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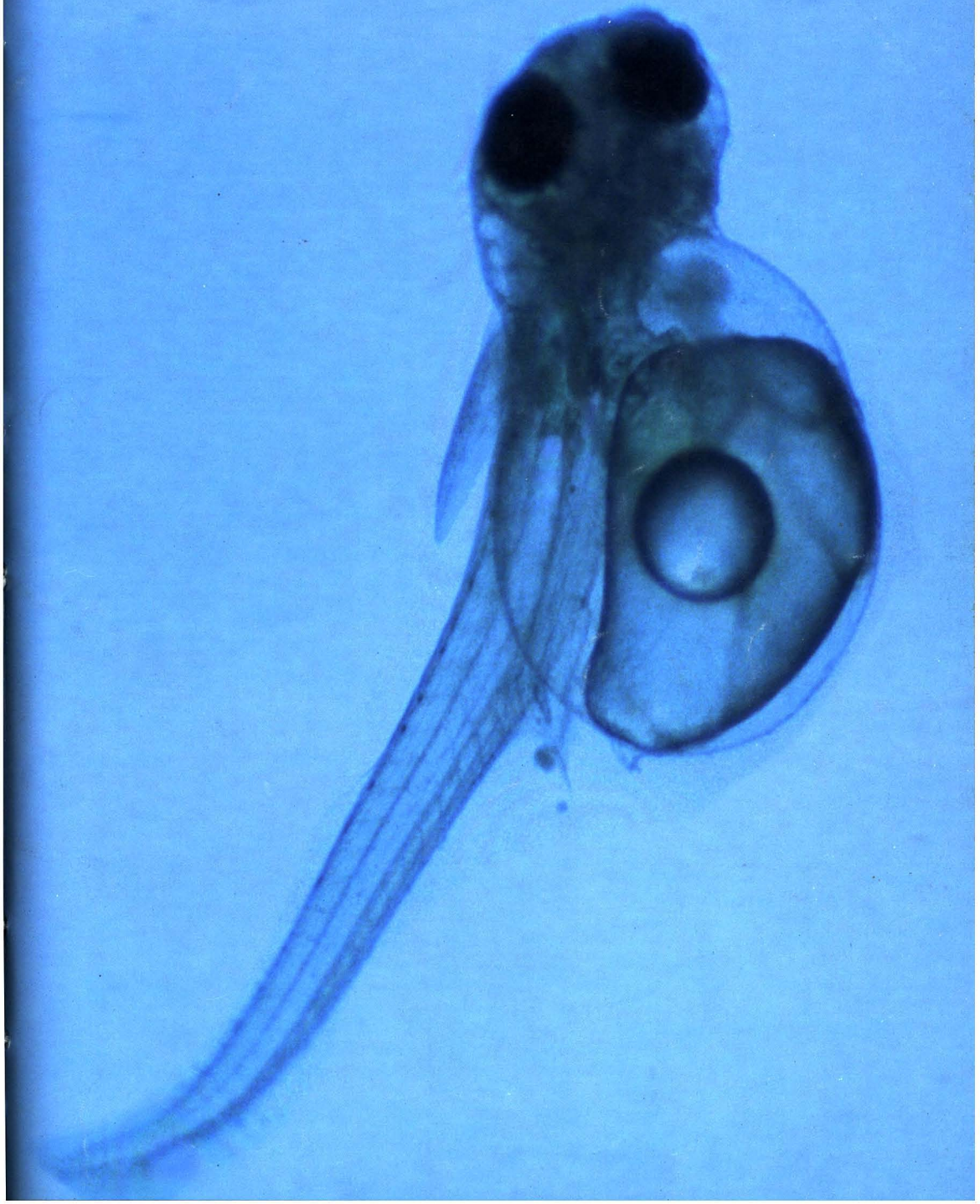

The National Institute of Environmental Health Sciences
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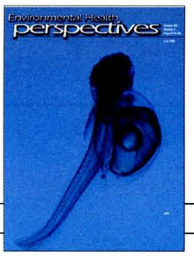
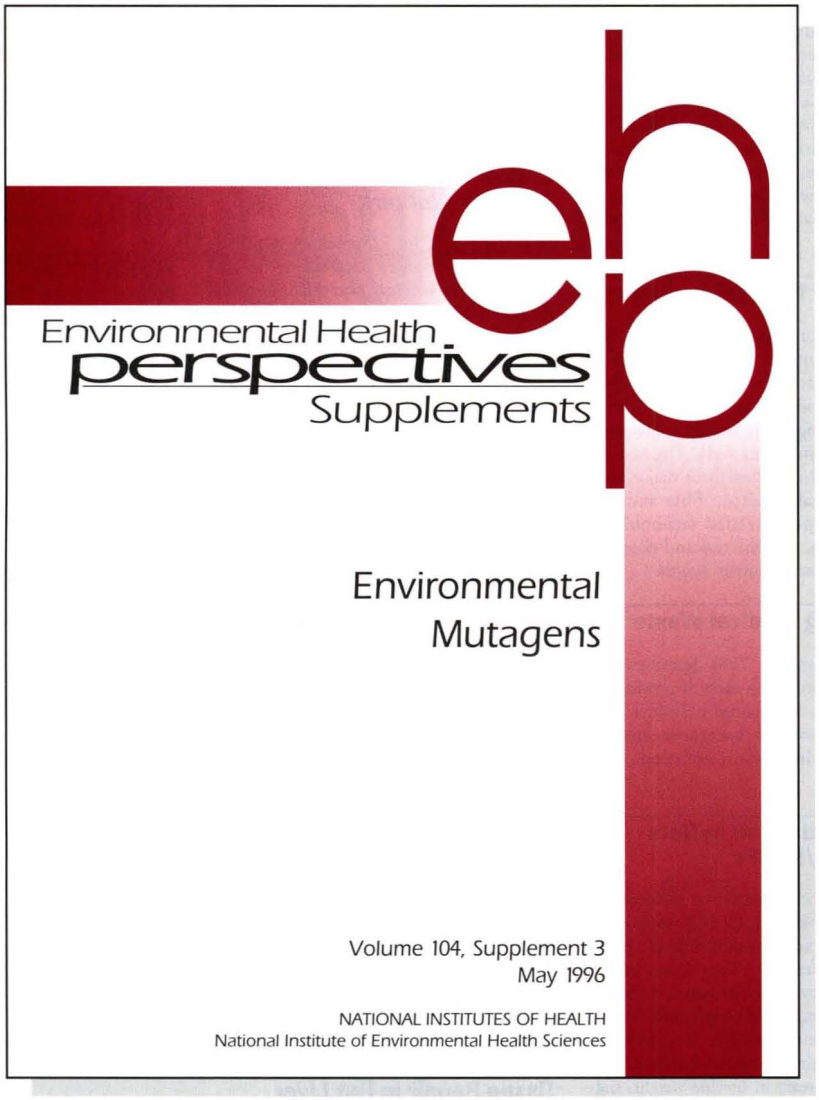
On The Cover: Medaka larva with severe abnormalities after treatment with trichloroethylene whole extract **(Villalobos et al., p. 734)**.





