCDD seen in experimental animals. Similar dose-dependent hepatic sequestration has been proposed in humans (*62*). These data suggest that elimination of these chemicals may not be a first-order process and the use of a single one-component half-life to estimate body burdens may not adequately predict these values.

Two different methods were used to estimate body burdens in experimental animals. One method involved classical pharmacokinetic calculations, and the second method used tissue concentration data presented in the papers. These methods resulted in similar body burden estimates for some cases where the appropriate data were available. For example, in mice receiving 1.5 ng TCDD/kg/day, estimated body burdens using classical pharmacokinetic

calculations were 14 ng TCDD/kg body weight and 23 ng TCDD/kg body weight using TCDD tissue concentrations. Body burden estimates from a tumor promotion study with rats receiving 125 ng TCDD/kg/day produces estimates of 3615 ng TCDD/kg body weight using pharmacokinetic calculations and 2582 ng TCDD/kg body weight using TCDD tissue concentrations. These results suggest that the use of either method to derive body burdens will result in reasonably accurate estimates.

In estimating human body burdens, we assumed that dioxins distribute solely to the lipid portion of the body and that the concentration of dioxins in serum lipid is directly correlated to the concentration of dioxins in total body lipid. Several studies have demonstrated direct correlation between lipid-adjusted serum and adipose tissue concentrations of dioxins from human biopsy samples for the lower chlorinated dibenzo-*p*-dioxins and dibenzofurans (*30–33*). This relationship is not as certain for the higher (six or more chlorine substitutions) chlorinated analogs. Furthermore, in humans exposed to background levels of dioxins, the absolute or lipid-adjusted concentrations of CDDs and CDFs in adipose tissue and liver are not directly related and liver/fat ratios vary between 1.22 and 15.42 depending on the congener and possibly on dose (*99*). The highly chlorinated dibenzo-*p*-dioxins and dibenzofurans are found in greater concentration in the liver compared to the fat (liver/fat ratio 7.4–15.42). In the same samples, TCDD had a liver/fat ratio of approximately 2 (*96*). The human liver appears to accumulate these chemicals in greater proportion than adipose tissue, similar to what has been observed in experimental animals. In experimental animals, liver/fat concentration ratios are not only different for different compounds, but they are dose dependent. As the dose of dioxins are increased, so is the liver/fat ratio (*95–98*).

Using the assumption that dioxins are equally distributed in the body lipid may underestimate the body burden of these chemicals due to chemical and dose-dependent sequestration in the liver. The magnitude of underestimation can be determined if several assumptions are used: that the liver/fat ratio for all dioxins is 15 and that liver is 10% of the body weight and is 10% lipid by weight. A liver/fat ratio of 15, as determined for the hexachlorodibenzofurans in humans, is used as a worst-case scenario for hepatic sequestration. Using these assumptions, the present estimate of dioxin TEQ body burdens in background populations will change from 13 to 21 ng TEQ/kg body weight. Hence, the assumption that dioxins are equally distributed in body lipid may slightly underestimate the body burdens of these chemicals, but the magnitude of error will be less than a factor of two. A better understanding of the pharmacokinetic properties for this class of compounds in humans is clearly indicated.

Chloracne has been described as the hallmark of dioxin toxicity in humans (*17*). Dioxin exposure in several animal species results in a chloracnegenic response and the body burdens which produce this response in animals are similar to the body burdens of dioxins in humans with chloracne. The chloracnegenic response has been thought to be a relatively high-dose phenomenon; however, the variation in human sensitivity to the chloracnegenic effects of TCDD is almost two orders of magnitude. For example, there are individuals who developed chloracne at body burdens approximately three times background (*51*). In contrast, there are subjects with body burdens of 1450 ng TEQ/kg body weight who have not developed chloracne (*51*). These data suggest that humans differ widely in sensitivity to the chloracnegenic actions of dioxins.

There are two points of caution when interpreting the chloracne data. First, human body burdens may not be an accurate measure of chloracnegenic potential if point-of-contact concentrations are important. For example, if dermal exposure results in a localized chloracnegenic response, body burdens estimated from serum or adipose tissue levels may not accurately reflect the concentration of dioxins at the site of effect. Also, the lack of chloracne in highly exposed patients does not necessarily indicate that these individuals are resistant to all the effects of dioxins. In mice, gene products, in addition to the Ah receptor, regulate the chloracnegenic response (*100*). It seems likely that multiple genetic factors may influence the relative susceptibility of individuals in a response-specific fashion.

Human responses to dioxins other than chloracne are not as obvious. In the Yu-Cheng poisoning incident, increased rates of toxic effects such as miscarriages, stillbirths, low birth weight infants, and developmental delays have been observed in offspring of women exposed to high levels of PCDFs and PCBs. However, it has been difficult to determine if the effects are due to the dioxins in the mixture, the non-dioxinlike PCBs, or to the combination of these chemicals. Researchers have tried to correlate effects with serum concentrations of either the PCDFs or PCBs (*56*). Birth weights were negatively correlated with PCDF levels in these individuals (*56*). Other effects such as induction of arylhydrocarbon hydroxylase activity, a marker for CYP1A1, were not correlated with either the polychlorinated dibenzofurans or the PCB concentrations, but decreased placental EGF receptor autophosphorylation was correlated with total PCB concentrations (*56*). However, due to the nature of the exposure, patients with high levels of dibenzofurans will likely have high levels of PCBs, making such correlations difficult to interpret. Also, the presence of dioxinlike and non-dioxinlike PCBs adds to the complexity of these correlations.

We compared the body burdens of dioxins in the Yu-Cheng population to body burdens in experimental animals to determine the role of dioxins in the toxic effects seen in these individuals. Women who were pregnant at the time of exposure or became pregnant thereafter had children with lower birth weights compared to unexposed women, and the decrease in size persisted years after birth (*63*). Body burdens in the Yu-Cheng mothers were estimated at 2130 ng TEQ/kg. In experimental animals the body burdens that result in decreased birth weights range from 400 to 2000 ng TCDD/kg, while decreased growth occurs in rats at 1,000 ng TCDD/kg. The similarities between the body burdens in animals and humans suggests that dioxins may play a role in the decreased birth weights.

The behavioral effects of dioxins have not been thoroughly studied in experimental animals. One study reported deficiencies in object learning in rhesus monkeys prenatally exposed to TCDD. Delayed developmental milestones were seen in children born to Yu-Cheng mothers, but the body burdens are approximately 51 times higher in humans than in the monkeys. There is recent evidence that some of the non-dioxinlike PCBs may have neurotoxic actions (*101*). The absence of studies in experimental animals examining the developmental behavioral toxicity of dioxins makes it difficult to assess the role of either the dioxins or the non-dioxinlike PCBs in the developmental effects of the children of the Yu-Cheng patients.

In experimental animals, some biochemical changes produced by dioxins occur at lower body burdens than do the toxic effects (*57–61,71*). Induction of CYP1A1 and decreased hepatic EGF receptor are two well-characterized biochemical responses to TCDD. Earlier studies comparing the induction of CYP1A1 and decreased EGF receptor in human placenta and rat liver suggested that humans may be more sensitive when compared on a tissue-dose basis (*18*). However, it is possible that the difference in sensitivity is not entirely due to species differences but due to altered tissue sensitivity. For example, induction of CYP1A1 is similar in lung, liver, and skin

of mice based on administered dose (*102*). In contrast, when the sensitivity of these tissues is compared on a tissue-dose basis, the lung is much more sensitive than the liver or skin (*102*). The present study indicates that humans and rats are equally sensitive to TCDD-induced biochemical changes when compared on a total body burden. Thus, when comparing the relative sensitivity of human or animal tissues to TCDD-induced biochemical changes, it may be more appropriate to compare body burdens than tissue concentrations. In addition, these data provide support for our approach.

TCDD is clearly carcinogenic in experimental animals. All species and both sexes of experimental animals that have been chronically exposed to TCDD exhibit a dose-dependent increased incidence of tumors (*5*). Several recent epidemiological studies have indicated an association between TCDD serum concentrations and increased incidence of tumors (*19–23*). Body burdens in rats and mice with increased tumors are comparable to the body burdens in the human cohorts that have increased incidence of tumors thought to be associated with dioxin exposure. Although these data are not conclusive, they are consistent with the hypothesis that exposure to TCDD was an important factor in the increased incidence of tumors in these cohorts. It is interesting to note that based on body burdens, mice are more sensitive to the carcinogenic effects of TCDD than are rats.

Carcinogenic responses are seen in hamsters, but the carcinogenic doses produce body burdens 46–1,300 times that seen either in humans, rats, or mice. Hamsters are insensitive to the lethal effects of dioxins, and they may also be less sensitive to the carcinogenic response. However, responses such as cancer are dose dependent as well as time dependent. Thus, the apparent differential sensitivity of the hamster may be due to differences in the dose–time regimens used in the hamster compared to the rat and mouse studies. It would be useful to compare these species under similar exposure protocols.

Decreases in serum testosterone have been reported in a National Institute of Occupational Safety and Health (NIOSH) cohort (*41*). There was a decrease in testosterone concentrations in individuals with serum concentrations of TCDD as low as 20 ppt at the time of tissue sampling, which is 3-4 times background TCDD levels and only a 33% increase over total average body burdens. Although the decrease in testosterone concentrations was statistically significant, the decrease was minor, and average levels were still within the normal range. In addition, a clear association between serum TCDD concentrations and effect was not readily apparent in the data (*41*). If differences in exposure patterns in the individuals are taken into account by back-calculating serum TCDD concentrations to the time of exposure, there is a clearer association between serum TCDD concentrations and lower testosterone concentrations. Here the lowest serum TCDD concentration associated with decreased testosterone concentration is 140 ppt (200 ppt TEQ). In experimental animals, high doses of TCDD decrease testosterone concentrations in rats at a body burden of 12,500 ng TCDD/kg body weight (*73*). These data suggest that some humans may be approximately 280 times more sensitive to the testosterone-decreasing effects of dioxins compared to rats. Alternatively, the decreased testosterone levels in the NIOSH cohort could be related to the concomitant exposure to other chemicals involved in the manufacturing process. Future studies examining the sensitivity of other species to the testosterone-decreasing effects of dioxins and epidemiological studies of other populations may provide additional information to adequately assess the association between dioxin exposure and decreased testosterone concentrations in some human populations.

Many of the effects of TCDD have been studied following an acute exposure in experimental animals. In contrast, humans receive low daily doses of these chemicals. One of the assumptions in extrapolating these effects to humans is that the effects are solely related to body burdens. For some of these endpoints, such as decreased testosterone, this assumption has not been adequately tested. Effects such as cancer are clearly related to both dose and time. It is possible that, in addition to dose and body burden, length of exposure may also have a significant effect on toxicity. Analysis of the area under the total body concentration–time curve may be a more appropriate marker for dose, and analysis of these data sets is ongoing.

The clinical significance of some of the endpoints studied is uncertain. Induction of CYP1A1 and CYP1A2 by TCDD are some of the most sensitive markers of dioxin exposure, yet their relevance to toxicity is unclear. Recent studies have suggested an association between PAH exposure and CYP1A1/1A2 induction for lung and colorectal cancer and atherelerosclerosis (*103–105*). However, these associations are speculative and not proven. At present, one could conclude that low doses of dioxins produce effects such as enzyme induction in experimental animals and that humans are exposed to levels of dioxins that induce CYP1A1/1A2 in experimental animals, but the relationship between these effects and disease are uncertain.

One of the most sensitive targets for TCDD toxicity in experimental animals is the immune system. Immune alterations, including increased viral sensitivity in mice and altered lymphocyte subsets in marmosets, have been reported at body burdens equivalent to human background exposures. However, the evidence for immunotoxicity of dioxins in humans is inconclusive. There are reports of subtle immune alterations in populations heavily exposed to dioxins. The incidence of intestinal and upper respiratory tract infections correlated with chloracne state and increased with increasing serum TCDD concentrations (*106*). One year after the Yu-Cheng poisoning episode, patients exhibited decreases in percentage of total T-cells, active T-cells, and T-helper cells, which recovered by the 3-year follow up study (*107*). Recent studies of occupationally exposed individuals with slightly elevated body burdens of approximately 72 ng TEQ/kg showed no alterations in lymphocyte subsets (*108*). However, in mice, a dose of TCDD that suppresses the antibody response to sheep red blood cells is not associated with alterations in lymphocyte subsets (*109*). Thus, immune function may be altered without altering lymphocyte subsets. Although some of these data suggest that the human immune system may be sensitive to the effects of dioxins, our present understanding of immunology does not support a conclusion that these alterations are or are not clinically significant.

The present study indicates that *in vitro* similar responses are seen in human and animal tissues after similar dioxin exposure. Human populations exposed to high concentrations of dioxins exhibit symptoms that are similar to the signs of toxicity seen in some experimental animals exposed to dioxins. These effects are seen at equivalent body burdens, strongly indicating that dioxins are responsible for some of these toxic effects in humans. For most of the toxic effects of dioxins, background exposure is well below those associated with overt toxicities. However, the background level used in this evaluation (13 ng TEQ/kg body weight) is an average background. Body burdens of dioxins appear to be log-normally distributed in humans (*110*), thus it would not be unusual to see populations with body burdens three to four standard deviations beyond the mean body burden. Recent studies in the Netherlands indicate that plasma TEQ concentrations in the 95th percentile of the

population are twice that of the mean (*113*), suggesting that at least 5% of the population has two times the mean body burden. In addition, there are subpopulations such as subsistence fishermen who are likely to have much greater body burdens. There are also some toxic effects, such as endometriosis and increased viral sensitivity, which occur in experimental animals at body burdens less than 10 times the average background exposures to humans. Finally, human exposures that result in adverse health effects, such as chloracne, decreased birth weights, developmental delays, and cancer are 3–540 times the present average background exposure to these chemicals. Nevertheless, the available data indicate that high-level human exposure to dioxins produce adverse health effects and that humans are a sensitive species to the toxic effects of dioxins. Whether these low-dose effects are occurring in the general population or the more highly exposed subpopulations remains to be determined.

Appendix. Table Notes

(Some notes appear in more than one table.)

Table 1

a) Apparent equilibrium binding dissociation constants are presented (*42*). Under conditions of infinite dilution, an apparent K_d of 9 pM has been determined for the Ah^b allele in the C57Bl/6 mice; this value is close to the estimated true K_d (*43*).

b) Splenic lymphocytes from C57Bl/6 mice and peripheral blood lymphocytes were isolated, cultured, and exposed to TCDD. Ethoxyresorufin-O-deethylase (EROD) activity, a marker for CYP1A1, was determined following TCDD exposure (*43*).

c) The authors (*47*) compared the cytotoxic effects of TCDD on organ culture of human, mouse and rat embryonic palatal shelves. Embryonic palates from human, mouse and rat were grown in the same organ culture system and exposed to TCDD. Cytotoxicity was detected using transmission electron microscopy.

d) Thymocytes were isolated from either murine or human sources and cocultured with either murine (*48*) or human (*49*) thymic epithelium culture. The incorporation of tritiated thymidine into DNA was determined in cells treated with TCDD following antigen stimulation.

e) Human tonsilar lymphocytes and murine splenic lymphocytes were used as a source of B-cells. Human and murine B-cells were grown under identical conditions and exposed to TCDD. Proliferation and IgM secretion were determined in response to different concentrations of TCDD ranging from 0.3 to 30 nM (*50*).

Table 2

f) The lower value, 96 ng TEQ/kg body weight, is the body burden estimate of a patient with the lowest reported adipose dioxin concentration for any patient with chloracne (*51*). This individual was exposed to a mixture of CDDs and CDFs in 1969 and developed chloracne. At the time of exposure this individual had adipose tissue CDD/CDF concentrations of 419 ng TEQ/kg adipose tissue (*51*). An additional 17 ng TEQ was added to this value to include the PCBs. The values of dioxins at the time of exposure were estimated by the authors (*51*). The higher of the two values represents the average body burden of dioxins (TEQs) in individuals from Yusho with chloracne (*52*). Estimates of body burdens from these individuals were determined by Ryan et al. (*52*).

g) Rhesus monkeys were administered 1 µg/kg TCDD, and it is assumed that essentially no TCDD was eliminated when the animal developed a chloracnegenic response. This is a LOEL dose; no lower doses were tested (*53*).

h) Assumes the rabbit and the rat have the same rate of elimination, a half-life of 23.7 days (*88*) and that the rabbits weighed 2.5 kg throughout the experiment. This is a LOEL dose; no lower doses were tested (*52*).

i) Assumes the half-life of TCDD in mice is 11 days and that the mice weigh 25 g. This is a LOEL dose; no lower doses were administered (*5*).

j) In highly exposed patients from the Yu-Cheng incident, there is a decrease in birth weights of children born from these patients compared to unexposed control populations (*18,56*). In addition, the Yu-Cheng mothers have altered levels of placental epidermal growth factor receptor (EGFR) and CYP1A1. The data indicate that the changes in placental EGFR and CYP1A1 in these patients were maximal. Body burdens determined based on levels of 2,3,4,7,8-pentachloro-dibenzofuran (TEF = 0.5) and 1,2,3,4,7,8-hexachlorodibenzofuran (TEF = 0.1) in placenta tissue. Lipid content of the placenta is estimated at 1% (*112*) and the average percent body fat of a women is assumed to be 22%. These body burden estimates were also used as body burdens of Yu-Cheng mothers whose children demonstrate decreased growth (*63*) and delayed developmental milestones (*64,65*).

k) In a rat liver tumor promotion study, rats initiated with diethylnitrosamine were exposed to doses of TCDD from 3.5 to 125 ng/kg/day. Statistically significant increases in numbers of altered hepatic foci were observed in rats treated with 125 ng TCDD/kg/day (*67*). At the end of the study, liver concentrations of TCDD were approximately 20 ppb (*60*); assumes 20% body weight is adipose tissue and that at this dose, the liver has three times the concentration of TCDD than adipose tissue. Body and liver weights were reported (*67*) for these animals. The body burden calculation assumes that liver and fat account for 85% of the body burden in these animals. For tumor promotion, 125 ng TCDD/kg/day is the LOEL and 35 ng TCDD/kg/day is the NOEL for tumor promotion (*67*). For induction of CYP1A1 (*60*) and downregulation of EGFR (*59*), 125 ng TCDD/kg/day was assumed to produce a maximal response.

l) Mice were administered 10 µg TCDD/kg and sacrificed 7 days after treatment. EGFR binding was determined in hepatic plasma membrane (*58*).

m) Animals received a single dose and were sacrificed 24 hr later. Assumes no TCDD eliminated at this time. CYP1A1 induction determined by RT-polymerase chain reaction (*60*). The LOEL for CYP1A1 induction was 1 ng/kg, a no observed effect level from this study is 0.1 ng/kg.

n) Animals received 1.5 ng/kg/day 5 day/week for 13 weeks (*61*). Mice were sacrificed 3 days after last dose. Hepatic, dermal, and pulmonary EROD activity were significantly induced at this dose. Tissue concentrations of TCDD were measured in liver, skin, and fat. Body burden estimates assumes 95% of the body burden is in liver, skin, and fat. This is the LOEL from this study; no lower doses were tested.

o) Body burdens are estimated by authors (*62*) for the increased accumulation of PCDD/PCDF in liver compared to adipose tissue using a pharmacokinetic model.

p) Assumes average level of dioxins and dibenzofurans in human serum ranges from 28 to 41 TEQ ppt and from 8 to 17 TEQ ppt for the PCBs. Thus, the average TEQ ranges from 36 to 58 TEQ ppt. Using 58 ppt as the average concentration of PCDDs, PCDFs, and PCBs in serum, a body burden of 12.76 ng TEQ/kg body weight was calculated.

For PCDD and PCDF concentrations, a body burden of 9 ng TEQ was determined. Average concentrations of TCDD in adipose tissue are 5 ppt (lipid adjusted) (*27*), resulting in a body burden of 1.1 ng TCDD/kg.

q) In control rats, PCDDs and PCDFs were determined at different ages; 200-day-old rats had approximately 78 ppt TEQs in liver (*67*). This is an equivalent liver concentration in 60-day-old rats 24 hr after administration of 1 ng TCDD/kg.

r) Liver, fat, blood, and skin concentrations of TCDD, 1,2,3,7,8-PCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PCDF, 2,3,4,7,8-PCDF, and OCDF were determined in 150-day-old female $B6C3F_1$ mice. The TEF methodology was used to estimate TEQ levels in these animals; assumes that 95% of the body burden is in liver, fat, and skin.