A Tradition of Progress

EHP Supplements continues its 24-year tradition of publishing monographs based on current environmental issues and conference proceedings, as well as an annual review of environmental health. The Environmental Mutagens issue highlights research achievements, future research directions, and health problems associated with environmental mutagens.

For subscription information, see p. 788.
For a listing of published volumes of *EHP Supplements*, see p. 793.

In This Issue

Emerging Disease Threats

Twenty-nine diseases and microbes have emerged since 1973 including HIV, *Cryptosporidium*, and *Vibrio* cholera, and 20 diseases have reemerged in the last two decades including tuberculosis, dengue, and malaria. The first **Focus** (p. 694) describes how environmental changes resulting from human activities may be contributing to the emergence and spread of disease worldwide.

Hazards of New Technologies

As society approaches the 21st century, technology seems to advance at an almost breathtaking pace. For centuries, however, people have realized that with the benefits technology brings there usually come problems or limitations as well. The second **Focus** (p. 700) describes three major areas of technological advances: fiber and fine particles, computer-related technologies, and transgenics, and the real and potential hazards they pose to human health.

Eliminating Medical Waste

The **Innovations** (p. 708) describes how new hospital products, such as gowns and surgical drapes, made from polyvinyl alcohol can cut down on hazardous medical waste, reduce incinerator emissions, and save money.

Enzyme Induction in Rats Given PCB/PCDFs

A 2-day prepubertal female rat bioassay was used by Li and Hansen (p. 712) to estimate both dioxinlike and non-dioxinlike biological responses from extracts of an Illinois landfill contaminated by polychlorinated biphenyls and polychlorinated dibenzofurans. Several metabolic enzymes in rat liver were induced, serum total thyroxine was reduced, and there was a 35% increase in uterine weight, suggesting that a comprehensive approach that uses both biochemical and endocrinological endpoints might improve assessments of risk for complex mixtures.

Global Warming and the Asian Sandfly

A discriminate analysis model was used to simulate global warming and predict seasonal and geographic distribution of the sandfly in Soutwest Asia. This insect serves as a vector responsible for the endemic transmission of leishmaniasis and sandfly fever. Cross and Hyams (p. 724) report that with an increase of 1°C, 14 locations could become endemic with disease transmission, 17 more with a 3°C increase, and 12 more with a 5°C increase. The model also predicted that seasonality for disease would be extended throughout 12 months in 7 locations with at least a 3°C rise in temperature and in 29 locations with a 5°C rise.

Cancer and Mortality in Alachlor Workers

Acquavella et al. (p. 728) examined mortality and cancer rates between 1968 and 1993 in Iowa workers potentially exposed for about 17,000 worker years to alachlor, the active ingredient in a family of preemergent herbicides.The authors report lower mortality than expected and cancer incidences no higher than the overall rate for Iowa, suggesting that there was no appreciable effect of alachlor even though pesticide exposure at the production facility greatly exceeded that characteristic of agricultural operations.

Dioxin Bioassays for Trichloroethylene Incineration Products

Medaka embryo cardiotoxicity, antiestrogenicity in trout liver cell cultures, and guinea pig Ah receptor assays were used by Villalobos et al. (p. 734) to evaluate dioxinlike responses to an incompletely combusted trichlorethylene aerosol, a toxic by-product that mimics transient incinerator emissions. Extracts of the soot aerosol caused embryotoxicity, induced cytochrome P450 and metabolic enzyme activity, reduced estradiol-dependent vitellogenin synthesis, and enhanced Ah receptor binding. TCDD and dibenzofuran were not detected in the by-products, suggesting that unknown components associated with incomplete combustion of trichloroethylene may pose health risks through dioxinlike mechanisms.

Tissue Repair in Rat Liver

Mangipudy et al. (p. 744) used an animal model to investigate thioacetamide toxicity and the role of cell proliferation in tissue repair. The authors report complete survival of thioacetamide-treated rats in the absence and complete mortality in the presence of colchicine doses that block cell division, but found sustained and stimulated tissue repair when colchicine doses are used that incompletely block cell division, emphasizing the critical role of tissue repair in toxicity responses.

Air Pollution and Lung Cancer Risk in Italy

Spatial models were used to evaluate sources of pollution around Trieste, Italy. Based upon autopsy registrys, Bigerri et al. (p. 750) report that there was a moderate risk for lung cancer in relation to the city center or to proximity of an incinerator and suggest that air pollution may have been a causal factor in tumor development.

PCDD/PCDF/PCBs in Norwegians Eating Contaminated Crabs

Johansen et al. (p. 756) determined whole blood concentrations of 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins and polychlorinated-*p*-dibenzofurans and 19 different PCB congeners from men ingesting different amounts of crab from a Norway fjord polluted with organochlorine compounds from a Mg-production plant. There were stronger correlations between crab consumption and the sum of PCDDs and PCDFs in blood and a weaker correlation between crab consumption and some of the highly chlorinated PCBs or with urine cadmium concentration.

PCB/DDT Metabolites in Human Milk

Norén et al. (p. 766) sampled human milk in Stockholm at seven intervals between 1972 and 1992 for methylsulphonyl metabolites of chlorinated biphenyls and methylsulphonyl metabolites of *p,p'*-DDE. Concentrations of metabolites of chlorinated biphenyls decreased from about 9 to 2 ng/g lipids, and those of *p,p'*-DDE from about 5 to 0.4 ng/g lipids, and were correlated with the levels of total PCB or of total *p,p'*-DDE. The major metabolite in milk was from *p,p'*-DDE, while PCB methylsulfones with 5 and 6 chlorine atoms were predominant.

P450 in Crabs in Japan

Ishizuka et al. (p. 774) measured cytochrome P450 content in hepatopancreas of the fresh water crab, *Eriocheir japonicus*, as well as activities of several metabolic enzymes, and found some correlation with polycyclic aromatic hydrocarbon (PAH) pollution in the Tone river. The activities of benzo[*a*]pyrene 3-hydroxylase, ethoxycoumarin *O*-deethylase, ethoxyresorufin *O*-deethylase, imipramine 2-hydroxylase, bunitrolol 4-hyroxylase, and metabolic activation of benzo[*a*]pyrene appeared to be useful indicators of levels of PAH in the environment.

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BASIC RESEARCH PROGRAM

The National Institute of Environmental Health Sciences

Editorial

Friendly Fire

"Chance favors the prepared mind"
Louis Pasteur

Progress in science has been marked through the ages by public airing of controversial issues. Indeed, the rewards for innovative progress have included banishment, imprisonment, casting out devils, and even burning at the stake (*1*). Those who dared to challenge prevailing concepts that matter was composed of four fundamental substances, earth, air, fire, and water (*2*), and that humors were elemental body fluids defining the physiologic and pathologic teachings of the Hippocratic school (*3*) often became scientific martyrs. Scientific debate continued unabated throughout the ages. One of the most famous controversies involved Copernicus, who challenged all Christendom when he proposed that the earth revolved around the sun casting doubt on the geocentric theory "proven" by Ptolemy (*4*). The concept that the earth was round instead of flat was decried by kings and rulers worldwide until Magellan sailed the oceans (*5*). Harvey's proposal that the blood circulated through the body in a closed system pumped by the heart was booed out of the lecture hall (*6*), while the rejection of spontaneous generation and the idea that unseen microbes transmitted disease almost cost Pasteur his tenured position (*7*).

Todays novel ideas and scientific hypotheses are debated with no less enthusiasm but with less risky consequences. The editors of *Environmental Health Perspectives* begin a series of articles designated **Friendly Fire** that will appear whenever controversial scientific issues would benefit from public debate. The title of the series derives from the military term denoting self-inflicted casualties that are unavoidable whenever conflict ensues. We hope that no mortality occurs, but we will accept minor wounds in the battles ahead.

Initially the editors of EHP will select topics that are of broad interest to the environmental community and of great importance for public health, such as risk assessment for arsenic (*8*), health effects of EMF exposure (*9*), linear versus nonlinear dose-responses for dioxin (*10*), long-term toxicity from lead exposure (*11*), value of metal chelation therapy (*12*), risks of radon exposure (*13*), multiple chemical sensitivity (*14*), and so on. Topics may be submitted by readers as well, in hopes that a public and open format for debate will maintain an objective and informative source of information for the broad readership of EHP.

The information for this new section will be added to the Editorial Policy and Instructions for Authors found in the back of each issue of EHP. Ordinarily, one of the editors of EHP will write a short introduction for the debate, followed by experts in the field who will write either a "pro" or "con" article citing the scientific references that justify their positions on the topic. A feature unavailable to other authors for EHP will be the opportunity for the experts to exchange articles and write one page rebuttals to be included in the published material. Occasionally a guest editor, with approval and review by EHP, may nominate expert authors and be responsible for production of the entire section.

Some of the current topics scheduled for debate include discussions of health risks from arsenic ingestion, policies used in risk evaluation of electromagnetic fields, relationships between exposure to electromagnetic fields and breast cancer, epidemiological evidence of estrogen toxicity, and evaluation of multiple chemical sensitivity. The protagonists are experts in their fields who will present brief reviews of the topic with substantive evidence for interpretation of the available experimental data. Each of these topics must inevitably deal with estimations of risk, which necessarily involve social and economic as well as scientific issues. The journal will strive to address such controversial subjects that have great impact on environmental health with the aim to educate the public and advance the science of environmental research.