Table 3

s) Estimated highest body burden at time of last exposure. Calculations based on measured TCDD levels in serum (lipid adjusted) and assuming a first-order elimination kinetics and a half-life for elimination of 7.1 years. Also assumes a body weight of 70 kg and 22% body fat. Calculations for estimated serum concentrations at last time of exposure performed by authors (*18,23*).

t) Animals administered 100 µg TCDD/kg 6 times every 4 weeks over a 24-week period; assumes a half-life of 14.9 days (*111*). Body burdens are estimated immediately after the last treatment with TCDD. The administration of 50 µg TCDD/kg 6 times every 4 weeks over a 24-week period did not increase the incidence of any types of tumors in 10 hamsters (*68*).

u) Assumes a single first-order elimination rate constant and a half-life for the whole body elimination of 23.7 days (*85*) and a gastrointestinal tract absorption of 86% (*85*). Increased incidence of hepatocellular carcinomas were observed at 100 ng/kg/day and 10 ng/kg/day is the NOEL (*69*). Decreased testis weight and testosterone concentrations were observed after 12.5 ng TCDD/kg 7 days later (*76*). Decreased serum glucose levels were observed in rats treated with 100 ng/kg/day for 30 days (*79*).

v) Assumes an apparent half-life of 11 days and a body weight of 20 g. Mice receiving 71.4 ng/kg/day for 2 years had a statistically significant increase in hepatocellular carcinomas (*70*).

w) Assumes neonatal rats and hamsters are exposed to an equal dose of TCDD as are the dams on a weight basis and assumes all alterations are due to the neonatal exposure. For decreased body weight in pups 400 ng/kg is the LOEL; a dose of 64 ng/kg to the dam was the NOEL for this response (*73*). For decreased sperm count the LOEL is 64 ng/kg and no lower doses were tested (*86*). In hamsters only one dose was tested (2000 ng/kg) for decreased sperm counts (*74*). Decreased growth in rats is indicated by decreased body weights up to postnatal day 63 (*75*). The incidence of fetal mortality was increased in hamsters at a dose of 18 µg/kg but not at a dose of 6 µg/kg (*81*).

x) Assumes a single first-order elimination rate constant and a half-life for the whole-body elimination of 400 days (*81*) and a gastrointestinal absorption of 86% (*88*). This is the LOEL from this study; no lower doses tested. Monkeys exposed to a diet of approximately 5 ppt had a daily intake of 0.151 ng/kg/day. Monkeys exposed to approximately 25 ppt in the diet had a daily intake of approximately 0.76 ng/kg/day. For animals with decreased object learning, the TCDD-exposed offspring were born after 16.2 months of maternal TCDD exposure of a diet of 0.151 ng TCDD/kg/day. Animals with increased incidence and severity of endometriosis had a daily intake of 0.151 ng/kg/day for 4 years, and body burdens were determined at the end of the exposure period. Monkeys exposed to 0.76 ng TCDD/kg/day for 16.2 months had significant decreases in offspring viability.

y) The authors extrapolated serum concentrations of TCDD at the time of sampling to initial exposures (*41*). Workers with serum TCDD concentrations of 140–496 ng/kg (lipid adjusted) have a greater incidence of low testosterone concentrations (*41*). Extrapolation assumed a half-life for TCDD of 7.1 years. To estimate body burdens in these workers, it was assumed that the background TEQ was 60 ng/kg, thus the total serum TEQ was 140 ng TCDD/kg + 60 ng TEQ/kg = 200 ng TEQ/kg (lipid adjusted).

z) Assumes that high-exposed group (>33 ng/kg) had a background of 60 TEQ ng/kg. This group had at least 93 TEQ ng/kg. Assumes average subject was male, weighing 70 kg with 22% body fat.

aa) Workers with increased glucose tolerance and diabetes have serum levels of 640 ppt TEQ (*24*).

bb) Guinea pigs received 30 ng TCDD/kg intraperitoneally and sacrificed 24 hr after dose. Assumes that no TCDD was eliminated at this time. This is a LOEL, no other doses tested (*78*).

Table 4

cc) Assuming a single first-order elimination rate constant and a half-life of 6–8 weeks. Body burdens calculated by authors (*82*). Animals treated with a single dose of TCDD were tested 2 weeks after treatment (*83*).

dd) Mice were treated with TCDD and challenged with influenza virus 7 days later (*84*).

ee) Mice were administered 100 µg/kg and examined 30 days after receiving the treatment (*77*).

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Electric and Magnetic Field Exposures for People Living near a 735-Kilovolt Power Line

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The purpose of this study was to assess the effect of a 735-kV transmission line on the electric and magnetic field exposures of people living at the edge of the line's right of way. Exposure of 18 adults, mostly white-collar workers, living in different bungalows located 190–240 feet from the line (exposed subjects) was compared to that of 17 adults living in similar residences far away from any transmission line. Each subject carried a Positron meter for 24 hr during 1 workday, which measured 60-Hz electric and magnetic fields every minute. All measurements were carried out in parellel for exposed and unexposed subjects during the same weeks between September and December. During measurements the average loading on the line varied between 600 and 1100 A. The average magnetic field intensity while at home was 4.4 times higher among exposed subjects than unexposed (7.1 versus 1.6 mG, $p = 0.0001$) and 6.2 times higher when considering only the sleeping period (6.8 versus 1.1 mG, $p = 0.0001$). Based on the 24-hr measurement, average magnetic field exposure was three times higher among the exposed. Electric field intensity was also higher among the exposed while at home (26.3 versus 14.0 V/m, $p = 0.03$). Magnetic field intensity among the exposed was positively correlated with the loading on the line ($r = 0.8$, $p = 0.001$). Percentage of time above a magnetic field threshold (2 mG or 7.8 mG) was a good indicator to distinguish the two types of exposure. Percentage of time above 20 V/m was significantly different, but percentage of time above 78 V/m was rare and comparable for the two groups. Variability of exposure was very low. This study demonstrates that a 735-kV line contributes significantly to residential 60-Hz magnetic field exposure and, to a lesser extent, electric fields for people living at the edge of the right of way. Because of the limited size of our sample, caution is recommended before generalizing these results. Nevertheless, due to the uncertainty on the risks associated with such an unusual high residential exposure, research is needed on its possible effects. *Key words:* electrical fields, electromagnetic fields, high-voltage power lines, magnetic fields. *Environ Health Perspect* 103:832–837 (1995)

The potential health effects of power frequency electric and magnetic fields (EMF) have been and are still the subject of numerous studies (*1–5*). The risk of cancer is under particular scrutiny, especially for workers highly exposed during their work, and for people living in the vicinity of distribution and transmission lines (*6,7*). Although transmission lines are a well-known source of residential exposure to magnetic fields, few studies have been done on the personal exposure of people residing close to these lines (*8,9*). Several epidemiological studies have considered fields produced by the distribution lines, but few have measured the specific exposure from transmission lines. Feychting and Ahlbom (*10,11*) performed spot measurements inside houses but could not predict the specific impact of the lines on personal exposure. McMahan et al. (*12*) performed only spot measurements at the entrance of houses and found that those living along the easement of 220-kV lines had much higher-than-average magnetic field levels than those living one block away. Kavet et al. (*13*) published the first study using personal exposure measurement to assess magnetic field exposure from high-level power lines. They found that people living close to 345-kV power lines had higher at-home exposures, and higher average 24-hr exposures than people living far away, but no attempt was made to separate the exposure during sleep from that during residential daytime activities. Morever, work exposure was not specifically assessed and few people were evaluated.

Magnetic fields emitted from power lines have received great attention because of their capacity to penetrate structures, but electric fields emitted from such lines have not been emphasized because they are usually shielded by buildings. Nevertheless, some reports found higher levels of electric fields in houses located near a high-voltage line (*8*). In fact, some people perceive transmission lines as one of the major environmental threats, and transmission lines are the focus of nearly all litigation on EMFs (*14,15*). Thus, a better understanding of their role in determining exposure of nearby residents is useful.

The objectives of the present study were to assess the impact of a 735-kV power line on the 60-Hz electric and magnetic fields exposure of working people living close to the line. Specifically, we examined the effect of the line on exposure during the time spent at home (wake and sleep periods) and on total exposure over a 24-hr period. We compared residential and occupational exposures and evaluated the use of the simplified version of the Wertheimer-Leeper wire coding scheme, developed by Kaune and Savitz (*16*), for this type of residential exposure to EMFs.

Methods

We identified single-floor bungalows located at the edge of a 735-kV line crossing a suburb of Québec City by visiting the study area. Another part of the suburb was selected as the unexposed area because of the presence of similar homes with identical exterior characteristics but without a transmission line nearby. In all, 63 exposed residences, located less than 250 feet from the transmission line, were registered, as well as 141 homes located more than 1200 feet from the line (unexposed group). All exposed residences and a random sample of the 115 unexposed residences were selected. We then dropped a letter in the mailbox of these residences inviting the occupants to participate in the study. Residents were then called by telephone to evaluate their interest in participating, and to check for the following selection criteria: occupants had to own the house, and participants had to work at least 4 hours per day away from their home. If more than one person was eligible per house, the participant was chosen at random using the following criteria: equal representation of both genders was desired and 50% of all participants had to be under 40 years of age. Twenty exposed and 20 unexposed persons who fulfilled our criteria and elected to participate were finally selected for the study. We had to exclude after the measurements two exposed and three unexposed persons because they had

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worked at home during the measurement periods. The final group included 18 exposed and 17 unexposed participants.

A technician visited each participant at home to explain how to wear the meter. Personal exposure to 60-Hz electric and magnetic fields was measured using six Positron model (Montréal) 378108 personal exposure monitors. This meter measures the three components of the magnetic and the electric field perpendicular to the body surface, at a specific rate, and stores measurements in memory (*17,18*). Meters were worn in a pocket, and measurements were done every minute. During sleep, subjects were asked to place the instrument close to their bed but far away from any electric outlet or electrical device. Exposure assessment was done for 24 hr during 1 workday. The six meters were randomly assigned to exposed and unexposed subjects. An equal number of exposed and unexposed subjects was assessed every week from September to mid-December 1993. Subjects were asked to fill out a log sheet of their main activities during the exposure period to separate the 24-hr period in three subperiods: the at-home period, the at-work period, and the period away from home and work. The at-home period, was then subdivided into waking and sleeping periods.

Meters were calibrated from the start and quality control procedures were ensured during the study. Accuracy of magnetic field measurements were assessed with different levels of emitted magnetic fields.

The data stored in the meters were copied to a microcomputer and arithmetic means of the electric and magnetic field measurements were calculated for each time period and for each subject. We then calculated geometric means of the arithmetic means to compare exposure levels between the two groups of subjects. All statistical analyses were done using the SAS system (*19*). Fisher's exact test was used to compare proportions, and Student's *t*-tests were used to compare the geometric means between the exposed and unexposed groups. We calculated 95% confidence intervals for the geometric means. Percentage of time above specific thresholds for electric and magnetic fields were calculated according to Armstrong et al. (*20*). Variability of exposure was estimated using the jagged metric proposed by Wenzl et al. (*21*).

Results

Characteristics of the participants are presented in Table 1. Exposed and unexposed subjects had similar characteristics. Age and gender were comparable by design, and there was also good comparability for the time spent at home and the time spent at work. Most of the participants were white-collar workers and only two in each group were potentially exposed at work based on recent occupational exposure data (*6,22*).

Characteristics of the residences of the participants are presented in Table 2. Age, purchase value, electrical heating, and roof characteristics were comparable between the two groups. Exterior metal covering was more frequent in the unexposed group (6/17 versus 1/18), and duration of ownership was shorter in the exposed group (13 years versus 20 years). The minimum temperature during the days of measurement was comparable between the two groups: average minimum of -4.3°C with a range of -13.6–0.6°C for the exposed, and -2.4°C with a range of -10.3–2.1°C for the unexposed.

Results of magnetic field measurements are presented in Table 3. Exposed and unexposed subjects had similar average magnetic field exposures for the at-work (1.1 versus 1.2 mG) and the away periods (1.8 versus 1.7 mG). In contrast, the average at-home exposure was significantly higher for exposed than for unexposed subjects (7.1 versus 1.6 mG, $p = 0.0001$). This difference was still statistically significant during the 24-hr average (4.9 versus 1.7 mG). The range of values at home for exposed subjects was 4.6–11.4 mG, whereas it was 0.4–3.8 mG for unexposed subjects. Average magnetic field exposure was higher during the waking period: 7.5 mG for the exposed versus 2.2 mG for the unexposed. However, the difference between exposed and unexposed subjects was higher during the sleeping period: average magnetic field of 6.8 mG for exposed and 1.1 mG for unexposed. In summary, the average at-home magnetic field exposure was 4.4 times higher for the exposed subjects. Based on the average 24-hr measurement, magnetic field exposure was 2.9 times higher in the exposed group than in the unexposed group. These values were not different when considering only residences without metal covering.

Electric field exposure was quite similar between exposed and unexposed subjects during the at-work and away periods. In contrast, the at-home exposure was 1.9 times higher for the exposed subjects (26.3 versus 14.0 V/m, $p = 0.03$; Table 4). The difference between exposed and unexposed subjects was maximum during the sleeping period, when electric fields were 2.8 times higher for exposed ($p = 0.03$). These differences were slightly lower when considering only residences without metal coverage: the ratio of geometric means of exposed to unexposed fell from 2.8 to 2.0 for the sleep period and from 1.9 to 1.7 for the total at-home periods.

Exposed residences were located between 190 and 240 feet from the middle of the power line, but no correlation was found between magnetic field measurements and these short distances from the line. Hourly loadings on the line were obtained for the period of measurements. Average loading of the current in the line during the at-home period was positively correlated with the magnetic field measurements (Fig. 1).

Table 1. Characteristics of exposed and unexposed subjects

	Exposed ($n = 18$)	Unexposed ($n = 17$)	p
Mean age (years)	42.5[a]	43.2	0.86
Female (%)	55.6	64.7	0.73
Mean time at home (hr)	14.4	14.2	0.81
Mean time at work (hr)	6.8	7.0	0.77
Possibly exposed at work (%)	11.1	11.8	1.00

[a]One missing value.

Table 2. Characteristics of exposed and unexposed residences

	Exposed $n = 18$	Unexposed $n = 17$	p
Mean age (years)	33.5[a]	31.3	0.21
Electrical heating (%)	55.6	64.7	0.73
Metal roof (%)	0.0	5.9	0.49
Metal exterior coverage (%)	5.6	35.3	0.04
Mean purchase value (×$1000)	95.1	103.1	0.20
Mean (years) duration of the ownership	13.0	20.1	0.04

[a]One missing value.

Table 3. Personal exposure to 60-Hz magnetic field (mG), geometric mean

Period of exposure	Group		Ratio[a]	p
	Exposed	Unexposed		
At work	1.1 (0.7–1.6)[b]	1.2 (0.8–1.7)	0.9	0.65
Away	1.8 (1.3–2.5)	1.7 (1.2–2.4)	1.1	0.83
At home				
Awake	7.5 (6.5–8.6)	2.2 (1.6–2.9)	3.4	0.0001
Asleep	6.8 (5.9–7.8)	1.1 (0.8–1.6)	6.2	0.0001
Total	7.1 (6.3–8.0)	1.6 (1.2–2.2)	4.4	0.0001
24 hr	4.9 (4.3–5.5)	1.7 (1.3–2.2)	2.9	0.0001

[a]Ratio of means of the exposed group over the unexposed group.
[b]95% confidence intervals in parentheses.

Electrical wires around the residences were coded according to the modified Wertheimer-Leeper method proposed by Kaune and Savitz (*16*). This coding considers transmission lines only if they are located less than 150 feet from a residence, but it can be used to assess the characteristics of the distribution system for the two groups. Among the exposed subjects 6 residences were coded high, 6 medium, and 6 low, but this was not the case for the unexposed subjects, where 16 residences were coded medium and 1 high. However, no difference was found among the exposed subjects for the average magnetic and electric fields at-home measurements between high, medium, and low categories of the modified Wertheimer-Leeper coding, as shown in Table 5.

Following Armstrong and colleagues (*20*), we calculated the percentage of time above a lower threshold (>20 V/m, >2 mG) and a higher threshold (>78 V/m, >7.8 mG) for the two groups. Results, shown in Table 6, demonstrate that the exposed subjects spent longer periods of time than the unexposed at levels above these thresholds. Especially during the at-home period, more than 50% of the exposed subjects spent at least 60% of their time above 20 V/m and 99% of their time above 2 mG. The higher threshold for magnetic fields (7.8 mG) was also exceeded by more than 50% of the exposed subjects at least 26% of the time. The proportion of time above these thresholds during the 24-hr period is still higher for the exposed subjects than the unexposed.

Exposure variability was assessed using the jagged metric proposed by Wenzl et al. (*21*) because the biologically active component of these fields may be their moment-to-moment variability. Because the Positron meter stores field measurements into 16 logarithmically scaled bins, with an interbin ratio of two (*18*), we modified the proposed Wenzl metric as follows: percentage of adjacent minutes with exposure measurements differing by at least one bin and with fields above 5 V/m or 2 mG. Values of this index of variability among exposed and unexposed subjects for the at-home period, subdivided into waking and sleeping periods, are presented in Table 7. Variability as measured by the jagged metric is low and mainly present for the electric field during the waking period. No significant difference was found between the two groups.

Discussion

Exposure to 60-Hz electric and magnetic fields is prevalent in modern societies, but few characterizations of personal exposure of the general population to these fields have been carried out. Much of the focus has been on assessing high exposure at workplaces, especially in electrical utilities (*8,9,17,22,23*). Because residential exposure to 60-Hz electric and magnetic fields can be influenced by the use of electrical appliances inside the house, our research protocol was designed to separate exposure during waking and sleeping periods. As 24-hr exposure might be influenced significantly by occupational exposure, our methodology seeked to assess the contribution of occupational exposure separately. Efforts were also made to have two groups

Table 4. Personal exposure to 60-Hz electric field (V/m), geometric mean

Period of exposure	Group		Ratio[a]	*p*
	Exposed	Unexposed		
At work	5.0 (3.2–8.0)[b]	4.1 (2.5–6.7)	1.2	0.51
Away	4.9 (3.0–7.9)	6.0 (3.3–10.9)	0.8	0.56
At home				
Awake	19.8 (15.6–25.2)	13.2 (9.8–17.7)	1.5	0.03
Asleep	27.3 (16.3–45.7)	9.9 (4.6–21.4)	2.8	0.03
Total	26.3 (18.4–37.6)	14.0 (8.9–22.1)	1.9	0.03
24 hr	18.9 (13.7–26.1)	11.4 (7.5–17.4)	1.7	0.05

[a]Ratio of means of the exposed group over the unexposed group.
[b]95% confidence intervals in parentheses.

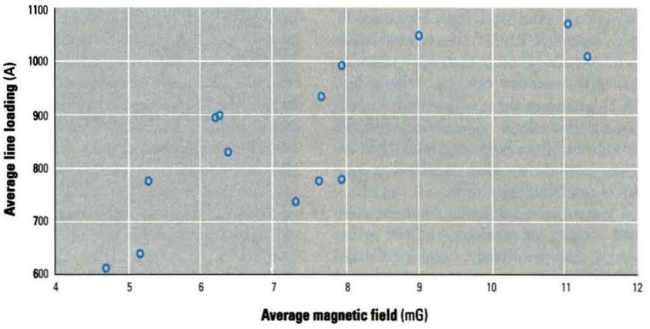

Figure 1. Correlation between the average magnetic field personal exposure at home and the average current loading in the power line during the measurement period for the exposed group ($n = 14$); $r = 0.80$; $p = 0.001$.

Table 5. Geometric means of intensity of electric and magnetic fields for the exposed group during at-home periods according to the coding of the distribution system[a]

	High	Medium	Low
Electric field (V/m)	27.7 (11.1–85.0)[b]	23.4 (6.4–79.8)	28.1 (10.5–71.0)
Magnetic field (mG)	7.5 (5.2–11.4)	6.2 (4.6–7.6)	7.8 (5.2–11.1)

[a]Modified Wertheimer-Leeper coding (*16*).
[b]Ranges in parentheses.

Table 6. Percentage of time above lower and higher thresholds and peak values for electric and magnetic fields during the at-home period

		Electric field			Magnetic field		
		Median	Min	Max	Median	Min	Max
% of time above lower threshold (>20 V/m, >2.0 mG)	Exposed	60.3	5.7	83.9	99.3	71.5	100.0
	Unexposed	13.2	1.6	98.1	17.7	0.6	85.3
% of time above higher threshold (>78 V/m, >7.8 mG)	Exposed	1.1	0.1	62.5	26.2	0.2	90.5
	Unexposed	0.4	0.0	58.4	0.4	0.0	8.2
Peak (V/m, mG), range of the values of the bin	Exposed	156.3–312.5	78.1–156.3	5,000–10,000	15.6–31.3	7.8–15.6	250.0–500.0
	Unexposed	78.1–156.3	19.5–39.1	312.5–625.0	7.8–15.6	3.9–7.8	125.0–250.0

of residences and two groups of subjects with similar characteristics, with the exception of exposure to the transmission line. The distribution system was not comparable for the two groups, but this did not lead to a bias because the distribution system appeared to have no influence on the fields measured inside the houses of exposed subjects. Exterior metal covering of homes was more common in the unexposed subjects but this was taken into account in the analysis and did not substantially affect our findings. Conditions of measurements were similar between the two groups, particularly for the minimum outdoor temperatures during the measurement periods.

Our results demonstrate that such a 735-kV line is a significant contributor to residential 60-Hz electric and magnetic field exposures for people living in residences located at the edge of the right of way (i.e., 190–240 feet from the line). The influence of the magnetic field exposure was considerable because the at-home measurement of the exposed group was 4 times higher, and the 24 hr exposure was 3 times higher than that of the unexposed subjects. The magnetic field exposure at home of the unexposed group (1.7 mG) was slightly higher than the usual residential exposure (≤1 mG) measured in the United States (*8*). The at-home magnetic field exposure for the exposed group was much higher than measured by Kavet et al. (*13*), who found a mean of 3.2 mG for personal exposure of five people living close to a 345-kV line. Our measurements were also higher than those of McMahan and colleagues (*12*), who found mean magnetic fields of 4.8 mG at the front door of 76 houses located at the edge of two 220-kV lines and two 66-kV lines. At-home average magnetic field exposure of exposed subjects was, in fact, in the range of the mean occupational exposure of many electrical workers (*17,22,23*).