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Michael P. Dieter
Science Editor, EHP

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 36th Annual Meeting of the Society of Toxicology to be held March 9–13, 1997 in Cincinnati, Ohio. General areas of research may include male or female reproductive toxicology, reproductive endocrine toxicology, teratology/developmental toxicology, and/or postnatal development. By November 8, 1996, candidates for these awards should send to the address below 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 in their evaluation. The abstracts and posters should describe the original research which may include applied studies, investigations of mechanisms of toxic response, or studies of basic mechanisms of action. Interested individuals may request Society information and abstract forms from the Society of Toxicology in Reston, Virginia (703) 438-3115 or sothq@toxicology.org). All submitted material will be treated as confidential. The Winning presentations will be announced at the Annual Meeting of the Specialty Subsection in Cincinnati. For further information, contact:

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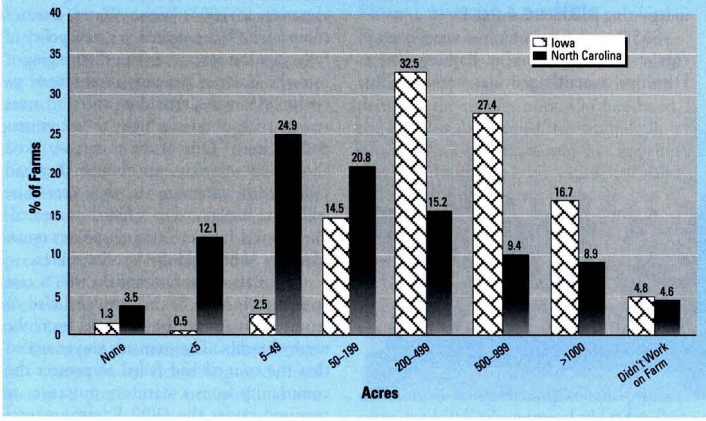


In the Commentary by Alavanja et al. published in EHP in Volume 104, Number 4, 1996, the legend for Figure 4, "Distribution of farm size in Iowa and North Carolina," was incorrectly labeled. The correctly labeled figure shown at left indicates that, in general, Iowa farms are larger than North Carolina farms.

Everything about microscopic life is terribly upsetting.
How can things so small be so important?
Isaac Asimov

Forum

Surfers Against Sewage

A ground swell of public concern about water pollution has undulated through Britain, thanks to Surfers Against Sewage (SAS). The organization, founded in 1990 by a group of Cornish surfers to protest local water pollution, now has over 20,000 members, including windsurfers, swimmers, and beach users, who lobby in protest of the 300 million gallons of sewage that are dumped daily and the 2 million tons of toxic waste that are dumped annually into the seas around Britain. Over half of the sewage dumped into the ocean is either raw or has received only preliminary treatment. Many beaches are littered with human excrement, tampons, condoms, and other sewage debris.

Not only is the pollution visually unpleasant, it also poses health risks to water users. High levels of pathogenic viruses and bacteria are contained within the sewage. SAS has developed a medical database that contains over 800 cases of individuals who have experienced adverse health effects stemming from activities in the ocean. The most common illnesses include gastrointestinal-problems and infections of the ear, nose, throat, eye, and skin. However, more serious illnesses such as hepatitis have also been attributed to water pollution. Over 68% of these cases have occurred at "Government Passed Beaches," says SAS. A study entitled *Health Risks Associated with Bathing in Sea Water*, which was commissioned by the Department of the Environment and published in the *British Medical Journal* in December 1991, found that there are increased health risks for those who enter British sea water. Surfers are 80% more likely to experience health problems than nonswimmers, while the risks for swimmers and waders are 31% and 25% higher, respectively, than for nonswimmers.

SAS has gained respect as a political pressure group from the organization's ability to blend the use of sound science, legal work, and media attention. The group has been described by the BBC as "some of government's most sophisticated environmental critics."

"Surfers Against Sewage is waging an effective and important campaign against senseless pollution of the seas. In their campaigns, SAS makes science accessible and relevant to people's experiences and, therefore, makes it matter," said Sue Mayer in the 1996 SAS annual report. Mayer is the former director of science at Greenpeace UK and currently works as a consultant on environmental science and policy issues. "By challenging the questionable assumptions in standard-setting, SAS makes politicians and institutions face up to their abuse of science in legalizing pollution," she said.

SAS is urging the reform of water quality regulations. The European Bathing Water Directive currently provides water quality standards for Europe. However, according to SAS, the United Kingdom enforces only the minimum legal standard, which meets only two out of 19 criteria set by the directive.

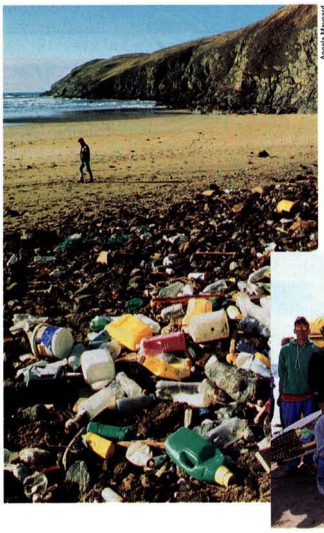
Standard practice for treating sewage involves administering a primary treatment and then sending the sewage through a long pipe out to sea. The idea is that harmful microorganisms will die as the sewage disperses throughout the water, and the sewage will not be harmful by the time it reaches shore. However, according to SAS, the current government system for measuring pathogens in the water is inadequate. And water users such as surfers and windsurfers, who venture farther from the shore, encounter sewage at the point of outfall, where it is most harmful.

SAS also pressures industry to change sewage treatment methods. The group is working to mandate that all sewage be fully treated before it is discharged into the sea and that both the liquid and sludge content be used as fertilizer. SAS also aims for the complete cessation of dumping of toxic waste into the oceans.

The SAS campaign has involved protesting, demonstrating, lobbying, and publicly pressuring the water industry. Demonstrators wearing wetsuits and gas masks have carried bags of toilet paper and panty liners collected from the beaches to the House of Commons, the European Commission in Brussels, and water industry conventions.

So far, SAS has experienced several major victories. In 1993, Welsh Water, a water company in Wales, agreed to a new policy of fully treating sewage before discharging it into the ocean. Company representatives credit SAS with persuading them to treat sewage with ultraviolet light to kill viruses and bacteria. "One of the things we liked about SAS was that even though they had colorful demonstrations . . . they were quite willing to explain to us what they wanted, like rational human beings," spokeswoman Margaret Abbett told the Associated Press.

This past April, two women won a case against their local council, which failed to require that sewage be removed from the nearby beaches. The women's lawyer argued that the council had failed to protect the community from a statutory nuisance, as required under the 1990 Environmental Protection Act. SAS members are hoping this


Angela Maynard

Chris Power

Trash warriors. A coalition of surfers, windsurfers, and beach users is fighting the dumping of sewage and trash into the seas around Britain.

case will set a precedent for other local governments to enforce sewage cleanup.

Carcinogens in Food

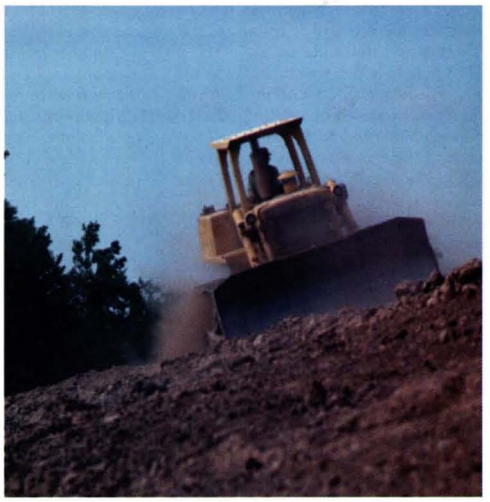
Labeled a "finding sure to appeal to anyone tired of washing vegetables in detergent to remove pesticides" by a *New York Times* health columnist, the National Academy of Sciences National Research Council's February report, *Carcinogens and Anticarcinogens in the Human Diet*, found little to be alarmed about concerning links between chemicals in food and cancer. "I've really been surprised at the great interest that has resulted from the study, and from the message that if you use common sense when you eat, you're alright," says Ronald Estabrook, a biochemistry professor at Southwestern Medical Center in Dallas who headed the 20-member panel that issued the report.

Specifically, the report found that, based on existing data, the great majority of naturally occurring and synthetic chemicals in the diet appear to be present at levels below which "any significant adverse biologic effect is likely, and [are] so low that they are unlikely to pose an appreciable cancer risk." Conversely, the varied and balanced diet needed for good nutrition "also provides significant protection from natural toxicants," the report says. The real cancer culprits in diet, the committee suggests—as other NRC reports have concluded—are excess fat and calories.

But others say there is much more to the story than appears beneath the "sigh-of-relief" headlines. Although the NRC committee made much of the fact that little scientific evidence exists on which to base their conclusions, this point was not adequately communicated to the public, according to committee member Bernard Weinstein, director of the Columbia-Presbyterian Cancer Center in New York. "I would have started the report emphasizing that we need much more intensive research in this area. There are a lot of open questions here and I wouldn't give a clean bill of health to these trace amounts of chemicals yet." As an example, Weinstein cited findings made public in April, after the report's release, that a gene known as *Shinga* can be transferred into bacteria and spread a toxin to humans from ground meat. "This is a minor compound, a natural chemical in beef. We should not be lulled into false security," he said.

There is also criticism of the committee's composition. According to Samuel Epstein, a professor of occupational and environmental medicine at the University of Illinois at Chicago, the group is "disproportionately weighted with industry consultants and others who trivialize the significance of avoidable exposures to industrial carcinogens in air, water, food, and the workplace, and who exaggerate the role of lifestyle risk factors and of naturally occurring carcinogens, particularly 'natural pesticides' in food." Epstein voiced such concerns to the NAS as far back as 1993 in his role as chairman of the Cancer Prevention Coalition, Inc., which bills itself as a coalition of independent experts in public health and cancer prevention. Al Meyerhoff, senior attorney with the Natural Resources Defense Council, agrees, saying that the conclusions suffer from "serious data gaps on toxins and exposures that make the report a dubious exercise. Increasingly, when dealing with cancer risk, 'science' is in the eye of the beholder," he says. "Different scientists reach fundamentally different conclusions."

Estabrook argues that the committee was unbiased and unanimous in its conclusions. But he concedes that the "database is shallow. We looked at what exposure data was available and we put it all into perspective. This is by no means the final word."



Getting tough on dumps? A new EPA rule includes stricter air pollution controls for large landfills, while a new law may exempt smaller dumps from ground water monitoring.

New Laws on Landfills

New environmental rules for landfills seem to be moving in opposite directions: more stringent for larger landfills and less burdensome for smaller ones. On one hand, the EPA has determined that landfills are a source of air pollution and has issued a new rule requiring large municipal solid waste landfills to control their emissions of certain gases. On the other hand, President Clinton has signed into law legislation allowing states to ease certain environmental requirements for small landfills, as long as human health and the environment remain protected.

The new EPA rule, promulgated under the Clean Air Act, aims to reduce landfill emissions of smog-creating volatile organic compounds (VOCs), some of which are also known or suspected carcinogens such as benzene, vinyl chloride, and chloroform. The rule will also cut methane emissions in half which, in terms of reducing greenhouse gases, is the equivalent of taking 20 million cars off the road, according to a statement issued by EPA Administrator Carol Browner. Methane is about 25 times more powerful than carbon dioxide (the primary greenhouse gas) in trapping heat in the earth's atmosphere, according to the EPA.

The rule applies to landfills for household waste—not hazardous waste—with a capacity of 2.5 million cubic meters or greater. Those landfills that are found to emit more than 50 megagrams per year of VOCs will be required to drill collection wells to contain the gas. In turn, the gas may be routed to either an energy recovery system, where it can be captured for use, or to a combustion device, where it can be safely burned.