Magnetic field exposure for the exposed group was only slightly lower during sleeping periods than during waking periods. This is in contrast with the unexposed group, for whom exposure during sleeping periods was half that of the waking period. These data support the hypothesis that an important part of the magnetic field exposure among unexposed subjects is related to activities during the waking period (directly by using electrical appliances or indirectly from distribution lines). For the exposed group however, exposure from the transmission line dominated during the waking and sleeping periods. At-home magnetic field exposure for the exposed subjects was also highly correlated with the loading of the current in the line. This was not surprising because magnetic fields are directly produced by the current in the line (*9*). The lack of correlation between the magnetic field exposure in the homes and the distance of the houses from the line could be explained by the low range of distances separating the exposed houses from the line (190–240 feet). Electric field exposures during the at-home period were also higher for the exposed subjects compared to unexposed. Although the at-work and away periods were comparable for the two groups, the average electric field exposure during the at-home period for exposed subjects was twice that of unexposed subjects. This was only slightly reduced when considering only houses without exterior metal covering. As stated earlier, our mean electric fields values for the exposed group are in the range of the mean occupational exposure of many electrical workers (*17,22*). There is no reason to believe that such a difference could be due to uncontrolled factors. Exposure in the yard near the home could be different between exposed and unexposed subjects, but this should not be the case here given the study season. Morever, this possibility could not explain the difference between our two groups during the sleeping period. Because of the comparability between our two groups for subject characteristics and residences, there is no reason to believe that the difference observed could be due to varied use of electrical appliances. In fact, the causal role of the transmission line seems possible, but this has not been investigated much previously (*8*).

The influence of the at-home exposure from the line for the 24-hr average exposure is not surprising because most of the subjects spent more than 60% of their time in their homes. Few studies have considered the effect of a specific source on the 24-hr exposure of people. Kavet et al. (*13*) found some persistent effect of the at-home magnetic field exposure on the 24-hr exposure, but few subjects were considered and no details were given on the time spent at home by their subjects. Deadman et al. (*17*) studied the overall weekly time-weighted (TWA) average for a pilot group of utility workers. The geometric mean of magnetic field exposure (weekly TWA) was 6 mG for the exposed workers compared to 1.7 mG for unexposed workers (*17*), which is comparable to our 24-hr results.

Sussman (*8*) and Kaune (*9*) recently argued that average exposure might be similar for completely different profiles of exposure. Accordingly, we studied other indices of exposure. The distribution of percentage of time above a certain threshold between the two groups was compared as in the Armstrong et al. study (*20*). The median percentage of time spent above 2 mG at home was 99.3% in the exposed compared to 17.7% in the unexposed. The median percentage of time above 7.8 mG was 26.2% in the exposed group compared to 0.4% in the unexposed. These two thresholds seem to distinguish quite clearly the exposed from the unexposed. In regard to electric fields, only the percentage of time above 20 V/m at home was significantly different: median 60.3% in the exposed group compared to 13.2% in the unexposed group. Percentage of time above 78 V/m was rare in the two groups. The magnetic field exposure according to these criteria was comparable to that of many electrical utility workers and even higher than the utility workers studied by Armstrong et al. (*20*). In contrast, electric field exposure was lower; especially peak exposure and percentage of time above 78 V/m are much higher among electrical utility workers.

Using the modified Wenzl metric showed little variability for magnetic fields. Only a small variation was found among unexposed subjects during the waking period. The variability was more frequent for the electric field but only during the waking period and with the same intensity for the exposed and unexposed. The cause of this variability is possibly related to short and intense electric field exposures from electrical appliances (*8,9*), but we also have

Table 7. Percentage of adjacent minutes with field measurements differing by at least one bin during the at-home period[a]

		Electric field			Magnetic field		
		Median	Min	Max	Median	Min	Max
Total home period	Exposed	3.9	0.7	17.3	0.1	0.0	1.8
	Unexposed	2.9	0.1	9.9	1.5	0.2	4.5
Sleeping period	Exposed	0.2	0.0	23.9	0.0	0.0	0.2
	Unexposed	0.2	0.0	11.4	0.0	0.0	4.1
Waking period	Exposed	7.6	2.6	12.8	0.4	0.0	3.1
	Unexposed	6.7	0.3	10.3	2.1	0.6	13.3

[a]With electric field above 5 V/m and magnetic field above 2 mG.

to consider that variability during the waking period is expected due to body movements which modified the electric field measurements (*18*).

Our measurements were made at the beginning of the winter when the average loading on the line varied between 600 and 1100 A. It is likely that exposure could be higher during the colder days of the winter, but from the line-loading data obtained during a year, the mean loading during the measurement period is representative of the range of daily means during a complete year.

We used a modified version of the Wertheimer-Leeper (W-L) coding because it is simpler to apply than the original W-L coding scheme and has shown good validity in classifying residences according to their magnetic fields (*16*). However, the coding scheme disregards transmission lines farther away than 150 feet, and in our sample of homes it failed to distinguish the exposed and unexposed homes. This would suggest that the 150-feet cutoff is not appropriate for a 735-kV transmission line. Indeed, magnetic field levels of about 6 mG can be measured at a distance of 230 feet from a 735-kV line. Thus, in our study the coding scheme characterized homes only by the distribution lines. An interesting pattern was observed: all three categories (high, low, medium) were found among our exposed homes, whereas most of the unexposed residences were classified into the medium category, due to a three-phase primary line passing within 150 feet. As houses were selected randomly however, we can think of no explanation, other than chance, for this pattern, and it does not appear to affect our findings.

Exterior metal siding was more common among the unexposed homes. Residences were quite comparable for their age and purchase value, but renovation may have been more frequent in the unexposed, since average duration of ownership was slightly longer among the unexposed. This did not, however, alter the results; the difference in electric field exposure remained substantial even when only houses without metal covering were considered.

The field intensities measured, while higher among the exposed than the unexposed, are still far from the maximum acceptable exposures for the general public of 5000 V/m (unperturbed root mean square) and 1000 mG, proposed by the International Radiation Protection Association (*24,25*). These guidelines are based on the effects of currents induced in the human body by acute exposures, with safety factors applied to the "low observed effect levels" to account for uncertainty in the estimates of risk for long-term exposure.

The risk of long-term exposure to elevated power-frequency magnetic fields, such as those observed here, is possible, but not proven (*3,6,26,27*). It is clear, though, that the exposures seen here are markedly higher than those found in most epidemiological studies of residential exposures, in which excess risks of certain cancers have been observed. Such exposure is present during at-home periods, especially for magnetic fields at night. This sleeping time is essential for the biological rhythm of the human body and some laboratory research on animals has found effects of such fields on the secretion of the pineal gland (*28*).

Nevertheless, there is no clear biologically established exposure metric that can be used to evaluate an internal effective dose. Recent experimental studies indicate that repeated short-term exposures could be more hazardous than constant exposure (*29,30*). Therefore, a cumulative exposure or a TWA may not be the most appropriate measure of exposure. The jagged metric we used here showed little variability of exposure from the line, but this may not be the appropriate measure of effective dose. Percentage of time above 2 mG or 7.8 mG was remarkably different between the two groups, but there is no biological basis to use such levels as thresholds. Finally, we have to consider that these results were generated by the study of only a limited number of subjects and that one has to be cautious before generalizing such findings to other populations.

Conclusion

Exposure to 60-Hz magnetic fields (and to a lesser extent electric fields) from a 735-kV power line appears to be significant for people near it. Mean levels of magnetic fields as well as percentage of time above 2 mG and 7.8 mG are considerably more important for the people living near a line. These levels are in the range of the occupational exposure of many workers. However, some characteristics of the exposure are different: peaks in electric fields were much lower, and the exposure appears more constant. Furthermore, in contrast to occupational exposures that cease or diminish after work, residents near transmission lines are continously exposed while at home and expecially during the night. Although it is unclear if these characteristics imply a lower risk, it appears that such exposures are unusually high for residential areas. It is not currently possible to estimate precisely any risk (*26*). Because few people live close to such a line, the possible attributable risk from this exposure in the general population is probably low, but the relative risk for the few exposed people might be important. Prudence is suggested in generalizing these results to other populations because of the relatively small sample size, but our results indicate the need to study the possible risk associated with such an unusual high residential exposure.

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Biological Monitoring of Polycyclic Aromatic Hydrocarbon Exposure in a Highly Polluted Area of Poland

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Air pollution in Poland and particularly in Silesia is among the worst in Europe. Many coal mines and coke oven plants are located in this area, representing a major source of carcinogenic polycyclic aromatic hydrocarbons (PAHs). We quantitated the PAH exposure level in air samples using personal sampling devices, collected urine samples from the same individuals, and measured 1-hydroxypyrene with high performance liquid chromatography. Samples were collected twice, once in February and once in September. Mean PAH level of samples collected at three different coke oven plants varied from 2.3 μg/m^3 to 12.3 μg/m^3; the lowest mean was in September. Mean levels of 0.15 μg/m^3 (September) and 0.44 μg/m^3 (February) were noted for the environmentally exposed group. Mean urinary 1-hydroxypyrene varied from 2.45 to 13.48 μmol/mol creatinine at the three coke oven plants. The corresponding variation between the three different environmentally exposed groups in Silesia was 0.41–1.54 μmol/mol creatinine. In the nonindustrialized area, the mean varied from 0.20 to 0.14 μmol/mol creatinine. Seasonal variation was found both at the coke oven plants and in the environmental exposed groups in Silesia. Both PAH levels and 1-hydroxypyrene varied seasonally among coke oven workers and the environmentally exposed group. Our study shows that PAH exposure in the industrialized area of Silesia is high compared to levels in Western Europe. 1-Hydroxypyrene excretion in environmentally exposed individuals in Poland is among the highest in Europe. *Key words*: air pollution, benzo[*a*]pyrene, biological monitoring, exposure monitoring, 1-hydroxypyrene, polycyclic aromatic hydrocarbons. *Environ Health Perspect* 103:838–843 (1995)

Silesia, a highly industrialized region of Poland, is one of the most polluted areas in Europe. The air pollution mainly comes from combustion of fossil fuels emitted by industrial plants and from burning of black coal for home heating during the winter. Assessment of polycyclic aromatic hydrocarbon (PAH) exposure in polluted regions is important for future epidemiological investigations. In addition, it is essential to compare several monitoring methods to validate these methods.

The standardized mortality rates (deaths per 100,000) for men who died from lung cancer in Silesia in 1990 was only slightly higher (73.4) than the average for Poland (71.1) (*1*). The highest mortality rates in Silesia were found in Swietochlowice (117.3) (*2*). The mortality rate from lung cancer was lower in the nonindustrialized region Biala Podlaska (63.1) (*1*). Monitoring data of air pollution in Silesia have shown that exposure to benzo[*a*]pyrene is high (*3*) compared to several European cities (*4*), but comparable to measurements in London in the 1950s (*5*).

Environmental PAH exposure in Silesia has been monitored by stationary samplers (*3*), and several studies have used diverse biomarkers of exposure to mutagenic and carcinogenic compounds (*6–9*). Exposure measurement is one of the key components in a dose–response assessment (*6*), and the sensitive urinary biomarker for PAH exposure, 1-hydroxypyrene (*10,11*), offers a good complement to standard ambient air monitoring. The literature contains little information on quantitative exposure data for individuals in exposed populations.

To investigate environmental exposure of individuals to PAHs in Silesia, air samples were collected by personal sampling devices. Urinary excretion of 1-hydroxypyrene was also analyzed to provide information on the amount of PAH absorbed.

Materials and Methods

Study subjects and data collection. The occupationally exposed group consisted of 66 workers from three different plants located in Silesia, which is a center of coal-based industries in Poland. The coke ovens are denoted plants B, D, and E; plants B and D have been studied previously (*12*). Plants B and E have side-filling of coal, whereas plant D has batteries with side-filling and batteries with top-filling. In addition we studied two environmentally exposed groups, one consisting of individuals living in an industrialized area, Silesia, and the other consisting of individuals living in a nonindustrialized area, Biala Podlaska. The environmentally exposed group in Silesia consisted of three subgroups (Table 1). For each participant, we collected data on lifestyle factors including smoking and medical history, age, and workplace description. Urine samples were collected in polyethylene tubes before and after shift for the occupationally exposed group and during morning and afternoon in the environmentally exposed groups. PAH breathing-zone samples and urine samples were collected the same day. Air samples were collected with personal sampling devices in only one of three environmentally exposed groups in Silesia. We were not able to collect air samples with personal monitoring devices in Biala Podlaska. The measurements were taken twice, once in summer (September) and once in winter (February and March). For a summary of groups and sample collection data see Tables 1 and 2. Samples in Silesia were collected in 1992 and samples in Biala Podlaska were collected in 1993. Most of the coke oven workers live near the plant.

PAH exposure assessment. We quantitated 48 PAHs with molecular weight from 128 to 302. The sum of the following 12 compounds were used for the calculations unless otherwise noted: fluoranthene, pyrene, benz[*a*]anthracene, chrysene/triphenylene, benzo[*e*]pyrene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene, benzo[*ghi*]perylene, and benzofluoranthenes (two isomers). In our method, three-ring and lighter PAHs are incompletely collected and therefore left out in this selection. The 12 compounds here consist of four rings or more, as outlined in the National Institute of Occupational Safety and Health method for PAH determinations (*13*).

Particulate PAHs were sampled on

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Versapor-800 (Gelman Sciences, Ann Arbor, Michigan) filters with Casella AFC 123 (Casella, London, England) and DuPont S2500 (Du Pont, Largo, Florida) at 2 L air/min for 6–8 hr. The standard 25-mm sampling cassette Nuclepore filter (Pleasanton, California) was made of polyethene with carbon black to minimize the effect of static electricity.

The method for sample preparation was modeled after that of Bjørseth (*14*). The filters were extracted (ultrasonic) with cyclohexane after addition of internal standards. Polar compounds and PAHs were extracted from the cyclohexane into *N,N*-dimethylformamide with 3% water. The *N,N*-dimethylformamide was diluted with an equal volume of water and extracted with cyclohexane that was dried (Na_2SO_4) and concentrated.

We analyzed the extracts by gas chromatography on a 25-m Cp-sil-8 CB column (inner diameter 0.25 mm, film thickness 0.25 µm) programmed from 120–320°C, 6°C/min. Splitless injection with a flame ionization detector was used. Internal standards were 3,6-dimethylphenanthrene and ß,ß′-binaphthyl. These standards were used to determine recovery and relative response factors. Quantitation was accomplished with Turbochrome 3 integration software (PE Nelson, Cupertino, California).

Data from PAH measurements with high-volume stationary samplers in Silesia were from Cimander et al. (*15*). The same method of analysis was used for subjects in Biala Podlaska and analysis was performed by the same labortory.

Determination of 1-hydroxypyrene. We determined 1-hydroxypyrene in urine essentially as described by Jongeneelen et al. (*10*). The samples were analyzed in sets together with five spiked urine samples containing 0.010, 0.020, 0.040, 0.100, and 0.250 µmol 1-hydroxypyrene/L. The spiked urine samples were treated as unknowns and used as standards in the quantitative determination of 1-hydroxypyrene. 1-Hydroxypyrene in the urine samples was enzymatically deconjugated and then transferred to primed C18 Sep-Pak cartridges (Millipore, Milford, Massachusetts), washed with water, and eluted with 4 mL methanol. This sample prepurification was performed with a Millilab lab robot (Millipore, Milford, Massachusetts). A 20-µl aliquot was injected in an HPLC with a Novapack C18 column (Millipore, Milford, Massachusetts) and quantitatively determined with a fluorescence detector LC 240 (Perkin-Elmer Ltd, Beaconsfield, England) with excitation wavelength 242 nm and emission wavelength 388 nm. Quantitation was accomplished with Millennium integration software (Millipore, Milford, Massachusetts). All values were corrected based on the creatinine content (*16*).

Statistical methods. Both urinary 1-hydroxypyrene and air measurements of PAH, pyrene, fluoranthene, and benzo[*a*]-pyrene were log normally distributed. Therefore, these data were log–transformed for *t*-tests and analysis of variance. In *t*-tests, the mean values were back-transformed, resulting in a geometric mean which was used in the Tables. The residuals after regression analysis gave the best fit to normal distribution when analyzed on log-transformed 1-hydroxy-pyrene and pyrene data. Coefficients for regression analysis were not back-transformed; therefore, the information in the coefficients are limited. For testing group differences with analysis of variance, Scheffe's method was used. The calculations were done with Statgraphics, version 5 (STSC, Rockville, Maryland).

Table 1. Characterization of the occupationally and environmentally exposed groups

Group	Mean age	Smokers (%)	*N*	Subtotals
Coke oven plants				
Plant B	45.9	41.2	17	
Plant D	46.4	76.5	17	
Plant E	36.7	71.9	32	66
Industrialized environment				
Gliwice	39.7	76.2	21	
Bytom	39.1	58.6	29	
Swietochlowice	54.3	18.8	16	66
Nonindustrialized environment				
Biala Podlaska	33.7	51.5	66	66

Results

In Silesia, environmental and occupational PAH levels were monitored both by personal carried sampling devices and stationary samplers (*15*). In Biala Podlaska, PAH levels were monitored only by stationary sampling. There were no unusual weather conditions during the sampling.

The concentrations of 48 PAHs were quantitated in each sample. The correlation coefficients between a selected number of these compounds were determined (Table 3), and there was good agreement between these variables. Therefore, in the following

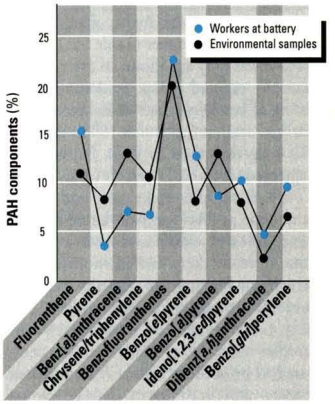

Figure 1. Relative proportion of 12 separated polycyclic aromatic hydrocarbon compounds. Chrysene and triphenylene and benzofluoranthenes were quantitated together.

Table 2. Number of samples collected from the participants in the study

Location	February 1-Hydroxypyrene[a] Morning	February 1-Hydroxypyrene[a] Afternoon	February PAH air measurements	September 1-Hydroxypyrene[a] Morning	September 1-Hydroxypyrene[a] Afternoon	September PAH air measurements
Coke oven plants						
Plant B	16	16	13	13	11	6
Plant D	13	13	13	11	13	8
Plant E	32	31	28	19	18	19
Industrialized environment						
Gliwice	17	17	12	13	12	10
Bytom	29	29	—	15	14	—
Swietochlowice	16	14	—	7	6	—
Nonindustrialized environment						
Biala Podlaska	5	31	—	—	45	—

[a]Morning = before shift; afternoon = after shift.

Table 3. Correlation coefficients between selected PAHs (sum of 12) and total PAHs (sum of all measured), fluoranthene, pyrene, and benzo[*a*]pyrene

	Total PAHs	Fluoranthene	Pyrene	Benzo[*a*]pyrene
February (*N* = 66)				
Selected PAHs*	0.96	0.94	0.91	0.98
September (*N* = 43)				
Selected PAHs*	0.96	0.96	0.94	0.96

*$p < 0.00005$ for all values; analysis performed on log-transformed data, Pearson product moment.

analysis we used 12 PAHs (including chrysene/triphenylene and two isomers of benzofluoranthene; Fig. 1), as well as pyrene and benzo[*a*]pyrene alone. There were no obvious systematic differences between the profiles of PAH compounds. PAH exposure levels are shown in Table 4, and a box plot of the PAH exposure level are shown in Figure 2. The PAH levels were higher in samples from coke oven workers than from environmentally exposed subjects, but there was great variation in samples from the various coke oven plants. The difference between arithmetic mean and median values shows that the data have a skewed distribution. The PAH levels in samples collected in winter were higher than samples collected in the summer (Table 5). The difference was only significant in the environmentally exposed group. Stationary monitoring of benzo[*a*]pyrene in Zabrze (industrialized area) in September 1992 was lower (10.4 ng/m^3) than in Biala Podlaska in September 1993 (20.4 ng/m^3) (*15*).

Urine samples were collected at the same time as PAH samples. Urine samples were collected before and after work for coke oven workers and in morning and afternoon for environmentally exposed subjects. Among coke oven workers, urinary 1-hydroxypyrene in the after-shift samples was lower or nearly constant compared to before-shift values. A summary of average values is shown in Table 6. For the following analysis, we used the data from after-shift or afternoon samples.

To analyze for a possible association between urinary excretion in wintertime and summertime, we calculated the correlation coefficient between winter and summer samples. The correlation between urinary 1-hydroxypyrene from winter and summer samples in the coke oven workers was relatively high (0.72; $p>0.00005$), and the values for the environmentally exposed subjects were lower (0.53; $p = 0.003$).

In Silesia, we found a higher level of urinary 1-hydroxypyrene in samples collected in the winter compared to samples collected in the summer both from coke oven workers and environmentally exposed subjects, but it was only among the environmentally exposed subjects that this difference was significant. In Biala Podlaska, the nonindustrialized area, we found no such seasonal difference (Table 7). A seasonal effect was found in environmental samples from the industrialized areas of Gliwice and Bytom, but not from the industrialized area of Swietochlowice (Fig. 3). Workers at coke oven plants are exposed to higher PAH levels than the environmentally exposed individuals. There was a significantly ($p<0.005$) higher level of 1-hydroxypyrene in individuals in the combined environmental group from Silesia compared to the values found at Biala Podlaska both in winter and summer (Table 7). In Silesia, the 1-hydroxypyrene level was higher in samples from coke oven workers than in samples from environmentally exposed subjects; these differences were also significantly different ($p<0.05$) except for the difference between Gliwize and plant E (Table 7).

In the environmentally exposed groups, both from the industrialized Silesia region and the nonindustrialized Biala Podlaska region, there was a significantly higher excretion of 1-hydroxypyrene among smokers than among nonsmokers. Among coke oven workers we also found a higher level of 1-hydroxypyrene in smokers, but this difference was not significant (Table 8).

We analyzed the associations among urinary 1-hydroxypyrene and exposure to pyrene, smoking, and age in both the winter and summer samples. The simple regression data between urinary 1-hydroxypyrene and pyrene exposure are shown in Table 9, and multiple regression analyses are shown in Table 10. The coefficients cannot be directly evaluated because both 1-hydroxypyrene and pyrene values are log transformed. Comparing the squared correlation coefficient in simple and multiple regression shows the importance of smoking as an explanatory variable. All individuals with data on urinary 1-hydroxypyrene and pyrene exposure (environmental summer samples) were smokers; therefore a multiple regression analysis could not be done.

Table 4. PAH exposure level from personal sampling devices at three coke oven plants and at Gliwice, an industrialized area

Time	Place	*N*	Exposure (μg/m^3), arithmetic mean (SD)		
			PAHs	Pyrene	Benzo[*a*]pyrene
February	Plant B	13	7.4 (7.1)	0.52 (0.48)	1.02 (1.06)
September	Plant B	6	2.3 (4.8)	0.15 (0.34)	0.31 (0.65)
February	Plant D	13	12.3 (28.3)	1.46 (4.13)	1.33 (2.8)
September	Plant D	8	11.4 (18.9)	0.72 (1.26)	1.51 (2.56)
February	Plant E	28	6.9 (13.4)	0.49 (1.16)	0.93 (1.92)
September	Plant E	19	5.3 (10.3)	0.41 (0.83)	0.72 (1.50)
February	Gliwice	12	0.44 (0.22)	0.012 (0.010)	0.041 (0.032)
September	Gliwice	10	0.15 (0.24)	0.009 (0.009)	0.009 (0.024)

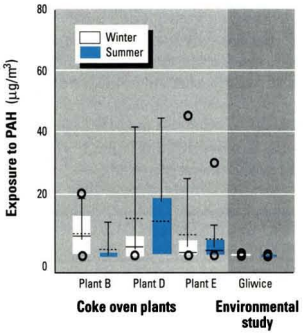

Figure 2. Box plot of PAH (sum of the 12 compounds listed in text and shown in Fig. 1) exposure in samples collected with personal monitors from coke oven workers and environmentally exposed subjects. All groups were sampled in winter and summer. Dotted line is arithmetic mean, solid line is median. The box encompasses 25th and 75th percentiles; whiskers extends to 10th and 90th percentiles. Values falling outside this range are indicated by circles.

Table 5. Seasonal variations in PAH levels

Sample type	Geometric mean (μg/m^3)		
	Winter (*N*)	Summer (*N*)	*p*-value[a]
Environmental (Gliwice)	0.40 (12)	0.06 (10)	<0.001
Occupational (sum of 3 coke oven plants)	2.08 (54)	1.38 (33)	0.33

[a]Student's *t*-test for difference between summer and winter samples, unpaired. Data log-transformed.

Discussion

Using two independent monitoring methods, we found higher exposure to PAHs in an industrialized region compared to a nonindustrialized region. Exposure via the environment in the industrialized region was high, although exposure was lower than for occupationally exposed coke oven workers. Urinary 1-hydroxypyrene depended both on pyrene concentration in the air and smoking habits.

The distribution of individual PAHs differs from previous data on PAHs collected from stationary samplers in the same area (*3,7*) although the major compounds like fluoranthene, benzo[*bjk*]fluoranthenes, pyrene, and benzo[*a*]pyrene are the most highly concentrated in all these analyses. The average concentration of benzo[*a*]-pyrene in Gliwice in September (0.041 μg/m^3; Table 4) is comparable to measurements in central London in the period

Table 6. Mean values (arithmetic) of urinary 1-hydroxypyrene before and after shift[a]

		Urinary 1-hydroxypyrene (μmol/mol creatinine)					
		Before shift (morning)			After shift (afternoon)		
Place	Time	Mean	SD	*N*	Mean	SD	*N*
Coke ovens							
Plant B	Winter	8.12	8.62	16	11.59	10.37	16
Plant B	Summer	8.67	10.55	13	4.69	4.71	11
Plant D	Winter	15.82	22.78	13	10.86	8.06	13
Plant D	Summer	14.64	14.72	13	13.48	9.91	11
Plant E	Winter	4.21	4.11	32	4.14	6.00	31
Plant E	Summer	2.35	2.25	19	2.45	1.94	18
Environmental exposure[a]							
Gliwice	Winter	1.25	0.93	17	1.54	1.20	17
Gliwice	Summer	0.90	0.60	13	0.84	0.57	12
Bytom	Winter	0.77	0.57	29	0.76	0.57	29
Bytom	Summer	0.32	0.24	15	0.38	0.21	14
Swietochlowice	Winter	0.47	0.24	16	0.36	0.17	14
Swietochlowice	Summer	0.44	0.28	7	0.41	0.36	6
Biala Podlaska	Winter	0.19	0.15	5	0.21	0.14	31
Biala Podlaska	Summer	—	—	—	0.27	0.20	45

[a]For environmental samples, morning and afternoon values are given.

Table 7. Seasonal variations of urinary 1-hydroxypyrene

	Geometric mean (μmol/mol creatinine)		
Sample type	Winter (*N*)	Summer (*N*)	*p*-value[a]
Environmental (Gliwice, Bytom, and Swietochlowice)	0.63 (60)	0.39 (32)	0.02
Occupational	3.81 (60)	3.19 (40)	0.62
Environmental (Biala Podlaska)	0.17 (31)	0.22 (45)	0.15
p-value[b]	<0.005	<0.005	

[a]Unpaired Student's *t*-test of log-transformed data.
[b]*p*-Value for all comparisons in winter and summer groups.

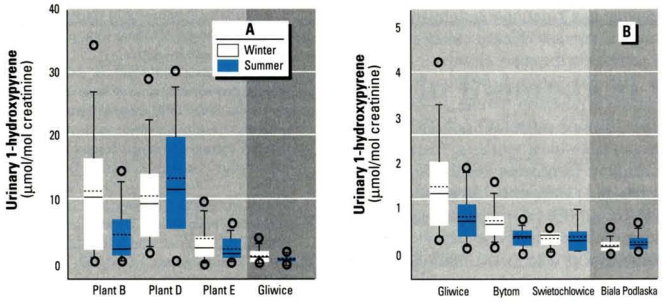

Figure 3. Box plot of urinary 1-hydroxypyrene levels in samples collected in Silesia and Biala Podlaska. Samples were collected in winter and summer. (A) Data from all coke oven plants (plants B, D, and E) and Gliwice, the most polluted area. (B) Data from all regions in the environmental study: industrialized areas: Gliwice, Bytom, and Swietochloweice, and Biala Podlaska, a nonindustrial area. All groups were sampled in winter and summer. Dotted line is arithmetic mean, solid line is median. The box encompasses 25th and 75th percentiles; whiskers extends to 10th and 90th percentiles. Values falling outside this range are indicated by circles.

1949–1951 (0.046 μg/m^3) (*5,17*), but these concentrations are not directly comparable because sampling procedures and quantitation methods differ. In a recent report, Brown et al.(*18*) studied lung cancer mortality in Poland and the significance of pollution, occupational exposure, and social factors as causation of disease.

An average of 0.8 m^3 of air was sampled by the filters; this volume is minimal for precisely measuring low PAH concentrations (i.e., environmental samples), but the volume is acceptable for occupational samples from coke oven plants. Low sampling volume can explain part of the deviations in our PAH profile and that of Chorazy et al. (*3*). But it is not unusual to find small differences in the composition of samples collected by portable samplers and those collected by stationary samplers. The advantage of personal sampling is that it reflects individual exposure at home and at work. For the occupationally exposed subjects, a difference between results from portable and the stationary samplers is likely because emissions from coke oven plants are only one of several PAH sources in the area.

Table 8. Effect of smoking habit on urinary 1-hydroxypyrene

	Geometric mean (μmol/mol creatinine)		
Sample type	Nonsmokers (*N*)	Smokers (*N*)	*p*-value[a]
Environmental, Silesia			
Winter samples	0.38 (29)	1.01 (31)	<0.0005
Summer samples	0.19 (12)	0.59 (20)	0.0023
Occupational, Silesia			
Winter samples	3.25 (21)	4.15 (39)	0.57
Summer samples	2.19 (15)	3.99 (25)	0.12
Environmental, Biala Podlaska			
Winter samples	0.13 (19)	0.25 (12)	0.01
Summer samples	0.14 (18)	0.30 (27)	<0.0001

[a]Unpaired Student's *t*-test of log-transformed data.

The differences in PAH concentrations of samples collected at the various plants can partly be explained by plant design like top-filling and side-filling, production capacity, and other technical factors. PAHs in air had been measured previously at plants B and D (*12*), and the highest benzo[*a*]pyrene levels were found in plant D. Plant D was the only plant which had batteries with top-filling of coal. The concentration of PAHs in air and levels of urinary 1-hydroxypyrene were highest in the winter samples. The seasonal difference was greatest among environmental samples. Two factors are important in explaining this result. During the winter, more coal and coke are used for heating, and an inversion layer forms, increasing local air pollution in cold days with light winds. Seasonal variations and high PAH levels during the winter have also been recorded in the UK in the 1950s and 1960s (*17,19*).

To study seasonal effects on PAHs in air and on 1-hydroxypyrene in urine, we used an unpaired *t*-test. Some subjects gave samples during both winter and summer. However, it is not likely that this overlap would affect our conclusion of a seasonal effect. In the cohort, there were too few samples to perform a reliable paired *t*-test. Seasonal variations have also been found in levels of PAH–DNA adducts (*8*) and sister chromatid exchanges in lymphocytes collected from the same volunteers who participated in this study (Pendzich J, unpublished data).

The urinary 1-hydroxypyrene concentrations vary in the same way as the pyrene

Table 9. Simple regression analysis of 1-hydroxypyrene association to pyrene exposure[a]

Group	Time	N	Intercept	Regression coefficient	p for coefficient	R^2
Occupational	Winter	54	2.02	0.26	0.002	0.18
Occupational	Summer	29	1.70	0.26	0.03	0.16
Environmental	Winter	11	-1.88	-0.41	0.10	0.27
Environmental	Summer	9	1.30	0.32	0.29	0.16

[a]Both 1-hydroxypyrene and pyrene values are log-transformed. Coefficients and intercepts are not back-transformed.

Table 10. Multiple regression analysis of 1-hydroxypyrene association to pyrene exposure, smoking, and age[a]

Group	Regression coefficient	p	R^2 (adjusted)
Occupational, winter (N = 54)			0.26
Intercept	0.89	0.07	
Pyrene	0.25	0.001	
Smoking	0.63	0.02	
Age	0.01	0.12	
Occupational, summer (N = 29)			0.07
Intercept	1.94	0.06	
Pyrene	0.24	0.07	
Smoking	0.12	0.80	
Age	-0.01	0.67	
Environmental, winter (N = 11)			0.73
Intercept	-3.94	0.01	
Pyrene	-0.74	0.01	
Smoking	0.91	0.01	
Age	0.01	0.46	

[a]Both 1-hydroxypyrene and pyrene are log transformed. Coefficients and intercepts are not back-transformed.

measurements. For instance, workers at plant D had the highest PAH exposure and the highest levels of 1-hydroxypyrene in urine. PAH concentrations in samples from coke oven plants are high compared to data from other coke oven plants published recently (*20,21*).

The 1-hydroxypyrene levels in urine of individuals from the nonindustrialized region were consistently lower than levels in individuals from industrialized Silesia. Urinary 1-hydroxypyrene levels in nonoccupationally exposed individuals have been published previously (*22*). Median values among smokers varied from 0.26 to 0.51 µmol 1-hydroxypyrene/mol creatinine in studies conducted in Western Europe and 0.76 µmol/mol creatinine in inhabitants of Beijing. The median values (Fig. 3B) are higher than 0.5 in Bytom winter samples and in Gliwice winter and summer samples. These samples were from smokers and nonsmokers. To our knowledge there is no similar environmental study of urinary 1-hydroxypyrene in a highly industrialized area in Western Europe. But in similar studies in China, urinary 1-hydroxypyrene among residents of cities (*23,24*) are comparable to values found in Silesia. In Silesia, several coke oven plants are located in a relatively small area and coal and coke are widely used for domestic heating.

Smoking contributes to excretion of 1-hydroxypyrene. Smoking 20 cigarettes a day increases the excretion of this metabolite by approximately 0.30 µmol/mol creatinine (*25*). In some studies of occupationally exposed individuals, the difference in urinary 1-hydroxypyrene between smokers and nonsmokers was greater than expected (*26*), indicating that smoking has an effect on uptake, excretion, or metabolism of 1-hydroxypyrene. In our data (Table 8), the difference in urinary 1-hydroxypyrene between smokers and nonsmokers was 0.9 and 1.8 µmol/mol creatine for coke oven workers in winter and summer samples, respectively. The difference in urinary 1-hydroxypyrene between smokers and nonsmokers is also elevated in environmentally exposed subjects from Silesia. Smokers may have induced P450 enzymes (*27,28*), resulting in a faster biotransformation, and smokers have less efficient ciliary clearance of particles in the upper airways.

The association between urinary 1-hydroxypyrene and pyrene or PAH and other relevant variables has been studied among coke oven workers. Correlation coefficients (r) between urinary 1-hydroxypyrene and pyrene in air are reported to be 0.58 (R^2 = 0.33) (*29*); the Spearman correlation coefficient for 1-hydroxypyrene after a shift and pyrene in air multiplied with exposure hours is 0.15–0.40 (R^2 = 0.02–0.16) (*20*), and the correlation coefficient between urinary 1-hydroxypyrene and PAH in air is 0.50–0.55 (R^2 = 0.25–0.30), before and after shift (*21*).

One explanation for these relatively low correlation coefficients may be uptake through the skin (*21*). In a study of creosote workers where skin uptake was estimated, R^2 was 0.79 in a multiple regression analysis (*22*). Multiple regression analysis of data from coke oven plants have given R^2 from 0.31 to 0.34 with variables including PAHs in air and thiocyanate in urine and plant type (coke oven or electrode plant) (*21*) and 0.44 with variables of pyrene in air, exposure hours, use of airstream helmet, and smoking habit (20). In a study of nonoccupationally exposed smokers, a high partial R^2 of 0.66 was found for PAHs from cigarette smoke, and the authors could explain 73% of the variability of 1-hydroxypyrene (*25*). In our study all regression coefficients were positive except for winter samples in environmentally exposed subjects. The number of samples in the environmental study are low, and for simple regression analysis the coefficients were not significant. Based on earlier studies and on our own data, one can conclude that pyrene in air is not a very strong predictor of excretion of 1-hydroxypyrene.

This study, together with previous studies, shows that Silesia is a region with high PAH exposure. These investigations have produced important data for current and future epidemiological investigation in this area. The interindividual variation in both PAH exposure and urinary 1-hydroxypyrene clearly shows the advantage of personal monitoring, either by portable sampling devices for air measurements or biological monitoring. There is a need for future exposure studies in this region, and urinary 1-hydroxypyrene is a biomarker well suited for monitoring of occupational and environmental PAH exposure.

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The E-Screen Assay: A Comparison of Different MCF7 Cell Stocks

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MCF7 human breast cancer cells have been studied extensively as a model for hormonal effects on breast cancer cell growth and specific protein synthesis. Because the proliferative effect of natural estrogen is considered the hallmark of estrogen action, it was proposed that this property be used to determine whether a substance is an estrogen. The E-screen assay, developed for this purpose, is based on the ability of MCF7 cells to proliferate in the presence of estrogens. The aim of our study was to characterize the response of four MCF7 cell stocks (BUS, ATCC, BB, and BB104) and determine which of them performed best in the E-screen test. The four stocks assayed were distinguishable by their biological behavior. In the absence of estrogen, MCF7 BUS cells stopped proliferating and accumulated in the G_0/G_1 phase of the cell cycle; estrogen receptors increased, progesterone receptors decreased, and small amounts of pS2 protein were secreted. Of all the MCF7 stocks tested, MCF7 BUS cells showed the highest proliferative response to estradiol-17ß: cell yields increased up to sixfold over those of nontreated cells in a 144-hr period. The differences between estrogen-supplemented and nonsupplemented MCF7 BUS cells were due mostly to G_0/G_1 proliferative arrest mediated by charcoal dextran-stripped serum. MCF7 BUS cell stocks and others showing a similar proliferative pattern should be chosen for use in the E-screen test, or whenever a proliferative effect of estrogen is to be demonstrated. *Key words*: bisphenol-A, cell type-specific proteins, estrogen sensitivity, hormone receptors, MCF7 cell variants, *p*-nonylphenol. *Environ Health Perspect* 103:844–850 (1995)

The use of pesticides in agriculture and the release of chemical compounds from manufacturing industries are common in southern Europe. Evidence of the estrogenic effects of some pesticides (*1*), alkylphenols (*2*), and plastic monomers (*3*) has raised concerns about environmental contamination by these chemicals. We became interested in the use of an estrogenicity test to assess the environmental and human health effects of estrogenic xenobiotics and to discriminate between estrogenic and nonestrogenic chemicals. Tests based on increased mitotic activity in tissues of the genital tract of female rodents after administration of chemicals have been proposed (*4*); but, although reliable, these methods are not suitable for large-scale screening of suspected estrogenic chemicals or for measuring the total estrogenic burden in human samples. We therefore adopted the biologically equivalent, easily performed E-screen assay described by Soto et al. (*5*). This bioassay compares the cell yield between cultures of breast tumor-derived MCF7 cells treated with estradiol and cultures treated with different concentrations of xenobiotics suspected of being estrogenic.

MCF7 cells were recommended as target cells because of their widely acknowledged estrogen-sensitivity (*6*).This cell line was initially established by Soule et al. (*7*) from a metastatic pleural effusion from a postmenopausal patient with metastatic, infiltrating ductal carcinoma of the breast: the patient was previously treated with radiotherapy and hormones. Although long-term established MCF7 cells are used worldwide, several MCF7 cell stocks with different sensitivities to estrogens have been developed during the last 20 years (*8*).

The purpose of this study was to characterize the response of four MCF7 cell stocks routinely held by different laboratories to assess which of them performs best in the E-screen test. We investigated the proliferative pattern and rate of estrogen-induced synthesis of cell type-specific proteins and the effects of *p*-nonyl-phenol and bisphenol-A on the four cell stocks.

Methods

Cell lines and cell culture conditions. Four stocks of MCF7 cells were used: MCF7 BUS cells were a gift from C. Sonnenschein (Tufts University, Boston), who cloned the cells (C_7MCF7) from passage 173 of the original MCF7 cells, received from C. McGrath of the Michigan Cancer Foundation; they were at post-cloning passages 70–103 at the time of our study. MCF7 ATCC cells at passage 147 were from the American Type Culture Collection (freeze no. 8655). MCF7 BB cells used at passages 580 to 595 were obtained in 1984 from G. Leclercq (Institut Jules Bordet, Brussels, Belgium), who received them from M. Rich of the Michigan Cancer Foundation. MCF7 BB104 cells were derived in our laboratory from MCF7 BB cells by keeping them in an estrogen-free medium for more than 24 months and were used at passages 12 to 21. For routine maintenance, cells were grown in Dulbecco's modification of Eagle's medium (DME) supplemented with 5% fetal bovine serum (FBS; PAA Labor und Forschungs Ges, MBH, Linz, Austria) in an atmosphere of 5% $C0_2$/95% air under saturating humidity at 37°C, except for MCF7 cells BB104, which were routinely maintained in 10% charcoal dextran-treated human serum (CDHS)-supplemented phenol red-free DME medium, prepared as described below.

Plasma-derived human serum was prepared from expired plasma by adding calcium chloride to a final concentration of 30 mM to facilitate clot formation. Sex steroids were removed from serum by charcoal-dextran stripping (*6*).

Cell proliferation experiments. We used MCF7 cells in the E-screen test according to a technique slightly modified from that originally described by Soto et al. (*5*). Briefly, cells were trypsinized and plated in 24-well plates (Limbro, McLean, Virginia) at an initial concentration of 10,000 cells per well in 5% FBS in DME. BB104 cells were seeded in 10% CDHS supplemented medium. The cells were allowed to attach for 24 hr, then 10% CDHS-supplemented phenol red-free DME was substituted for the seeding medium. A range of concentrations of the test compound were added, and the assay was stopped after 144 hr by removing the medium from wells, fixing the cells, and staining them with sulforhodamine-B (SRB).