Although the rule is an important step in reducing ozone-forming VOCs, its primary benefit will be in methane reduction, said Dan Lashof, a senior scientist for the Natural Resources Defense Council (NRDC). "Landfills are an important, but relatively small, source of ozone-forming compounds," Lashof said. "But they are one of—if not the—biggest sources of methane." The process of capturing the VOC emissions will also net significant amounts of methane, Lashof said. In addition, the rule requires landfills to monitor surface methane on a quarterly basis and expand their collection wells if these emissions exceed 500 parts per million.

Of 7,000 landfills nationwide, the EPA estimates the rule will affect up to 280. Total costs nationwide are estimated at $778 million in one-time capital costs and $93 million annually, which the EPA estimates will translate into customer costs between $0.20 and $0.40 monthly. These customer costs could be offset by landfills selling the energy generated through the recovery systems.

Industry representatives are generally supportive of the new rule. "Lots of private landfills are already collecting methane, and this will just require more fine-tuning," said Ed Repa, director of environmental programs for the National Solid Waste Management Association.

"It's a workable rule," said Chris Voell, director of technical services for the Solid Waste Association of North America, "though, as a direct public health concern, we don't think EPA had all the data they needed to say methane has an impact on health."

For small landfills, defined as those that accept 20 tons of solid waste or less per day, amendments to the Solid Waste Disposal Act, signed into law on March 26, could mean less stringent regulations. One provision, authored by Senator Pete Domenici (R-New Mexico), requires the EPA to develop guidelines that afford states flexibility in regulating small landfills while still protecting human health and the environment.

The guidelines, which must be developed within two years, will address four areas: frequency of cover application, frequency of monitoring, infiltration layers for final cover, and means of demonstrating financial assurance. Domenici's office said that, while states currently have a good deal of flexibility to design solid waste regulations to fit local needs, the rules in these four areas are too rigid. According to EPA staff, the current rules require landfill operators to cover solid waste with dirt every day, monitor methane on a quarterly basis, install a final cover of 24 inches of earthen material, and be able to demonstrate they have the money to provide closure and post-closure care for the landfill. The amendments will likely only apply to landfills in dry, remote areas, according to the EPA, where groundwater contamination is less of a potential problem.

Another provision in the amendments exempts small landfills from groundwater monitoring requirements if they are located in an area that receives less than 25 inches of precipitation annually, unless the state finds such monitoring necessary to protect groundwater resources. An earlier EPA attempt to create this exemption by regulation was overturned by the U.S. Circuit Court of Appeals for the District of Columbia, which found the agency did not have authority to issue the exemption.

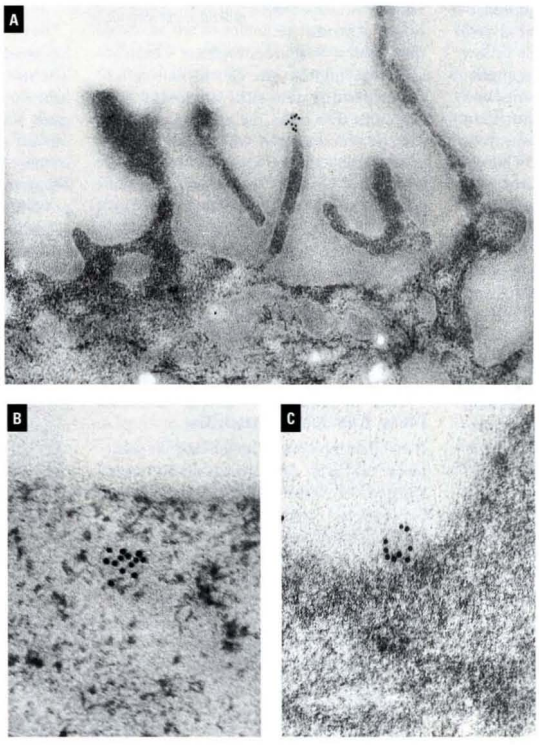
"The irony is, a landfill's not eligible for the exemption if you have evidence of groundwater contamination, but without groundwater monitoring you can only prove the contamination if it shows up in someone's well," said David Lennett, an attorney who has represented the NRDC.

Proponents of the small-landfill measures say they are necessary because the stringent requirements for large landfills in some cases are simply unnecessary—and unaffordable—for small landfills in arid climates. For example, the New Mexico Environment Department estimates Domenici's provision could save the state $50 million over the next 10 years.

"It's just adding some common sense to the process," said Tom Kennedy, executive director of the Association of State and Territorial Solid Waste Management Officials. If an area doesn't get enough rainfall to create leachate, measures aimed at reducing and monitoring leachate are not needed, he said.

However, other industry groups remain skeptical. "It doesn't make sense from a public health perspective," Repa said. "It is in arid, remote areas where people are drinking groundwater from wells, rather than municipal treatment systems," he said. "So it makes sense to require monitoring wells there." Repa estimated the cost of installing a monitoring well to be about $4,000, plus an annual $1,000 to monitor it. "If you look at the cost, it's small compared to remediation," he said.

"Subtitle D [of the Resource Conservation and Recovery Act] was supposed to close down the small landfills, and create larger ones with more environmental protections through economy of scale," Repa said. "This [exemption] would allow the status quo at those smaller facilities." However, according to Domenici's office, in large states like New Mexico, consolidation of small landfills may not always be a cost-effective option.



Working outside the cell? Micrographs (A, B, and C in successively higher magnifications) showing granules apparently secreting BRCA1 protein outside the cell suggest that it may be possible to design drugs to mimic the protein's effects. Source: Jensen RA et. al., BRCA1 is secreted and exhibits properties of a granin. *Nature Genetics*, 12:303–308, (1996).