The fixation and staining technique was modified from that described by Skehan et al. (*9*). Briefly, cells were treated with cold 10% trichloracetic acid and incubated at 4°C for 30 min. Then the cells were washed five times with tap water and left to dry. The fixed cells were stained for 10 min with

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0.4% (w/v) SRB dissolved in 1% acetic acid. Wells were rinsed with 1% acetic acid and air dried. Bound dye was solubilized with 10 mM Tris base (pH 10.5) in a shaker. Finally, aliquots were transferred to a 96-well plate to be read in a Titertek Multiscan apparatus (Flow, Irvine, California) at 492 nm. We evaluated linearity of the SRB assay with cell number for each MCF7 cell stock before each cell-growth experiment. Alternatively, cells were lysed and nuclei counted on a ZM Coulter Counter apparatus (Coulter Electronics, Luton, England) according to a previously described technique (*6*).

We used the E-screen test to determine, for all four MCF7 cell stocks, the relative proliferative potency (RPP), defined as the ratio between the minimum concentration of estradiol-17ß needed for maximal cell yield and the minimum dose of the test compound needed to obtain a similar effect, and the relative proliferative effect (RPE); that is, the ratio between the highest cell yield obtained with the chemical and with estradiol-17ß × 100 (*5*).

Results are expressed as the means plus or minus standard deviations. In proliferation yield experiments, each point is the mean of three counts from four culture wells. Mean cell numbers were normalized to the steroid-free control, equal to 1, to correct for differences in the initial plating density. Differences between the diverse groups were calculated with Student's *t*-test.

Estrogen and progesterone receptor measurements. We seeded MCF7 cells in T-25 flasks in 5% FBS-supplemented DME. The next day, the medium was changed to 10% CDHS-supplemented DME medium, and estradiol-17ß or the chemicals to be tested were added. One group of cells received vehicle alone. After 72 hr, the culture medium was discarded and cells were frozen in liquid nitrogen. To extract receptor molecules, cells were incubated at 4°C for 30 min with 1 mL of extraction buffer (0.5M KCl, 10 mM potassium phosphate, 1.5 mM EDTA, and 1 mM monothioglycerol, pH 7.4) according to a previously described technique (*10*). The cell debris were pelleted, and estrogen receptors and progesterone receptors were measured in a 100-µL extract aliquot by enzyme immunoassay using the Abbott estrogen receptor and progesterone receptor-enzyme immunoassay monoclonal kits (Abbott Diagnostic, Wiesbaden, Germany) according to the manufacturer's instructions.

Cell type-specific proteins and compounds tested. Cathepsin-D and pS2 proteins were measured in culture media with the ELSA-CATH-D and ELSA-pS2 immunoradiometric assays (CIS BioInter-

Table 1. Cell cycle distribution and estimated doubling time (T_D) of the four MCF7 cell stocks[a]

Treatment/stock	Distribution (% total cells) G_0/G_1	S	G_2/M	$T_{D\,(hr)}$
10% FBS–DME				
MCF7 BUS	69.0 ± 2.6	19.2 ± 2.1	11.9 ± 1.4	32 ± 1.7
MCF7 ATCC	59.4 ± 3.9	32.4 ± 3.6	7.4 ± 2.5	49 ± 2.7
MCF7 BB	66.2 ± 3.4	22.3 ± 1.8	11.5 ± 1.6	27 ± 1.7
CDHS–PhR-DME				
MCF7 BUS	93.5 ± 4.2	4.0 ± 1.2	2.6 ± 0.5	46 ± 3.0
MCF7 ATCC	68.1 ± 4.7	19.7 ± 3.0	12.9 ± 2.0	59 ± 3.1
MCF7 BB104	60.8 ± 4.1	36.6 ± 2.7	4.2 ± 1.9	37 ± 2.2

Abbreviations: FBS, fetal bovine serum; DME, Dulbecco's modification of Eagle's medium; CDHS, charcoal dextran-treated human serum; PhR, phenol red-free.
[a]All data are means ± SD of at least three separate determinations.

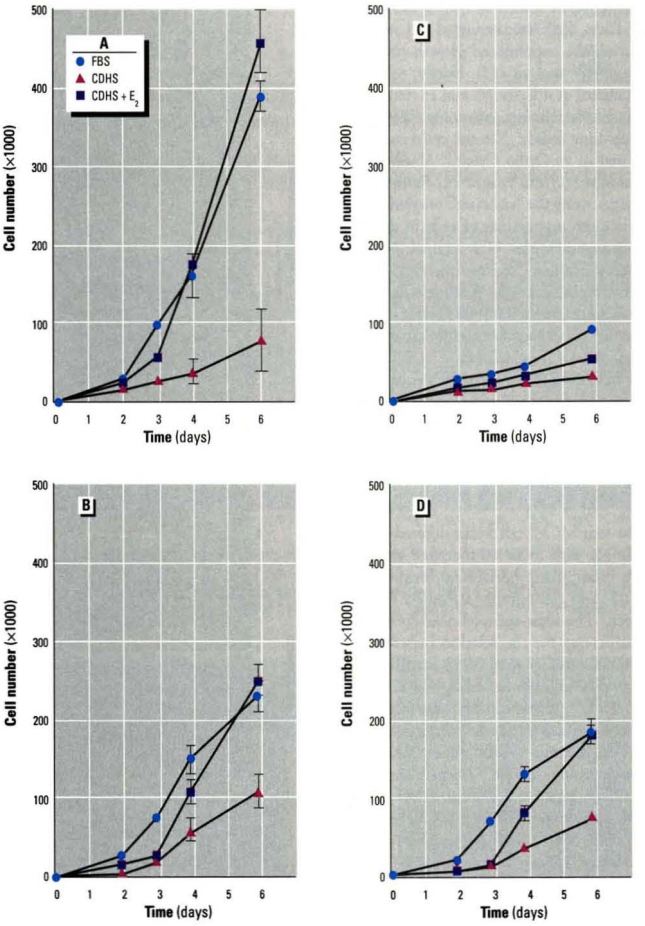

Figure 1. Growth curves of MCF7 cells cultured in the presence of 10% fetal bovine serum (FBS), 10% charcoal dextran-treated human serum (CDHS), or 10% CDHS plus 1 nM estradiol-17ß (CDHS + E_2). Each point is the mean of three counts from four culture wells; bars indicate SDs. (A) BUS cells, (B) BB cells, (C) ATCC cells, (D) BB104 cells.

national, Gif-sur-Yvette, France). The culture medium was centrifuged at 1000*g* for 10 min to eliminate floating and detached cells. Samples were kept frozen at -80°C until the assays were done.

Estradiol-17ß was obtained from Sigma (St. Louis, Missouri). Bisphenol-A (BPA) and *p*-nonyl-phenol (NP) were obtained from Aldrich-Chemie (Albuch, Germany). Chemicals were dissolved in ethanol to a final concentration of 1 mM and stored at -20°C; all were diluted in phenol red-free DME immediately before use. The final ethanol concentration in the culture medium did not exceed 0.1%.

Flow cytometry studies. MCF7 cells grown in 10% FBS-supplemented DME medium were seeded by quintuplicate in T-25 flasks. Cells were harvested and processed during the exponential growth phase for cytometry analysis (*11*). Briefly, cells were incubated at 4°C for 30 min in darkness in Vindelov's solution containing RNAse and propidium iodide. The cell cycle was determined in an Ortho Cyteron Absolute flow cytometer (Ortho Diagnostic, Raritan, New Jersey), using the "cell cycle" program to calculate the proportions of cells in different phases of the cycle. As an internal DNA reference, stained chicken blood cells were added to each sample. Alternatively, MCF7 cells were grown in 10% CDHS-supplemented phenol red-free medium in the presence of 10 nM estradiol or its vehicle for 72 hr before harvesting, then processed as described above.

Results

Growth Characteristics and Light Microscopy

The four MCF7 cell stocks differed in their staining with SRB; we therefore evaluated the relationship between optical density (OD) and cell number separately for each stock. The least-square linear correlation coefficients for the relation between OD and cell number were 0.998 for BB, 0.996 for BB104, 0.996 for BUS, and 0.996 for the ATCC stock. From the best-fit parameters, we estimated the correleation between cell number and OD by solving the following equations: MCF7 BB cell number = $[(1.5 \times 10^{-6}) \times OD] + 0.038$; MCF7 BB104 cell number = $[(1.38 \times 10^{-6}) \times OD] + 0.040$; MCF7 BUS cell number = $[(1.31 \times 10^{-6}) \times OD] + 0.048$; and MCF7 ATCC cell number = $[(2.45 \times 10^{-6}) \times OD] + 0.020$. Sulforhodamine-B staining was clearly more intense in MCF7 ATCC cells than in the other three cell stocks.

We measured the growth rate and cell cycle distribution for MCF7 cells grown in 10% FBS-supplemented DME medium. Table 1 shows the distribution of phases in the cell cycle and the estimated doubling time (T_D) for all clones studied.

MCF7 BUS and ATCC cells were easily distinguishable from BB and BB104 cells by light microscopy. The first two stocks had rounded edges, and were smaller and more refractive than the latter two. Cells from BB and BB104 stocks had extensive intercellular contacts, showed greater cell density at confluence, and attached more strongly to the plastic surfaces.

Proliferative Patterns

MCF7 cells maintained for 6 days in 10% CDHS-supplemented DME behaved differently depending on the stock tested. MCF7 BUS cells underwent two doublings and then stopped proliferating. In contrast, the other three MCF7 clones either slowed their proliferation rate (MCF7 BB104 and BB cells) or were not disturbed at all (MCF7 ATCC cells) in estrogen free-medium (Fig. 1). Flow cytometry studies confirmed the high proportion of arrest in MCF7 BUS cells cultured for 72 hr in estrogen-depleted medium. Switching ATCC cells to an estrogen-free medium did not significantly modify the distribution of cell cycle phases (Table 1).

The addition of estradiol-17ß to CDHS-supplemented medium increased

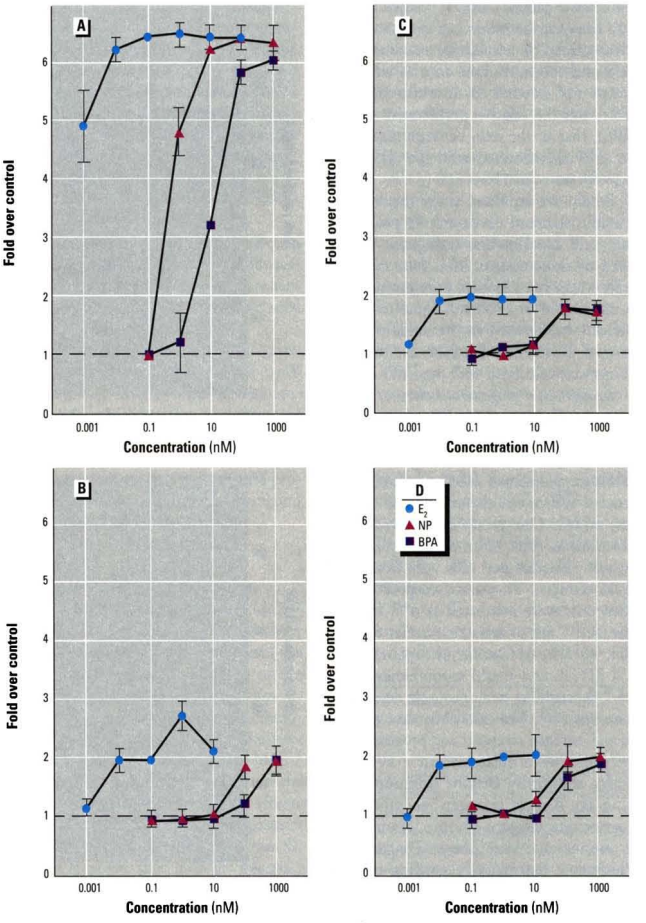

Figure 2. Cell proliferation yields of MCF7 cells. (A) BUS, (B) BB, (C) ATCC, and (D) BB104 cell stocks growing in 10% charcoal dextran-treated human serum supplemented medium were exposed for 144 hr to different amounts of estradiol-17ß (E_2), *p*-nonyl-phenol (NP), and bisphenol-A (BPA). Each point is the mean of three counts from four culture wells; bars indicate SDs.

cell yields in all MCF7 stocks. In MCF7 BUS cells, the proliferative effect was greatest with ≥0.01 nM estradiol-17ß (Fig. 2). The cell yield was sixfold greater than in controls (6.67 ± 1.21; $p < 0.001$). Estradiol-17ß also increased cell yield in BB and BB104 cells by up to twofold compared to controls ($p < 0.05$). In ATCC cells, the effect of estradiol-17ß was almost negligible (<1.5-fold increase, not significant). As expected from the preceding data, estradiol-17ß treatment also modified the proliferation of these cells differently. When 0.1 nM estradiol-17ß was added to 10% CDHS-supplemented DME medium, MCF7 BUS cells showed the shortest doubling time (T_D = 21 ± 3.8 hr) and ATCC cells the longest T_D (54 ± 4.2 hr).

In all cell stocks, NP and BPA increased cell yields to values similar to those obtained with estradiol-17ß. However, NP and BPA were much less potent than estradiol-17ß (i.e., MCF7 BUS cells showed maximal proliferation at concentrations of nonylphenol of 10 nM and higher (Fig. 2). The RPP values are shown in Table 2.

We also studied the response of MCF7 cells to estradiol-17ß in medium supplemented with different amounts of serum. MCF7 BUS and BB104 cells were cultured in DME medium with 5–50% CDHS. Figure 3 shows the effect of 0.1 nM estradiol-17ß on cell yield. In MCF7 BB104 cells; estradiol-17ß consistently increased proliferation (approximately twofold over control values), regardless of the concentration of CD serum added. In MCF7 BUS cells, differences in cell yield between estradiol-17ß-treated and nontreated cells decreased as the concentration of serum increased. The effect of 0.1 nM estradiol-17ß was maximal when 10% serum was added to the medium. At 50% of serum replacement, 0.1 nM estradiol-17ß had no apparent effect on MCF7 BUS cells.

Cathepsin-D and pS2 Secretion

Cathepsin-D and pS2 protein accumulation in the culture medium reflected increases in cell number (Fig. 4). MCF7 BB and BB104 cells secreted the largest amounts of pS2 after 144 hr of subculture in estrogen-free medium (BB, 508 ± 101 ng/10^6 cells; BB104 612 ± 133 ng/10^6 million cells). Estradiol had little effect on the secretion of pS2 in both cell stocks (~1.7 increase over control values in BB104). However, pS2 secretion by MCF7-BUS cells was significantly increased by concentrations of estradiol-17ß 0.1 nM and higher (~3.5-fold increase over controls). Interestingly, MCF7-BUS cells showed the lowest basal levels (53.9 ± 16.7 ng/10^6 cells) of protein secretion and the greatest effect of estradiol-17ß on pS2 secretion (Fig. 4A). Differences in cathepsin-D protein secretion between the four MCF7 cell stocks were smaller; basal levels ranged from 8.9 ± 4.0 pmol/10^6 cells in the BUS stock to 19.5 ± 7.8 pmol/10^6 cells in the BB104 clone. Estradiol-17ß treatment slightly increased cathepsin-D accumulation in the culture medium. The greatest effect was seen in BB cells, in which 1 nM estradiol-17ß raised cathepsin-D protein levels 1.8-fold (Fig. 4B).

Table 2. Estrogenic response of MCF7 cells to estradiol-17ß (E_2), *p*-nonylphenol (NP) and bisphenol-A (BPA)

Cell stock	Compound	Concentration (nM)[a]	PE[b]	RPE (%)[c]	RPP (%)[d]
BUS	E_2	0.01	6.7	100	100
	NP	10	6.8	103	0.001
	BPA	100	6.3	97	0.0001
ATCC	E_2	0.01	1.6	100	100
	NP	100	1.6	100	0.0001
	BPA	100	1.5	95	0.0001
BB	E_2	0.01	2.2	100	100
	NP	100	2.0	95	0.0001
	BPA	1000	1.9	90	0.00001
BB104	E_2	0.01	2.3	100	100
	NP	100	2.1	98	0.0001
	BPA	1000	2.1	98	0.00001

[a]Lowest concentration needed for maximal cell yield.
[b]Proliferative effect: ratio between the highest cell yield obtained with the chemical and the hormone-free control.
[c]Relative proliferative effect: (PE of the test compound/PE of E_2) 100.
[d]Relative proliferative potency: (dose of E_2/dose of test compound needed to produce maximal cell yield)100.

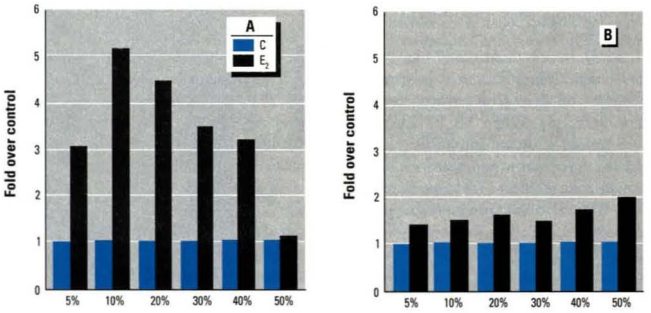

Figure 3. Effect of increasing concentrations of charcoal dextran-treated human serum on the growth of MCF7 (A) BUS and (B) BB104 cells. MCF7 cells were grown for 6 days in estrogen-depleted medium supplemented with different concentrations of charcoal dextran-treated human serum (from 5% to 50% as indicated along the X-axis). Estradiol-17ß (0.1 nM) was added to cultures (E_2); controls (C) received vehicle alone.

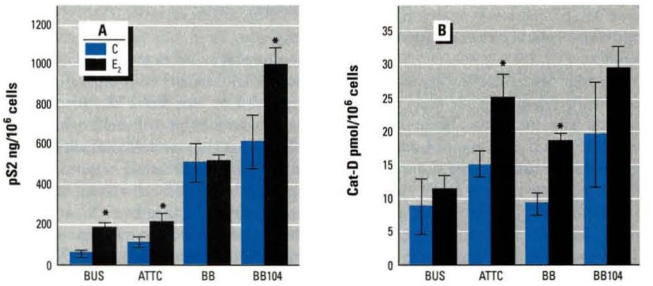

Figure 4. pS2 (A) and cathepsin-D (B) accumulated in the culture medium. MCF7 BUS, ATCC, BB, and BB104 cell stocks were grown in 10% charcoal dextran-treated human serum-supplemented medium (C) and exposed for 144 hr to 1 nM estradiol-17ß (E_2). *Values significantly different from control ($p < 0.05$). Bars indicate SDs.

Hormone Receptors

MCF7 cells bear receptors for estradiol-17ß and progesterone. The highest value for estrogen receptor (400 ± 55 fmol/mg protein) was found in the BB104 stock; these cells are routinely kept in estrogen-free medium. In BUS cells, estrogen receptor expression was 183 ± 29 fmol/mg of extracted protein. Treatment with estradiol-17ß decreased estrogen receptor levels and increased progesterone receptor levels. The lowest basal progesterone receptor value (7.9 ± 1.3 fmol/mg protein), which approached the lower limit of detection of the monoclonal antibody assay, was observed in the MCF7 BUS stock, which also showed the largest estradiol-mediated increase in progesterone receptor (~12-fold increase) (Fig. 5). Basal levels of progesterone receptor were 24 ± 7, 75 ± 12, and 83 ± 7 fmol/mg of protein in BB104, BB, and ATCC cells, respectively. In all the three stocks, estradiol-17ß increased progesterone receptor levels in a dose-dependent manner; however, the effect was smaller than that observed in BUS cells (Fig. 5).

We did another set of experiments to investigate the effect of estrogens on the "disappearance" of estrogen receptors (Table 3). In cells treated for 72 hr, estradiol-17ß significantly increased progesterone receptor and decreased estrogen receptor concentrations. When MCF7 BUS cells were treated with concentrations of ≥100 nM NP there was a significant increase in progesterone receptor (Table 3). Treatment with BPA also increased progesterone receptor, but the effect was weaker at higher concentrations (>1000 nM). However, estrogen receptor levels were unchanged when the medium contained NP or BPA.

Discussion

A bioassay can be effectively assessed only with the help of a standardized set of parameters that measure reproducibility. In evaluations of the E-screen test, uniformity of the MCF7 cell stock used is the most important variable that affects reproducibility.

The four MCF7 cell stocks we assayed were distinguishable on the basis of their biological behavior. In the absence of estrogen, MCF7 BUS cells stopped proliferating; they accumulated in the G_0/G_1 phase, estrogen receptor receptor levels increased, progesterone receptor decreased, and low levels of pS2 protein were secreted. Of the MCF7 stocks we tested, MCF7 BUS cells showed the highest proliferative response to estradiol-17ß, with cell yields increasing up to sixfold over nontreated cells in a 144-hr period. This increase was of the same order of magnitude as that described previously

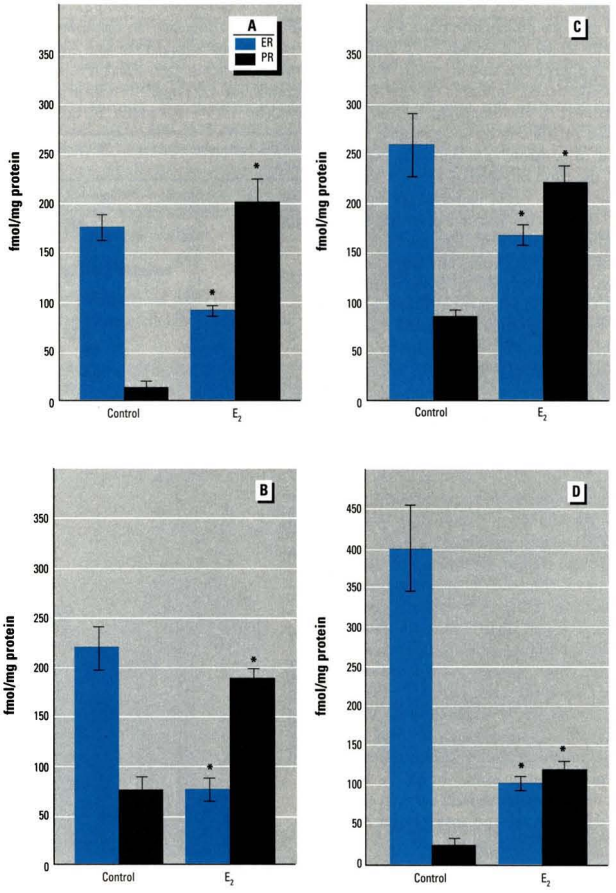

Figure 5. Estrogen and progesterone receptors in MCF7 cells: (A) BUS, (B) BB, (C) ATCC, (D) BB104. Cells in T-25 flasks were incubated in 10% charcoal dextran-treated human serum for 72 hr with 1 nM of estradiol-17ß (E_2). Controls received the vehicle alone. Estrogen receptors (ER) and progesterone receptors (PR) were measured in the extracted cells with the monoclonal antibody technique as decribed in Methods. Results are expressed as femtomole per milligram of extracted protein ± SD (bars). *Values significantly different from control ($p < 0.05$).

in monolayer cultures of MCF7 cells (*5,12–16*).The other three cell stocks responded to estradiol-17ß with a much smaller increase in cell yield, which was never higher than twofold over control values. Similar proliferative responses were reported in MCF7 cell stocks tested in media supplemented with different amounts of charcoal dextran serum, which ranged from 20% to 0.5% (*17–30*), and in serumless medium (*31–33*). Poor proliferative responses to estradiol-17ß were described when nonstripped, serum-supplemented media were used (*34–36*).

Differences in culture condition (*29–37*), the type of serum-supplemented medium (*17,18*), serum lots (*17*), the presence of phenol red (*38*), insulin (*27,39,40*), cell passage (*41*), and cell density or culture matrices (*20,33,41*), may explain the poor proliferative effect of estradiol-17ß in some MCF7 cell stocks. The disparate effects of estradiol-17ß, other so-called mitogens, and growth inhibitors on cell proliferation have also been attributed to heterogeneity of the uncloned cells (*39,42,43*) or to differences in the MCF7 cells used (*8,13,16,25,44,45*). The four cell stocks we assayed were cultured in the same medium (phenol red-free DME), which was supple-

Table 3. Effect (means ± SD) of estradiol-17ß (E_2), *p*-nonyl-phenol (NP), and bisphenol-A (BPA) on estrogen and progesterone receptors in MCF7 BUS cell stock

	Concentration (nM)	fmol receptor/mg extracted protein: Estrogen receptors	Progesterone receptors
Control		174 ± 12 (100)[a]	17 ± 12 (100)
E_2	0.1	195 ± 35 (112)	131 ± 12 (770)*
	1	91 ± 11 (52)*	202 ± 21 (1188)*
	10	87 ± 15 (50)*	211 ± 15 (1241)*
	100	87 ± 07 (50)*	154 ± 70 (906)*
NP	1	179 ± 30 (103)	23 ± 10 (135)
	10	207 ± 12 (119)	29 ± 13 (171)
	100	189 ± 15 (109)	161 ± 35 (575)*
	1000	164 ± 13(94)	191 ± 11 (1123)*
BPA	1	164 ± 10 (94)	23 ± 21 (135)
	10	229 ± 18 (132)	23 ± 12 (135)
	100	228 ± 30 (131)	56 ± 33 (329)
	1000	167 ± 18 (96)	167 ± 21 (982)*

[a]Numbers in parentheses are the percentage of variation versus controls.
*Values significantly different from control ($p < 0.05$).

mented with equal concentrations of human serum (10%) from the same source (healthy voluntary donors). Experiments with all four cell stocks were always run in parallel. Obviously, we could not use exactly the same number of passages (*41*). Nevertheless, MCF7 BUS cells, which showed the greatest proliferative response to estradiol-17ß, were at passage 100 (+173 passages before cloning) and MCF7 ATCC cells, which showed the poorest response to estradiol-17ß, were at a similar number of passages (received at passage 148, tested after 150–170 passages in our laboratory). Our results therefore suggest that differences in the response cannot be attributed to culture conditions or to passage number.

In experiments designed to test the influence of serum concentration on the effect of 17ß-estradiol, serum seemed to counteract the effect of estradiol-17ß in MCF7 BUS cells. Increasing serum concentration from 5% to 50% reduced cell yield despite the presence of 0.1 nM of estradiol-17ß. We found it necessary to increase the concentration of estradiol-17ß to maintain the differences between treated and nontreated cells as serum supplementation increased.

Differences in cell yield between estradiol-17ß-treated and nontreated cultures were significantly higher in cell stocks that showed arrest of growth in serum-supplemented, estrogen-free medium. The differences between estrogen-supplemented and nonsupplemented MCF7 BUS cells were mostly due to arrest in G_0/G_1 mediated by CD serum in the absence of estrogen (Table 1).

The minimal effect of estradiol-17ß on MCF7 ATCC cell yields was notable; similar results have been described by others (*30,32,35*). Because ATCC cells are from a different patient than the one from which MCF7 cells originated (*8*), this stock should be used with caution in cell proliferation tests such as the E-screen bioassay.

Estradiol-17ß affected MCF7 BUS cell yield, cell cycle distribution, and specific protein synthesis. In this stock, the hormone reduced estrogen receptor content, increased the amount of measurable progesterone receptor, and increased pS2 protein secretion. We found no significant effect of estradiol-17ß on cathepsin-D protein synthesis (*25*). Although some MCF7 cell stocks respond to estradiol-17ß with a higher increase in cathepsin-D protein secretion than others (*46*), all four MCF7 cell stocks tested here showed the same poor response. In MCF7 BUS cells, the presence of estradiol-17ß in the culture medium had a net stimulatory effect on pS2 protein production, mainly because of the low amounts of this protein secreted in estrogen free-medium.

p-Nonyl-phenol and BPA were found to be estrogenic, increasing cell yield and progesterone receptor concentration in MCF7 cells (*2,3,47*). These compounds mimicked the proliferative effect of estradiol-17ß and increased progesterone receptor levels, albeit to a lower extent than did the hormone. However, the effects of estradiol-17ß and these chemicals on the disappearance of estrogen receptor differed. An increase in progesterone receptor levels was not associated with estrogen receptor downregulation. Interestingly, Schutze et al. (*19*) showed that catecholestrogens, which increase the rate of MCF7 cell proliferation and progesterone receptor levels, evoked estrogen receptor processing only during the first 8 hr after treatment; thereafter, estrogen receptor increased, reaching basal levels at 24 hr. We are now investigating whether differences in the ability to evoke processing are due to an early phenomenon occurring before 72 hr, when estrogen receptor was routinely evaluated, or whether these differences are related to the use of exchange assays or the immunological detection of estrogen receptor (*48*).

Validation of the RPE and RPP of the chemicals tested here seems to depend on the cell stocks used in the E-screen bioassay. Although RPE was only slightly different in MCF7 BUS, BB, and BB104 cells, it may not be easy to detect partial estrogen agonists with cells other than BUS. The differences of less than twofold between estradiol-17ß-treated cells and controls when MCF7 BB, BB104, and ATCC cells were used defined a narrow range of sensitivity. It seems evident that the limited ability of BB and BB104 cells to grow in the presence of NP and BPA resulted in underestimation of RPP. The ATCC cell stock seems to be the least appropriate for both purposes.

In summary, it is now clear that induction of cell proliferation is the hallmark of estrogen action. The effects of estrogens on cell type-specific protein synthesis (whether induction or downregulation) and cell hypertrophy are variable and may be evoked by nonestrogenic agents. Our results suggest that the ability of estrogens to make cells proliferate can be proved *in vitro* using an appropriate bioassay such as the E-screen test. MCF7 BUS cell stocks and others showing a similar proliferative pattern should be chosen for use in the E-screen test, or whenever a proliferative effect of estrogen is to be demonstrated.

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XIVth World Congress on Occupational Safety and Health
April 22–26, 1996
Madrid, Spain

The XIVth World Congress on Occupational Safety and Health will be held in Madrid from April 22 to April 26, 1996. The organizers are the Spanish Ministry of Labour and Social Security, through the National Institute for Occupational Safety and Health (INSHT), the International Labour Office (ILO), Geneva, and the International Social Security Association (ISSA), Geneva.

These World Congresses, of which the first was held in Rome in 1955 and the last in New Delhi in 1993, have had such venues as Brussels, Paris, London, Zagreb, Vienna, Dublin, Bucharest, Amsterdam, Ottawa, Stockholm and Hamburg.

The XIVth World Congress, to be held in Madrid, aims to be an open forum for all persons involved in risk prevention at work, safety and health safety specialists, occupational health physicians, labour inspectors, persons directly concerned with safety and health at work, including entrepreneurs and managers in enterprises, trade union representatives, manufacturers and importers, as well as heads of public administration and social security administrators.

The main focus of this Congress will be on the consequences for occupational safety and health of processes of international and regional integration (e.g. EU, NAFTA) and of the globalization of economic relations, on an in-depth analysis of chemical risks and on new proposals for cooperation and participation within enterprises. Other specific issues will also be dealt with, such as training and information, control of working conditions or new responsibilities. Special emphasis will be placed on small and medium-sized enterprises and sectors facing specific problems with regard to safety and health at work, such as the construction sector and agriculture.

In addition, as part of this Congress, the International Section "Electricity" of the ISSA will be organizing the 3rd International Film and Video Festival on Occupational Safety and Health.
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Risk Assessment of Environmentally Influenced Airway Diseases Based on Time-Series Analysis

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Threshold values are of prime importance in providing a sound basis for public health decisions. A key issue is determining threshold or maximum exposure values for pollutants and assessing their potential health risks. Environmental epidemiology could be instrumental in assessing these levels, especially since the assessment of ambient exposures involves relatively low concentrations of pollutants. This paper presents a statistical method that allows the determination of threshold values as well as the assessment of the associated risk using a retrospective, longitudinal study design with a prospective follow-up. Morbidity data were analyzed using the Fourier method, a time-series analysis that is based on the assumption of a high temporal resolution of the data. This method eliminates time-dependent responses like temporal inhomogeneity and pseudocorrelation. The frequency of calls for respiratory distress conditions to the regional Mobile Medical Emergency Service (MMES) in the city of Leipzig were investigated. The entire population of Leipzig served as a pool for data collection. In addition to the collection of morbidity data, air pollution measurements were taken every 30 min for the entire study period using sulfur dioxide as the regional indicator variable. This approach allowed the calculation of a dose–response curve for respiratory diseases and air pollution indices in children and adults. Significantly higher morbidities were observed above a 24-hr mean value of 0.6 mg SO_2/m^3 air for children and 0.8 mg SO_2/m^3 for adults. Using the derived threshold value, the attributable risk for respiratory disease for children exposed to an increase, for example, from 0.6 to 1.2 mg SO_2/m^3 air (24-hr mean) (i.e., a doubling of the threshold level) was 30/10,000. For adults this risk was 2/10,000. *Key words*: airway diseases, epidemiology, risk assessment, threshold value, time-series analysis. *Environ Health Perspect* 103:852–856 (1995)

Much is known about the acute (*1*) and chronic respiratory health effects (*2*) associated with exposure to ambient air pollution. Studies are designed to address important issues associated with evaluating health effects of low-level exposure and actual exposure levels, while ensuring that independent effects of individual pollutants as well as their interactions in complex mixtures are detected (*3*).

The adverse effects of individual air pollutants such as total suspended particulates (TSP), total SO_4 (TSO_4), and total SO_2 (TSO_2) on lung function parameters have been investigated extensively (*4*). The results of these studies indicate that children are suffering from bronchial hyperreactivity, especially as a consequence of TSO_4. The harmful effects of PM_{10} (particulate matter < 10 µm in diameter) at levels below 150 $µg/m^3$ (24-hr standard mean) on the peak expiratory flow (PEF) have also been documented (*5*). The 5-day moving-average PM_{10} analysis indicated a strong association with adverse effects on PEF (*5*). These findings are supported by a reanalysis of the Steubenville, Ohio, study which evaluated the forced expiratory volume in 0.75 sec ($FEV_{0.75}$) and the forced vital capacity (FVC) (*6*).

Several studies report significant associations between air pollution and adverse health effects in susceptible individuals and groups, such as people suffering from chronic obstructive pulmonary disease (*7–10*). Many studies use hospital admissions or visits to the emergency unit with exacerbations of symptoms of respiratory illness as indicators of the health effects of pollution. Lag periods of one to several days depending on the pollution component have been observed between time of exposure and hospital admissions, visits to emergency units, or both.

All the studies cited above linked exposure [SO_2, TSO_4, SO_4, NO_x (nitrogen oxides), O_3] to adverse respiratory health effects without addressing possible threshold effects or the magnitude of change in the risk associated with concentrations of pollutants beyond this threshold value. The aim of the present investigation was to examine the quantification of risk using a dose-effect relationship assuming some of the same conditions as examined in those previouse studies (e.g., indicator component, measure of effect, lag time between exposure and effect, populations at risk).

Locality-dependent analysis of morbidity (incidence and prevalence of diseases) influenced by the environment may identify variations in the relationship between exposure and disease and may thus be relevant in assessing any increased risk. Ideally, the study group and the control group should be subject to the same environmental conditions, but should differ in the characteristic variable of the pollutant under investigation. However, in order to assess increased or decreased risk of disease due to a pollutant, the threshold limit beyond which the rate of disease increases above that occurring by chance (background frequency) must be known.

An increased risk of adverse health effects above this threshold level, measured by the frequency of visits to emergency units or hospital admissions, is assessed by comparing above-threshold days with data obtained on days when pollution levels are below the threshold. This type of analysis would not necessitate, as do classical case–control studies, an investigation of two or more population groups in which the control group is not exposed to the pollution variable under investigation. A quantitative difference in the exposure burden alone would be sufficient.

Methods

The study was conducted in Leipzig, a city of approximately 500,000 people, located in the most highly industrialized area of east Germany. The frequency of calls to the Mobile Medical Emergency Service (MMES) was investigated retrospectively for the 6-year time period of 1981–1987, with prospective follow-ups in 1988 and 1990.

Records of daily calls to the MMES for respiratory distress conditions were the basis for data collection and analysis. All reported respiratory distress conditions occurring within Leipzig were covered by the study because during the study period, the MMES was the only such service responding to medical emergency events in the city. However, the study focused only on respiratory illnesses among children and young adults. These illnesses included bronchitis, asthma, and croup.

Data from adults are presented for comparison only when appropriate. Overall, 358,000 calls to the MMES were registered and analyzed during 1981–1987. Of those,

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90,800 calls were for acute respiratory distress. On average, this amounts to 40–60 calls daily (*11*).

Relevant ambient air pollution measurements were conducted routinely during the same time span. Five monitoring stations distributed within the city limits measured SO_2 levels. SO_2 was (and still is) the indicator variable for air pollution in this region. During the winter months, domestic coal-burning heating units contributed approximately 65% of the total emissions measured within Leipzig. At times of extreme peak events (smog episodes), this could increase to 90%. Thus, these pollutants are not generated in other regions and transported but are emitted locally.

One monitoring station collected, in addition to SO_2, suspended particulates (PM_{10}) and carbon monoxide (CO). A previous investigation indicated that data collected at this particular monitoring station were representative for the yearly average conditions within Leipzig (*12*). All other monitoring stations were located in such a way to ensure that the data were representative and allow generalizations of the results for the entire city.

Measurements were taken continuously at each monitoring station. A central register collected the data automatically; each station produced 30-min mean values, for a total of 48 measurements per day. These were combined to obtain one 24-hr mean. These 24-hr mean values were then compared with the number of calls to the MMES. Figure 1 shows the SO_2 concentrations (24-hr means) for 1 year. In addition, using data from all stations, a 24-hr mean was calculated for the entire city. This value was calculated because the precise origin of the MMES calls could not be identified for each call. Therefore, an average citywide mean value was more representative for the analysis. During extreme pollution episodes (smog), the exposure measures collected at each station were virtually identical.

Time-Series Analysis

To analyze the data, the Fourier method, a time-series analysis, was applied. In using this method, two general problems have to be addressed: 1) stray data, which tend to lead to an inhomogenous data distribution and 2) pseudocorrelations, which tend to occur whenever deviations from the otherwise normal distributions of both the independent and the dependent variable temporally coincide, but actually depend on other parameters (e.g., temporality of association).

Clusters of data (temporal inhomogeneities) tend to occur whenever an increase in frequency is recorded that cannot be attributed to those external influences already under investigation, yet they coincide temporally. For example, it was noticed that the number of calls to the MMES increased on weekends or holidays, a time when the family physician was not available. For this reason and other minor incongruities, data collected on holidays were completely omitted from the analysis; Saturdays and Sundays were individually averaged according to the weekly average number of calls, Monday through Friday.

Many investigations relate the actual relevant air pollution event (smog) to the average duration of the entire event, which is actually comprised of a pre-event, actual event, and post-event time interval. First, this practice results in loss of information about the independent (air pollution) as well as the dependent variable (change in morbidity rate). Averaging any data contributes to loss of information as extreme values disappear, i.e., they are "averaged out." This will influence the outcome, especially when extreme events contribute to the effect. Second, this practice results in arbitrarily defining the length of the time intervals. Third, this practice results in effects being dependent on the length of each time interval, which may result in a shift of association between the three time intervals depending on the arbitrarily chosen duration of the total time period (i.e., the sum of the three time intervals).

This last point often makes comparison of studies impossible, as the length of the pre-event, actual event, and post-event time intervals strongly influences the remaining variance of the data. The duration of the three time intervals is essential in determining if a significant difference exists between the various time intervals (pre- or post-event, or whatever time interval is under investigation) and in relation to the independent and the dependent variable. The method applied in this study does not lend itself to such subjective manipulations.

To eliminate this time effect, all time periods for at least one variable (generally the dependent variable, i.e., changes in morbidity rate) were analyzed, using the Fourier analysis. To assess a possible association, the time-adjusted morbidity data were correlated with measures of the air pollution indicator component SO_2.

The time-adjusted morbidity, ΔM, is the difference between the raw morbidity data, $M(t)$, which were adjusted for temporal inhomogeneity, and the Fourier-analyzed morbidity data, $Mf(t)$ (Fig. 2). This means that the observed increase in the number of calls on weekends and holidays (not due to the independent variable, namely, an increase in the level of SO_2, but due to the lack of other available services)

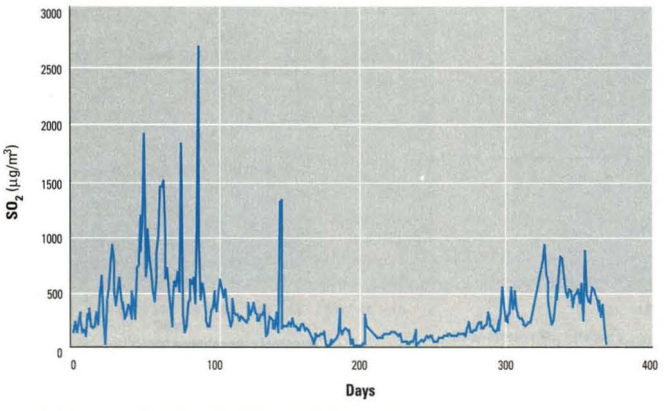

Figure 1. Daily mean sulfur dioxide levels for 1984 (24-hr averages).

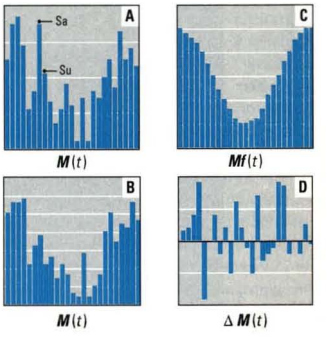

Figure 2. Controlling for temporal inhomogeneities and pseudocorrelations using morbidity measurements: (A) original data (Sa, Saturday; Su Sunday), (B) normed data, (C) Fourier-transformed data, and (D) deviation.

was adjusted by the daily average number of calls (averaged according to the weekly average number of calls). The adjustment was based on the mean of the relationship between the weekly average (Monday–Friday) and the mean value on Saturday and Sunday, respectively. Adjusting these data to the weekly average assured that extreme values remained in the data pool. This process prevents a pseudocorrelation caused by the normal, yearly temporal course of the dependent and independent variables. Using the Fourier analysis, it does not matter which of the variables, the independent, the dependent, or both, are used. In this study, the hypothesis was posed so that the Fourier analysis determined the morbidity to derive at threshold values. This requires that the data for the independent variable (pollutant concentration) are not transformed but numerically maintain their true value.

Figure 2 illustrates the different steps of the method. Figure 2A shows the raw daily morbidity data, i.e., the daily frequency of responses to calls to the MMES. Figure 2B shows the adjusted data, with weekends adjusted according to the weekly mean. The study also revealed that the frequency of calls to the MMES remained fairly constant throughout the week (Monday to Friday) with no significant difference in the frequency of calls between the different week days. Figure 2C shows the Fourier curve. Figure 2D shows the difference between the adjusted and the Fourier-analyzed data and corresponds well to the actual variance of the now time- and season-independent morbidity rate. These data were then used to determine the threshold values.