Using *BRCA1* to Treat Cancer

In the two years since the gene for inherited breast cancer, *BRCA1*, was identified, researchers have been trying to understand how the gene normally works. A team from Vanderbilt University and the University of Washington has now shown that *BRCA1* suppresses the formation and growth of breast tumors in mice. Their results, published in the March issue of *Nature Genetics*, suggest that the gene or drugs that mimic its protein product might someday be used to treat human breast and ovarian cancer.

"This is what everybody had hoped—that there would be a

gene found that actually would retard the development of breast tumors—and it seems to do that," said Anne Bowcock, a breast cancer researcher and associate professor of pediatrics at the University of Texas Southwestern Medical Center in Dallas. "I think it's a significant step toward the treatment of at least some breast cancers." Most of the 184,000 new breast cancer cases diagnosed annually are not the inherited type. But the new research suggests that the protein produced by normal *BRCA1* genes may be effective against the more common noninherited forms of breast cancer.

Researchers led by Jeffrey Holt of Vanderbilt first implanted normal *BRCA1* genes into human breast and ovarian cancer cells and found that cell growth was inhibited *in vitro*. Next, the researchers stably transferred either normal or mutant *BRCA1* genes into breast cancer cells and injected the cells into mice. Tumors developed in all 15 mice given mutant *BRCA1*, but in none of the 20 mice given normal *BRCA1*. Finally, the researchers injected viruses carrying the *BRCA1* gene into the abdomens of 10 mice with established breast cancer tumors; half the mice got normal *BRCA1*, the others got mutated *BRCA1*. The mice with mutant *BRCA1* all died of cancer within two weeks. Those with normal *BRCA1* survived 15 to 41 days, and their tumors either shrank or disappeared.

All the experiments used a cell line derived from noninherited breast cancer, suggesting that the treatment might work against the more common types of the disease. Against hereditary breast and ovarian cancer, "presumably it would have an even more dramatic effect—at least that's what we hope," said Roy Jensen, an assistant professor of pathology and cell biology at Vanderbilt.

Translating the results into treatments for human cancers will take time. "It's going to be a long time before this is taken to the bedside," said Bowcock. "The problem is actually getting *BRCA1* into breast cells—it's not going to be easy."

It's easier to get *BRCA1* into ovarian tumor cells, and Vanderbilt researchers recently began clinical trials using *BRCA1* on about 20 ovarian cancer patients, a move that concerns some of their colleagues. While he finds the results of the mouse and cell culture experiments "intriguing" and "encouraging," Roger Wiseman, head of the Comparative Carcinogenesis Group of the NIEHS Laboratory of Molecular Carcinogenesis (part of the team that identified *BRCA1*) feels the human gene therapy trials are "premature, based on the data that have been presented so far." Wiseman would like to see more animal studies performed before *BRCA1* is used in patients.

A related study led by Jensen found evidence that the BRCA1 protein may be secreted and do its work outside the cell. If this is true, it would be much easier to design and deliver drugs that mimic the protein's effects. However, this finding is controverted, and other research groups are convinced that the BRCA1 protein works from inside cells.

"What we need to do to confirm our theory is purify recombinant *BRCA1* and put it onto cells and see if it actually has a growth inhibitory action," said Jensen. If it does, and if it works only on breast and ovarian cancer cells and not other cells, "then that's pretty good evidence that there are specific receptors for this protein. Our efforts then would be focusing on trying to find those receptors."

EHPnet

Genetics and Biosafety

With the pounding pace at which research in genetics and biotechnology is progressing, it has become increasingly difficult for scientists to stay abreast of all the advances and ensure that the new technology is being used in a safe and cautious manner. In an effort to improve communications among scientists and promote the safe use of biotechnology, the United Nations established the International Centre for Genetic Engineering and Biotechnology (ICGEB).

The center maintains two main research laboratories, one in Trieste, Italy, and another in New Delhi, India, with several other smaller labs scattered around the world. These laboratories distribute their findings along with other biology-related information over the Internet via the ICGEB home page and ICGEBnet, an information resource network for molecular biologists.

The ICGEB home page, located at **http://base.icgeb.trieste.it/**, provides information on ICGEB-sponsored meetings and symposia, access to biology-related databases and newsgroups, and information on accessing ICGEBnet. A second biology-related network available from the home page called BIN21 is still being developed by the ICGEB and will focus on issues of biodiversity.

Among the databases accessible from the ICGEB's home page is one relating to P450 proteins and P450-containing systems, an on-line directory of biologists around the world, and SBASE (a sequence database of protein domains). An extensive library of biosafety-related rules and regulations from various nations, organizations, and research institutions is also available, along with lists of experts and databases that can be contacted to help researchers in dealing with biology-related legal issues. For further assistance on biosafety and legal questions, a link is provided to the Stockholm Environment Institute's Biotechnology Advisory Board home page. This international board of scientists will answer questions and give advice on any issue relating to ecology, biochemistry, genetics, biotechnology, pathology, environmental law, or economics.

Access to ICGEBnet and BIN21 will be free of charge but generally limited to scientists and policymakers with a pertinent interest in biology. Currently, ICGEBnet gives scientists around the world access to a variety of databases and provides a computer environment that allows molecular biologists to analyze nucleotide and protein sequences. Analysis software, including three major program packages, is distributed over ICGEBnet. In addition, information services such as electronic mail and bulletin boards are also available. The center's goal is to distribute these services to areas of the developing world where they are not yet widely available.

Fueling the Gas Debate

New findings continue to fuel the debate over the safety and effectiveness of gasoline additives, such as methyl tertiary butyl ether (MTBE) and ethanol, being used to reduce air pollution. The Clean Air Act Amendments of 1990 required that, beginning in 1992, areas that fail to meet air quality standards must use oxygenated fuels. Not only has the effectiveness of the oxygenates in reducing carbon monoxide (CO) emissions been questioned, the additives have also been accused of causing health problems including headaches, dizziness, nausea, and rashes. However, a recent study has found that the additives are successful in reducing CO emissions and do not appear likely to substantially increase health risks when compared to normal gasoline. The report

emphasizes that further research is needed, but recommends that the use of the additives should not be abandoned at this time.