Results

Threshold Values

Pilot studies have revealed a temporal delay of human health effects associated with extreme pollution exposure levels. If these adjustments are applied to the raw morbidity data, health effects or variations in morbidity are observed 1 day (24 hr) later (*7–10,13*). This 1-day lag period is based on the assumptions that a response has to take place first and that health effects are only recorded on a 24-hr basis.

Taking this lag period into account, the exposure interval day 1 to day 14 and the effect intervals are temporally offset by 1 day, resulting in an integration interval. The observed effect is then the average deviation of the morbidity rate, calculated for the integration interval using the Fourier-transformed curve. Assuming a dose–response effect, a nonlinear dependence can be expected between frequency of morbidity and dose of exposure. This dependency is also supported by the results obtained from environmental epidemiologic studies shown in Figure 3.

The time-independent morbidity rate and exposure measurements (with SO_2 as the indicator variable) were further analyzed by calculating continuous means. For example, for every 2 days, means were calculated by averaging the exposure measurements. These 2-day means were then sorted into a series of exposure concentration levels. Each 2-day mean was then compared with the corresponding observed 1-day delayed morbidity variation and sorted according to the 2-day-averaged concentration levels. This resulted in a table that

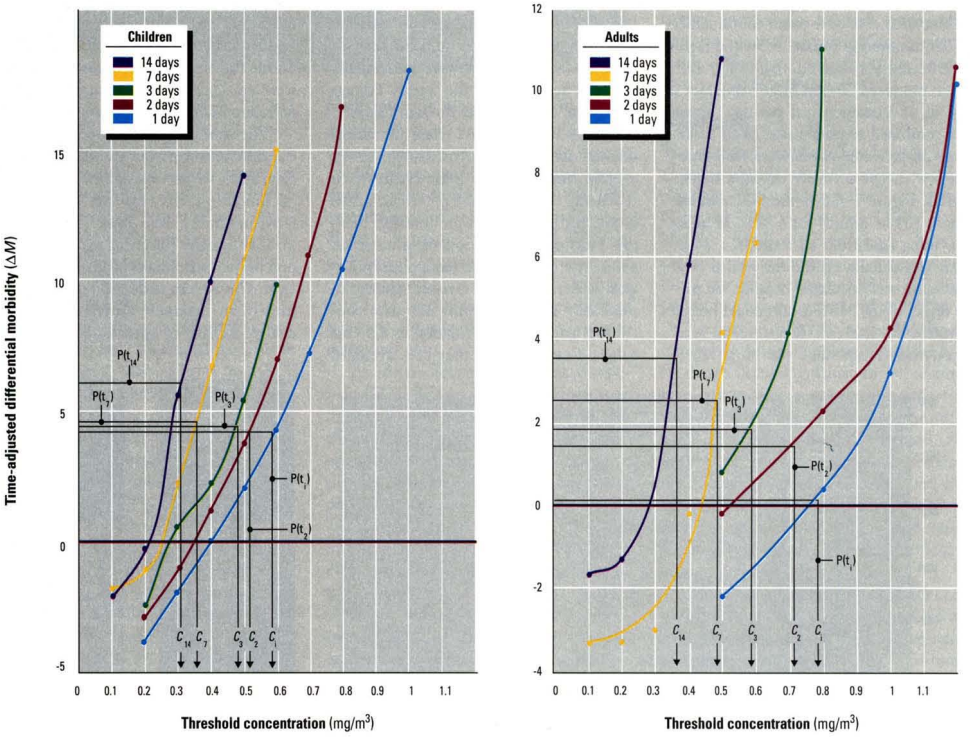

Figure 3. Derivation of threshold values (derived from the time-series analysis of time-adjusted morbidity). ΔM, time-adjusted differential morbidity [$M(t) = \Delta Mf(t) - M(t)$]; $Mf(t)$, Fourier-analyzed data; $M(t)$, raw data; $P(t_i)$, test variable for the ith integration interval; t_i, integration interval with length i; c_i, threshold concentration (if $c > c_i$ the greater the chance of increase in morbidity at exposure time $t = t_i$).

could be used to derive the (1-day delayed) morbidity rate for a specific exposure level. The same procedure was applied to the 3-day, 7-day, and 14-day averaged exposure measurements. Figure 3 illustrates this method. The expected trend is apparent: increased morbidity with increasing concentration within the same temporal integration interval and increased morbidity with increasing duration of the integration interval but constant level of exposure. Assuming a dependence between morbidity and exposure concentration allows one to derive threshold values.

Having established threshold values, one problem remains: above which point does the deviation from the mean morbidity rate become significant? To determine whether a significant deviation from the normal morbidity frequency occurred, a test variable was introduced. This test variable corresponds to the upper limit of the confidence interval of the morbidity frequency not attributable to the air pollution exposure. As the frequency of cases contributing to the estimate determines the Student's t-value, each temporal integration interval requires an individual test variable, $P(j)$:

$$P(j) = \Delta M_{\text{summer},\,j} + t_{\alpha} \cdot \delta(\Delta M_{\text{summer},\,j})/\sqrt{n}$$

where δ is the standard deviation and n is the number of variables.

Results indicate that effect-dependent threshold values for respiratory tract diseases differed in children and adults (Fig. 4). With the help of the test variable, $P(t_j)$, the exposure levels corresponding to the integration intervals were determined for the data depicted in Figure 3. Figure 4 presents the corresponding exposure concentrations and the integration intervals, which are essentially based on the exposure duration. Children were found to have an increase in morbidity at a level of 0.6 mg SO_2/m^3 air, whereas for adults this level was 0.8 mg SO_2/m3 air.

The shaded area in Figure 4 corresponds to the noncritical zone. As long as the level of exposure dependent on the duration of the exposure remains within this zone, the morbidity frequency is not expected to increase above background. However, should the concentration level measured and the duration of the exposure change and move outside this area, an increase in the morbidity of respiratory illnesses above normal background. Figure 4 also indicates that a child is more sensitive to lower concentrations of the pollutant than adults. This can be observed within all integration intervals.

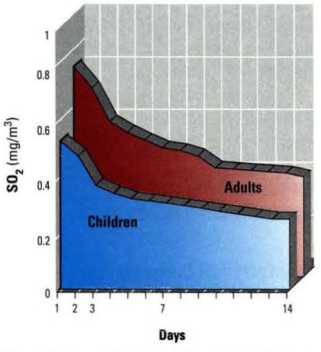

Figure 4. Dose–effect curve of respiratory illnesses for adults and children. The red and blue areas are the noncritical zones for adults and children, respectively.

Assessment of Attributable Risk

After determining threshold values, the next questions are what is the attributable risk associated with a pollution level beyond the threshold level and what increase in the burden can be expected?

Environmental epidemiologic studies always necessitate certain assumptions. The most critical assumption is associated with the confounding variables. In determining the threshold level, it was assumed that any confounding effect was virtually eliminated because the Fourier analysis rendered the morbidity time independent, particularly since cases not related to environmental effects were eliminated (e.g., respiratory distress conditions due to aspiration of foreign objects, not a negligible event in small children).

Allowing again for a lag period of 1 day and temporally adjusting the morbidity with the respective exposure measures, the number of cases were sequentially categorized according to concentration levels, for example, 0.6–0.8, 0.8–1.0, and 1.0–1.2 mg SO_2/m^3 air. Because the MMES was the only such service in Leipzig during the study period and because in case of respiratory distress the MMES was called without exception, the number of all children under 18 years of age living in Leipzig were used as a reference group. Like the frequency of calls (morbidity) among the population under investigation, the risk of respiratory disease increased above 0.6 mg SO_2/m^3 air (threshold level for children), as shown in Figure 5.

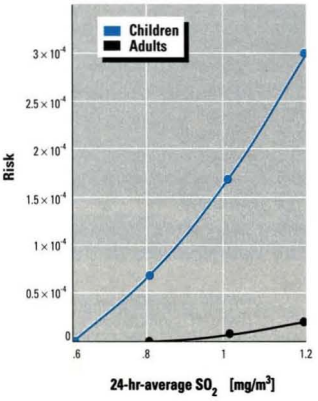

Figure 5. Attributable risk of respiratory illnesses (attributable risk indicates that risk above the expected level).

If the concentration doubles from 0.6 to 1.2 mg SO_2/m^3, respiratory diseases are increased above background to 3/10,000. It should be emphasized that this result is based on the number of all children under the age of 18 in Leipzig.

Discussion

Results of the methodology described here show that environmental epidemiologic studies can be used to quantify threshold values after controlling for confounding variables. The threshold value for excess respiratory illness among children due to environmental exposure was determined to be 0.6 mg/m^3 air (24-hr mean) for the indicator variable SO_2.

An attributable risk associated with a given change in the level of the pollution burden was estimated. The attributable increased risk associated with a doubling of the exposure burden from 0.6 to 1.2 mg SO_2/m^3 air was 3/10,000 children.

This method obviously has its limitations. For one, measuring an indicator variable precludes the determination of causality. An indicator variable does point toward an association between exposure to a pollutant and subsequent adverse respiratory health effects, but the potential of other air pollution constituents that may contribute to the same effect cannot be excluded. Nevertheless, the epidemiologic investigation presented here has the advantage that it occurs under real conditions in comparison to toxicological experiments.

Assessing threshold values and attributable risks should be considered when setting regulations. The preliminary model for smog regulation in Germany (*14*) established criteria for an early warning situation. One criterion is the presence of a low-exchange inversion weather front during which the level of SO_2 rises above 0.6 mg SO_2/m^3 (3-hr mean). Another one is that the low-exchange weather front should remain in effect for 24 hr, which virtually

establishes a 24-hr mean of 0.6 mg SO_2/m^3 air. Since this is an early warning stage, no consequences are expected. This study, however, shows that levels at and above 0.6 mg SO_2/m^3 air do indeed affect the health of susceptible population groups, such as children. Whether these levels affect only predisposed or vulnerable individuals cannot be determined with this method. Nevertheless, to prevent any undue adverse health effects among any individuals of the general population, the public should be advised that physical exertion (e.g., outdoor activities) be avoided, especially by vulnerable population groups such as children, when the early warning stage has been reached.

The purpose of this study was twofold: to investigate the methodological problem of determining threshold values and to derive at attributable risks based on environmental epidemiological studies. The results are a function of the same assumptions and limitations inherent in other studies. This also applies to problems associated with using an indicator component. Nevertheless, if the same conditions are applied as in other environmental epidemiology studies, such as correlation and regression as measures of the strength of the presumed association, identification of a temporal and spatial sequence in the relationship between the agent and morbidity, and comparability of the results with other studies, the statistical method presented here appears to be valid for determining threshold values and assessing the increased risk of respiratory tract morbidity associated with an increase in an air pollution constituent. Under these assumptions, the results show that environmental epidemiology can be used to assess a dose–response relationship and to estimate the attributable risk associated with a relative increase in the air pollution level.

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Third Congress of Toxicology in Developing Countries
Cairo, Egypt
November 19-23, 1995

Together for Human and Environmental Welfare

Organized by the National Research Centre, Cairo, Egypt, in collaboration with the Egyptian Society of Toxicology

Sponsored by the Academy of Scientific Research and Technology, Egypt, and many other national and international organizations

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Vice President, Abdell Khalek H.El-Sebae, Professor of Toxicology, Faculty of Agriculture, Alexandria University, Egypt
Vice President and Secretary General, Sameeh A. Mansour, Professor of Environmental Toxicology, National Research Centre, Cairo, Egypt

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Phones: (202) 3371211 / 3371362 / 3371433 / 3371499

New Books

Basic Documents on International Law and the Environment
P.W. Birnie, A.E. Boyle
Oxford: Oxford University Press, 1995, 500 pp. ISBN: 0198763204 (cloth), $75. 0198763212 (paper), $29.95.

East Asia's Environment; Principles and Priorities for Action
Jeffrey S. Hammer, Sudhir Shetty
Washington, DC: World Bank, 1995. ISBN: 082133297X, no price available.

The Ecology of the Chernobyl Catastrophe; Scientific Outlines of an International Programme of Collaborative Research
V.K. Savchenko
Pearl River, NY: Parthenon Publishing Group, 1995, 250 pp. ISBN: 1850706565, $85.00.

The Economics of Sustainable Development
Ian Goldin, L. Alan Winters, eds.
New York: Cambridge University Press, 1995, 314 pp. ISBN: 0521465559 (cloth), $59.95. 0521469570 (paper), $19.95.

Environmental Guide to the Internet
Jonathan F. Schupp
Rockville, MD: Government Institutes, Inc., 1995, 250 pp. ISBN: 0865874492, $49.

English–Spanish Glossary of Environmental Terms and Abbreviations
Milton Vasquez, Angelina Cazoria
Houston, TX: Gulf Publications, 1995. ISBN: 0884152545, $75.

Environment and Society; Human Perspectives on Environmental Issues
Charles L. Harper
Englewood Cliffs, NJ: Prentice Hall, 1995. ISBN: 0136690866, $24.

Environmental Epidemiology; Exposure and Disease: Proceedings of an International Workshop on Priorities in Environmental Epidemiology
Roberto Bertollini, ed.
Boca Raton, FL: CRC Press, 1995. ISBN: 1566700671 (alk. paper), $59.95.

Environmental Justice; Issues Policies, and Solutions
Bunyan Bryant
Washington, DC: Island Press, 1995, 300 pp. ISBN: 1559634170 $45.

Global Environmental Politics
Gareth Porter, Janet Welsh Brown
Boulder, CO: Westview Press, 1995, 230 pp. ISBN : 081332596X (cloth, alk. paper), $49.95. 0813321824 (paper, alk. paper), $13.95.

Handbook for Environmental Risk Decision Making; Values, Perceptions, and Ethics
C. Richard Cothern
Boca Raton, FL: Lewis Publishers, 1995. ISBN: 1566701317 (alk. paper), $69.95.

Handbook of Toxicology
Michael J. Derelanko, Manfred A. Hollinger, eds.
Boca Raton, FL: CRC Press, 1995, 1056 pp. ISBN: 0849386683, $125.

The Human Face of the Urban Environment; Proceedings of the Second Annual World Bank Conference on Environmentally Sustainable Development
Ismail Serageldin, Michael Cohen, eds.
Washington, DC: World Bank, 1995. ISBN: 0821333208, $7.95.

Medicine, Health, and Risk; Sociological Approaches
Jonathan Gabe, ed.
Cambridge, MA: Blackwell Scientific Publishers, 1995. ISBN: 0631194843, no price available.

Modulation of Cellular Responses in Toxicity
Corrado L. Galli, Marina Marinovich, Alan M. Goldberg, eds.
New York: Springer Academic Publishers, 1995, 371 pp. ISBN: 3540600108, $185.

Naturally-Produced Organohalogens
Anders Grimvall, Ed.W.B. deLeer, eds.
Boston, MA: Kluwer Academic Publishers, 1995. ISBN: 0792334353 (alk. paper), $177.50.

The Parents' Guide to Poisons in the Home; The Essential Sourcebook on Toxic Household Products
Gideon Koren
New York: Gramercy Books, 1995. ISBN: 0517148129, $5.99.

Placental Toxicology
B.V. Rama Sastry
Boca Raton, FL: CRC Press Inc., 1995, 304 pp. ISBN: 0849378125, $129.95.

Science, Politics, and the Pharmaceutical Industry
John Abraham
New York: St. Martin's Press, 1995. ISBN: 0312128738, no price available.

Summary of Contraindications to Oral Contraceptives
S.C.M. Knijff, et al.
Pearl River, NY: Parthenon Publishing Group, 1995, ISBN: 1850706913, no price available.

Toxic Microcystics
M.F. Watanabe, K.I. Harada, W.W. Carmichael, H. Fujiki
Boca Raton, FL: CRC Press, 1995, 400 pp. ISBN: 0849376939, $189.95.

The Wealth of Oceans: Environment and Development on Our Ocean Planet
Michael L. Weber , Judith A. Gradwohl,
New York, NY : W.W. Norton & Co., 1995. ISBN: 0393037649, $25.

2nd World Congress on Alternatives and Animal Use in the Life Sciences

October 20–24 1996, Utrecht, The Netherlands

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Calendar

October

10–11 October, Tue–Wed. Second Annual Gene Therapy Technology, Washington, DC. Information: Cambridge Healthtech Institute, 1037 Chestnut Street, Newton Upper Falls, MA 02164, (617) 630-1300 FAX: (617) 630-1325, e-mail: chi@world.std.com

11–13 October, Wed–Sun. European Conference on Combination Toxicology, Congress Centre Koningship Veldhoven, The Netherlands. Information: Secretariat Flora de Vrijer TNO Toxicology, PO Box 360, 370 AJ Zeist The Netherlands, 31 3404 44218, FAX: 31 3404 52224, e-mail: vrijer@voeding.tno.nl

12–13 October, Thu–Sun. Advances in Tissue Engineering, Washington, DC. Information: Cambridge Healthtech Institute, 1037 Chestnut Street, Newton Upper Falls, MA 02164, (617) 630-1300 FAX: (617) 630-1325, e-mail: chi@world.std.com

12–13 October, Thu–Sun. Third Annual Cell Adhesion Molecules, San Diego, California. Information: Cambridge Healthtech Institute, 1037 Chestnut Street, Newton Upper Falls, MA 02164, (617) 630-1300 FAX: (617) 630-1325, e-mail: chi@world.std.com

16–17 October, Wed–Thu. Advances in Signal Transduction and Gene Transcription, San Diego, California. Information: Cambridge Healthtech Institute, 1037 Chestnut Street, Newton Upper Falls, MA 02164, (617) 630-1300 FAX: (617) 630-1325, e-mail: chi@world.std.com

19–20 October, Thu–Fri. Arkansas Toxicology Symposium: New Horizons in Chemical-Induced Liver Injury, The Doubletree Hotel, Little Rock, Arkansas. Information: Jack A. Hinson, Director, Division of Toxicology University of Arkansas for Medical Sciences, Little Rock, AR 72205, (501) 686-5766, FAX (501) 686-8970

19–22 October, Thu–Sun. Eighth International Conference of the Society for Human Ecology: "Livelihood and Liveability", Lake Tahoe, Tahoe City, California. Information: Nancy L. Markee, University of Nevada Reno, MS 199, Reno, NV 89557, (702) 784-1674, FAX (702) 784-1142, e-mail nlmarkee@scs.unr.edu

21–25 October, Sat–Wed. Mechanisms and Prevention of Environmentally Caused Cancers, Santa Fe, New Mexico. Information: Alice M. Hannon, The Lovelace Institutes, 2425 Ridgecrest Drive S.E., Albuquerque, NM 87108-5127, (505) 262-7255, FAX (505) 262-7043

22–27 October, Sun–Fri. Fifth International Conference on the Chemistry and Biology of Mineralized Tissues, Kohler, Wisconsin. Information: L. Keller, The University of Texas, Health Sciences Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284-7823

29 October–1 November, Sun–Wed. Thirteenth International Neurotoxicology Conference: Developmental Neurotoxicity of Endocrine Disrupters, Hot Springs, Arkansas. Information: Joan Cranmer, Conference Chairman, Department of Pediatrics #512, University of Arkansas Medical Center, 4301 West Markham Street, Little Rock, AR 72205, (501) 320-2986, FAX (501) 320-4978

29 October–3 November, Sun–Fri. The XVIII Symposium of the International Association for Comparative Research on Leukemia and Related Diseases, Kyoto International Conference Hall, Kyoto, Japan. Information: Secretariat, The XVII Symposium of IACRLRD, Laboratory of Molecular Oncology, The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako, Saitama 351-01, Japan, 81-48-462-1111 ext. 3161, FAX 81-48-462-4686

November

3–5 November, Fri–Sun. Living in a Chemical World–The Second Decennial Symposium, Hotel Omni-Shoreham, Washington, DC. Information: David Rall, 5302 Reno Road, Washington, DC 20015, (202) 244-5380, FAX (202) 966-3093

5–10 November, Sun–Fri. International Symposium: 66 Years of Surfactant Research, Vienna, Austria and Budapest Hungary, with poster sessions on board ship from Passau, Germany. Information: B. Lachmann Department of Anesthesiology, Erasmus University, Post Bos 1738, 3000 DR Rotterdam, The Netherlands, 31 10 4087312, FAX 31 10 4367870

6–9 November, Mon–Thu. Susceptibility and Risk: The Third Annual Symposium of the Health Effects Research Laboratory, Raleigh, North Carolina. Information: 1995 HERL Symposium Susceptibility and Risk, c/o RSD Conference Coordinator, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Mail Drop 70, Research Triangle Park, NC 27711, (919) 541-5193, FAX (919) 541-4002, e-mail:meetings$mail@ herl45.herl.epa.gov

9–10 November, Thu–Fri. Cell Cycle Therapeutics, McLean, Virginia. Information: Cambridge Healthtech Institute, 1037 Chestnut Street, Newton Upper Falls, MA 02164, (617) 630-1300, FAX: (617) 630-1325, e-mail chi@world.std.com

14–15 November, Tue–Wed. Ash VIII on Ash Management and Utilization, Stouffer Renaissance Hotel, Crystal City, Arlington, Virginia. Information: Richard Will, The Coordinate Group, Inc., Box 3356, Warrenton, VA 22186-1956, (800) 627-8913 or (703) 347-4500, FAX (703) 349-4540

14–16 November, Tue–Thu. The American College of Veterinary Pathologists, Marriott Marquis, Atlanta, Georgia. Information: Sue Parker or Nick A. Montana, ACVP Executive Office 875 Kings Highway, Suite 200, Woodbury, NJ 08096-3172, (609) 384-6287, FAX (609) 853-0411

15–17 November, Wed–Sun. Second Annual Nucleic Acid Technologies, Amsterdam. Information: Cambridge Healthtech Institute, 1037 Chestnut Street, Newton Upper Falls, MA 02164, (617) 630-1300, FAX: (617) 630-1325, e-mail chi@world.std.com

16–17 November, Thu–Fri. Environmental Enhancement through Agriculture, Boston, Massachusetts. Information: William Lockeretz, School of Nutrition, Tufts University, Medford, MA 02155, (617) 627-3223, FAX (617) 627-3887, e-mail: wlockeretz@infonet.tufts.edu

17–21 November, Fri–Tue. American Society of Tropical Medicine and Hygiene 44th Annual Meeting, San Antonio, Texas. Information: Paulette Anderson, ASTMH Headquarters, 60 Revere Drive, Suite 500, Northbrook, IL 60062, (708) 480-9592, FAX (708) 480-9282

19–23 November, Sun–Thu. Third Congress of Toxicology in Developing Countries, Cairo, Egypt. Information: Sameeh A. Mansour (V-P & SG/3rd CTOX-DC), National Research Centre, Dokki, Cairo, Egypt, (202)3371211/3371362/3371433/3371499 FAX (202)-3370931/3498353

20–23 November, Mon–Thu. International Conference on Health Consequences of the Chernobyl and Other Radiological Accidents, International Conference Centre Geneva, Switzerland. Information: T. Kjellström, Director, EHG, World Health Organization, 1211 Geneve 27, Switzerland, 41 22 791 3756, FAX 41 22 791 4123, e-mail: johnsonj@who.ch

28 November–1 December, Tue–Fri. New Ocular Therapeutics and Drug Delivery, Atlanta, Georgia. Information: Cambridge Healthtech Institute, 1037 Chestnut Street, Newton Upper Falls, MA 02164, (617) 630-1300, FAX: (617) 630-1325, e-mail chi@world.st.d.com

December

9–13 December, Sat–Wed. The American Society for Cell Biology Thirty-Fifth Annual Meeting, Washington Convention Center, Washington, DC. Information: The American Society for Cell Biology, Bethesda, MD 20814-3992, (301) 530-7153, FAX (301) 530-7139, e-mail: ascb.info@ascbfaseb.org

10–15 December, Sun–Thu. International Conference on Food Factors: Chemistry and Cancer Prevention, Act City Hamamatsu, Hamamatsu, Japan. Information: ICoFF Secretariat, Japan Institute for the Control of Aging, Nikken Foods Co. Ltd., 723-1, Haruoka, Fukuroi, Shizuoka 437-01, Japan, 81 538 49 0125, FAX 81 538 49 1267

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17–22 December, Sat–Fri. International Symposium on Environmental Biomonitoring and Specimen Banking, Honolulu, Hawaii. Information: K.S. Subramanian, Environmental Health Directorate, Health Canada, Tunney's Pasture, Ottawa, Ontario CK1A OL2 Canada (613) 957-1874, FAX (613) 941-4545

1996

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

8–14 February, 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

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

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

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

10–16, 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

April

28 April–1 May, 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

May

19–22 May, Fri–Mon. 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

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.

ISSX 1996 European Spring Workshop
Food Toxins and Host Mechanisms Conditioning Toxic Responses

Sitges, Spain
June 1–4, 1996

This European ISSX Workshop will take place Saturday, June 1–Tuesday, June 4 in the lovely seashore city of Sitges, located 30 km south of Barcelona. Workshop attendance will be limited.

The objective of the workshop is to bring together both senior and young scientists to present and discuss their latest contributions in diverse areas of host mechanisms, such as mechanisms of toxicity, role of biotransformation enzymes, and inhibitory and inducing effects which condition the response of xenobiotics. There will be particular emphasis on compounds present in diet. In addition to the opportunity for poster and oral presentations, the following subjects will be covered in scientific sessions:

- mechanisms of toxicity
- role of biotransformation enzymes
- inhibitory and inducing effects
- natural and artificial food toxins

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Angel Messenguer, CID, CSIC, Barcelona (Chairman)
Josefina Casas, CID, CSIC, Barcelona
Maria-Jose Gomez-Lechon, Hospital "La Fe", Valencia
Margarita G. Ladona, IMIM Barcelona
Antonio Martinez-Tobed, Lab. Almirrall Barcelona

For further information please contact:

Prof. Angel Messeguer
Department of Biological Organic Chemistry, CID (CSIC)
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Telephone: (34) -3-4006121
FAX: (34)-3-2045904
E-mail: issx96@cid.csic.es

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.

Society of Toxicology Reproductive and Developmental Toxicology Subsection Graduate/Postdoctoral Student Award

We announce our intention to make awards of recognition for the best platform and/or poster presentation by graduate students or postdoctoral fellows in the areas of reproductive and developmental toxicology at the 1996 Annual Meeting of the Society of Toxicology, which will be held in Anaheim, California on March 10–14. General areas of research can include female or male reproductive toxicology, reproductive endocrine toxicology, teratology/developmental toxicology, and/or postnatal functional assessment. Candidates for these awards should send to the address listed below, by November 1, 1995, a copy of the abstract that is being submitted to the Society for this meeting. An outline of the talk or a copy of the poster material should also be included if possible, to assist the judges.

The abstracts and posters should describe original research which may include applied studies, investigations of mechanisms of toxic response, or studies of basic biochemical, physiologic, or genetic mechanisms of action. Interested individuals may request Society information and abstract forms from the address below. All submitted material will be treated as confidential. The winning presentations will be announced at the Annual Meeting of the Specialty Subsection in Anaheim.

Contact: Robert J. Kavlock, Ph.D., Developmental Toxicology Division (MD-71), Research Triangle Park, NC 27711, Health Effects Research Laboratory, U.S. Environmental Protection Agency.

Predoctoral Fellowships for Minorities/Disabled Persons

Fellowships to provide up to five years of support for research training in the biomedical or behavioral sciences are available to minority students under a new initiative of the National Institutes of Health. Applications may be submitted by November 15. Applicants must be pursuing the PhD or equivalent research degree, a combined MD/PhD degree, or some other combined professional doctorate/research PhD. Support is not available for those enrolled in medical or other professional schools unless they are in a combined professional doctorate/PhD degree program in biomedical or behavioral research. In addition, only members from those minority groups that are underrepresented in these fields are eligible.

Contact: Dr. Walter Schaffer, Research Training Officer, National Institutes of Health, (301) 496-9743, e-mail: wsllq@nih.gov Reference: PA-95-029.

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 November 1, 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.

National Cancer Institute

Proposals to conduct clinical research employing new agents, concepts or strategies for the treatment of cancer are invited by the National Cancer Institute. This solicitation especially seeks new clinical investigators who have not previously had independent grant funding.

Approximately 10 awards totaling $2-million per year for four years are expected to be available. Letters of intent are requested by September 1 while full proposals will be due October 20.

For solicitation copy, contact: Diane Bronzert, Division of Cancer Treatment, National Cancer Institute, Executive Plaza North, Room 734, Bethesda, MD 20892. (301) 496-8866, FAX: (301) 480-4663, e-mail: bronzerd@dct.nci.nih.gov.

U.S. Energy Department's Outstanding Junior Investigator Program

Applications for grants under the U.S. Energy Department's Outstanding Junior Investigator Program—which supports non-tenured academic faculty who are involved in experimental and theoretical high energy physics or accelerator physics research—should be submitted by November 1.

Five to 10 awards averaging $40,000—$50,000 are expected to be available in FY 1996. This program seeks to identify exceptionally talented new high energy physicists early in their careers, facilitate the development of their research programs, and help maintain excellence in the teaching of physics at the university level.

For the application guide, contact: DOE, Division of High Energy Physics, Office of Energy Research, ER-221, Attn: Dr. Jeffrey Mandula, 19901 Germantown Rd., Germantown, MD 20874-1290. (301) 903-4829. Reference: Program Notice 95-09. CFDA No. 81.049.

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: 1-202-482-2443.

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 productivity, should be submitted to the National Institutes of Drug Abuse, Mental Health, or Alcoholism and Alcohol Abuse by either October 1 or 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 multi-year 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.

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 non cancer 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.

The University of Michigan, Occupational Health Program–Research Position Available

The Department of Environmental and Industrial Health at the School of Public Health is part of a federal training grant from the National Institute of Environmental Health Sciences to the United Auto Workers. The U-M evaluates the UAW Hazardous Materials Worker Training Program. The Department has one position available August 1, 1995–October 1, 1995, with the expectation of an additional three-year period of employment pending renewal of funding. A faculty appointment as a research scientist is expected for those with appropriate educational qualifications and experience.

The person who will fill this part-time position will assist in the development and production of final project reports of the evaluation activities and research of the past several years on a large-scale union-based occupational safety and health education project. This person will also co-author articles for publication. Pending funding beyond October 1, 1995, the position will in addition entail the design, planning and execution of a new evaluation research study.

Candidates for this position should posses:

- Master's degree or higher (PhD preferred) in public health, Environmental and Industrial Health Organizational Psychology.
- demonstrated research, writing and programmatic experience in training and evaluation, evaluation methodologies; experience or interest in behavioral research.
- demonstrated experience in data analysis, especially an ability to understand and interpret quantitative data and experience in analyzing interviews; experience with worker populations
- significant experience in writing documents, such as research findings, reports, manuscripts for publication
- flexibility in scheduling.

There is some flexibility with respect to percentage of appointment. Consultation arrangements for the short term may also be made.

For information, please contact:
Dr. Thomas Robins at (313) 936-0757
e-mail: trobins@spu.umich.edu
or Robin Graubarth at (313) 747-4457
e-mail: RobnGrau@umich.edu

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.

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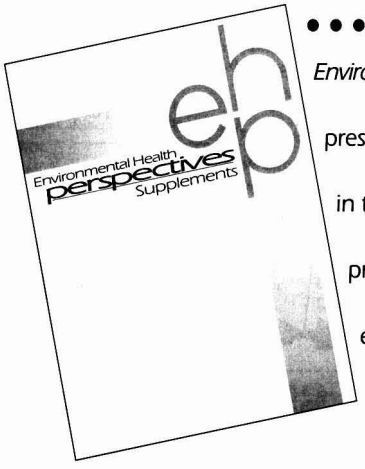

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Editorial Policy

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 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 policy is to give the corresponding author of each published article 200 free reprints.

Scientific Research

Scientific articles are subject to rigorous peer review. Two formats are available for the publication of scientific articles:

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. Lengthy historical perspectives are not appropriate in this category. Clarity of presentation is of primary importance because these articles are intended to be educational though targeted to the expert audience.

Opinions, Ideas, 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. Several 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.

Reviews & Commentaries are up-to-date, narrowly focused review articles that may present commentaries offering perspective and insight on a particular topic. Only recent developments in a field should be addressed.

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. Letters to the Editor cannot exceed 1200 words.

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 have focused on the development of a series of monographs that have generally arisen from symposium or conference proceedings. We continue to publish monographs, but they now appear as supplements to the main journal. Six to eight supplements are published per year. Four to six of these consist of conference, workshop, or symposium proceedings, and two issues are dedicated to the publication of solicited and unsolicited comprehensive reviews on environmental health. All articles published in the supplements, regardless of their source, are peer reviewed.

Each supplement resulting from a conference, symposium, or workshop should address a specific problem, an area of concern, a research problem, or a particular scientific issue. Supplements will, in general, be dedicated to scientific issues and not programmatic themes. It is intended that each collection of manuscripts form a landmark statement for a particular subject. Each supplement must be an up-to-date, balanced source of reference material for researchers, teachers, legislators, and the informed public. Publication of conference proceedings in *Environmental Health Perspectives 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 *Environmental Health Perspectives Supplements* set aside for the publication of reviews in environmental health sciences. Perspective reviews are in-depth, comprehensive review articles that address developments in specific scientific areas. Perspective reviews must not be simply a compilation of the literature. Perspective reviews 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

To ensure fairness, objectivity, and timeliness in the review process, we routinely request three reviews. Therefore, authors must submit four copies of each manuscript. All manuscripts must conform to the instructions to authors; those that do not will be returned without review.

All manuscripts must be typed, double-spaced, in English. Type the article on white paper, 216 ×279 mm (8.5 × 11 in) or ISO A4 (212 × 297 mm), with margins of at least 25 mm (1 in). Type only on one side of the paper. Number pages consecutively, beginning with the title page. If the manuscript is accepted for publication, a computer disk copy must be submitted along with two hard copies of the revised manuscript. Organizers of conference, symposium, or workshop proceedings will receive 25 free copies of the published supplement. Corresponding authors will receive 100 free reprints after publication.

Organization of Manuscripts

Research Articles are manuscripts reporting scientific research and discovery in the broad field of environmental health and may come from any field of scientific research. Criteria for publication are weighted toward quality and environmental significance.

Title Page: List title, 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).

Second Page: Provide a short title (not to exceed 50 characters and spaces) that can be used as a running head. List 5–10 key words for indexing purposes. List and define all abbreviations. Nomenclature and symbols should conform to the recommendations of the American Chemical Society or the International Union of Pure and Applied Chemistry (IUPAC). Include acknowledgments and grant information.

Abstract: Place a double-spaced abstract on the third page. The abstract should not exceed 250 words. The abstract should state the purpose of the study, basic procedures, main findings, and the principal conclusions. Emphasize new and important aspects of the study or observations. The abstract should not include details of materials and methods or references.

Introduction: Begin the introduction on a new page. State the purpose of the research and give a brief overview of background information. Do not include data or conclusions from the work being reported.

Methods: Begin on a new page. Describe the materials used and their sources. Include enough detail to allow the work to be repeated by other researchers in the field or cite references that contain this information.

Results: Begin on a new page. Present your results in logical sequence in the text. Do not repeat materials and methods, and do not repeat data in tables or figures. Summarize only important observations. Results and Discussion may be combined if desired.

Discussion: Begin this section on a new page. Emphasize new and important aspects of the study and the conclusions that follow. Relate results to other relevant studies. Do not simply recapitulate data from the Results section.

References: Begin this section on new page. References are to be numbered in order of citation in the text and should be cited in the text by number in parentheses. The style for references is as follows:

Journal Article:

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.

Book:

3. Harper R, Smith ECB, Jones DB. Odour description and classification. New York: Elsevier, 1968.

Editor as Author:

4. Doty RL, ed. Mammalian olfaction, reproductive processes, and behaviour. New York: Academic Press, 1976.

Conference Proceedings:

5. Ames B, Shigenaga MK, Gold LS. DNA lesions, inducible DNA repair, and cell division: three key factors in mutagenesis and carcinogenesis. In: Proceedings of the conference on cell proliferation, 14–16 May 1992, Research Triangle Park, NC. New York:Xavier, 1993; 35–44.

Government Report:

6. Melvin DM, Brooke MM. Laboratory procedures for the diagnosis of intestinal parasites. Report no. 75-8282. Atlanta, GA:Centers for Disease Control, 1974.
7. U.S. EPA. Status of pesticides in reregistration and special review. EPA 738-R-94-008. Washington, DC:Environmental Protection Agency, 1994.

Other Publications:

8. IARC. Arsenic and arsenic compounds. In: IARC monographs on the evaluation of carcinogenic risk of chemicals to man, vol 23. Some metals and metallic compounds. Lyon: International Agency for Research on Cancer, 1980;39–141.
9. Spiegelhalder B, Preussmann R. Nitrosamines and rubber. In: N-nitroso compounds: occurrence and biological effects (Bartsch H, O'Neill IK, Castegnaro M, Okada M, eds), IARC scientific publications no. 41. Lyon:International Agency for Research on Cancer, 1982;231–243.

Abbreviate journal names according to *Index Medicus* or *Serial Sources for the BIOSIS Previews Database*. List all authors; do not use et al. in the bibliography. Include the title of the journal article or book chapter and inclusive pagination. References to papers that have been accepted for publication but have not yet been published should be cited in the same manner as other references, with the name of the journal followed by "in press." Personal communications, unpublished observations, manuscripts in preparation, and submitted manuscripts should not be listed in the bibliography. They are to be inserted at appropriate places in the text, in parentheses, without a reference number.

Figures and Legends: Three sets of publication-quality figures are required. Graphs and figures should be submitted as original drawings in black India ink, laser-printed computer drawings, or as glossy photographs. Electronic versions of figures are encouraged, but should be submitted in addition to, not in lieu of, hardcopies of the figures. Dot matrix computer drawings are not acceptable as original art. The style of figures should be uniform throughout the paper. Letters, numbers, and symbols must be drawn to be at least 1.5 mm (6 points) high after reduction. Choose a scale so that each figure may be reduced to one-, two-, or three-column width. Identify all figures on the back with the authors' names and figure number; indicate TOP. Color figures will be considered for publication if the color facilitates data recognition and comprehension.

Figure legends should be typed on a separate page following the references. Legends should be numbered with Arabic numerals.

Tables: Each table must be on a separate page. Tables should be numbered with Arabic numerals. 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 *, **, #, ##. Type footnotes directly after the table. Complex tables should be submitted as glossy photographs.

Computer Disks: Electronic copies of initially submitted manuscripts are not required. Revised manuscripts resubmitted after acceptance for publication must be sent in electronic form together with two hard copies.

Submit electronic formats on 3.5" disks suitable for reading on either PC or Macintosh platforms. Macintosh is the preferred platform, although PCs are acceptable. The file should contain all the parts of the manuscript in ONE file.

Label the outside of the disk with the title of the manuscript, the authors, and the number it has been assigned. Name the computer used (e.g., IBM, IBM compatible, Macintosh, etc.) and the operating system and version (e.g., DOS 3.3). Identify the word processing program and version. Microsoft Word format is preferred, and its use will greatly facilitate publication; however, we can convert other formats, including: Microsoft Word, WordPerfect, ASCII, Text Only.

Research Advances are concise articles intended to address only the most recent developments in a scientific field. Lengthy historical perspectives are not appropriate. Begin with the title

page and continue as described for research articles. References, abbreviations, figures, and tables should be handled as described for research articles. Clarity of presentation is of primary importance and the use of color figures is encouraged. Include a photograph (black and white or color) of the author together with a brief biography. If multiple authors or groups are involved, up to three biographies with photographs may be included.

INNOVATIONS are short articles that describe novel approaches to the study of environmental issues. Prepare initial pages as described for Research Advances. Maintain text in a clear and precise manner and wherever possible include color photographs to illustrate strategy and clarify conceptual problems. Some degree of speculation regarding the potential usefulness of a new technique or novel process in other areas of environmental health may be included. References should not be included, but a suggested reading list is required.

COMMENTARIES are short articles offering ideas, insight, or perspectives. Begin with a title page and second page as described for research articles. Include a brief abstract.

REVIEWS & COMMENTARIES are brief, up-to-date, narrowly focussed, review articles with commentaries offering perspective and insight. Begin with a title page and second page as described for Research Articles. Include an abstract and handle references, tables, figures, and abbreviations as described for Research Articles.

MEETING REPORTS should not exceed 2400 words in length. Begin with the title of the meeting and authorship of the report and start text on the next page. Detail when and where the meeting was held, how many people participated, who sponsored the meeting, and any special organizational arrangements. Meeting sponsors and principal participants, such as session chairs, may be listed on a separate page. The report should summarize the contributions of the meeting to scientific knowledge, insight, and perspective; this should not take the form of comments of participants or personalized perspectives. Space is limited, so only the highlights should be mentioned. Novel ideas, perspectives, and insights should be emphasized. Do not describe social aspects of the meeting. Send an electronic copy and four hard copies.

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 it is appropriate that the major source of funding be from a disinterested source or that organizational safeguards be set in place to minimize the intrusion of institutional bias.

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.

Submission of Manuscripts and Proposals

Submit all manuscripts and proposals in quadruplicate to:

Editor-in-Chief
Environmental Health Perspectives
National Institute of Environmental Health Sciences
PO Box 12233
111 Alexander Drive
Research Triangle Park, NC 27709 USA

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.

Permission to reprint figures or tables from other publications must be obtained by the author prior to submission of the manuscript.

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
111 Alexander Drive
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.

Persons interested in free-lance writing opportunities with Environmental Health Perspectives should submit a cover letter, resume, and writing samples to the address above. For inquiries call the associate news editor at (919) 541-5377.

National Institute of Environmental Health Sciences
Postdoctoral Research Opportunities

Listed below are outstanding opportunities to conduct research with leading scientists in Research Triangle Park, North Carolina.

- Molecular Mechanisms of DNA Repair
- Molecular Neurobiology
- Ion Homeostasis and Cell Injury
- Molecular Dosimetry and Epidemiology
- Molecular and Cellular Biology
- Mechanisms by Which Organisms Produce Mutations
- Mechanisms of DNA Replication
- Reproductive Biology and Toxicology
- Cell Adhesion in Metastasis
- Molecular Mechanisms of Respiratory Diseases
- Epitope Mapping
- Molecular Biology and Fatty Acid Biochemistry
- Laboratory of Reproductive and Developmental Toxicology
- Gametogenesis
- Signal Transduction
- Molecular Biology of Renal Transport
- Molecular Biomarkers of Risk
- Ion Channel Physiology and Modulation

For more information on these positions please see the Position Announcements Section, p. 863

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