The Potential Health Effects of Oxygenates Added to Gasoline: A Review of the Current Literature was released in April by the Cambridge, Massachusetts-based Health Effects Institute, a cooperative effort of the EPA and the auto industry created to examine the health effects of motor vehicle emissions. HEI conducted a review of existing research, public complaints, and occupational exposures concerning gasoline additives.

According to the report, potential health effects from exposure to gasoline containing MTBE include headaches, nausea, and sensory irritation; acute, reversible neurotoxic effects (based on studies with rats at high exposure levels); and cancer (based on increased frequency of tumors in rats and mice at high exposure levels). Exposure to ethanol by ingestion of moderate to large quantities has been found to increase the risk of cancer, adversely affect embryos, and produce neurotoxicity. However, the report points out that these effects are unlikely to occur at low levels of inhalation.

The report concludes that possible short-term and cancer-causing effects of exposure to gasoline without oxygenates are similar to those from exposure to gasoline with oxygenates. Adding oxygenates to gasoline reduces the emission of carbon monoxide and benzene from motor vehicles, which may lower health risks for some people, the report stated. However, the process may increase exposure to oxygenates and aldehydes, which may have other health risks. The report concluded that an immediate reduction in oxygenate use is not warranted at this time because adding oxygenates is unlikely to significantly increase health risks associated with fuel use.

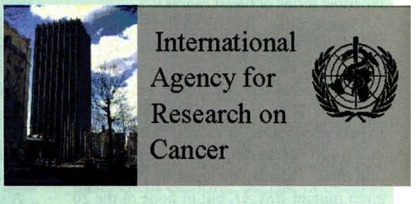
The report recommends that further research be conducted and outlines several priorities, including comprehensive assessments of personal exposure to oxygenates, human environmental chamber studies to evaluate the health effects of MTBE and MTBE-gasoline mixtures, epidemiologic and animal studies to evaluate cancer risks of MTBE, and comprehensive assessments of other oxygenates.

The study was commissioned by the EPA and the Centers for Disease Control and Prevention (CDC) as part of a broad review of oxygenated fuels being conducted by the White House Office of Science and Technology Policy, which will examine air quality benefits, engine performance, fuel economy, and costs of the fuels. The HEI study's conclusions are similar to those of a recent National Science and Technology Council report conducted by an interagency

IARC on Tamoxifen

Tamoxifen, an antiestrogenic compound that has been recognized by the World Health Organization as an essential drug for the treatment of breast cancer, is itself carcinogenic, according to the International Agency for Research on Cancer. IARC researchers, who met in February in Lyon, France, reviewed evidence on the potential carcinogenicity of 13 pharmaceuticals. Though they found evidence that tamoxifen increases a woman's risk of developing endometrial cancer, they emphasized that this does not abate the drug's benefits.

"No woman being treated for breast cancer should have [her] treatment stopped because of the conclusions of the [IARC] working group," the researchers concluded. "The risk of endometrial cancer is far lower than the benefits women with breast cancer receive from tamoxifen."



Tamoxifen has been prescribed to women with metastatic breast cancer for over 20 years and it is registered for use in nearly 100 countries. It has been used as both a curative agent and a secondary cancer-preventive agent, and it is also being evaluated for use as a primary preventive agent for healthy women at an increased risk of developing breast cancer, IARC director Paul Kleihues said in a 17 February 1996 article in *The Lancet*.

The IARC group, which consisted of 19 scientists from 8 countries, reviewed all the published scientific data on second primary tumors reported in patients who were given tamoxifen as treatment for breast cancer. In a draft of the study results, the group concluded that there was "sufficient evidence in humans for the carcinogenicity of tamoxifen in increasing the risk of endometrial cancer." However, the group also recognized that "there is conclusive evidence that tamoxifen reduces the risk of contralateral breast cancers" (second cancers in the other breast), and that "there is inadequate evidence tamoxifen affects the risk of other cancers." The results of the study will be published in volume 66 of the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*.

Though statements on a drug's benefits are not normally included in the *Monographs* series, Kleihues told *The Lancet* that the working group would probably make an exception for tamoxifen. Such a statement may help quell the concerns of those worried that an overzealous reaction to IARC's findings could stop the use of a very beneficial medication. Criticism of the agency's decision to evaluate the carcinogenicity of tamoxifen began late last year when the state of California, in response, considered listing tamoxifen as a carcinogen under its Proposition 65. Critics point out that IARC's report could cause tamoxifen to be quickly replaced by any of a number of new antiestrogens currently being introduced. With comparatively little human data available on the new drugs, the danger exists that tamoxifen will be uncritically replaced by a less effective or more toxic medicine.

Kleihues said that IARC received many letters concerning its study on tamoxifen, but that the agency did not cancel or reschedule the evaluation as a matter of principle. "It is important that women have access to scientific opinion on the low risks of endometrial cancer," Kleihues and working group chairman George Lucier stated in a press release, "so that they can make informed decisions on the treatment they will accept."

Tamoxifen is one of three triphenylethylene antiestrogenic compounds that will be reviewed in volume 66. The other two, droloxifene, a drug also used in the treatment of breast cancer, and toremifene, which is just being introduced, were found to be "not classifiable" as to their carcinogenicity to humans due to inadequate data.

Similar results were found for six of a group of seven benzodiazepines and benzodiazepine analogues that are used in the treatment of insomnia, anxiety disorders, and alcohol withdrawal. One, oxazepam, was found to be "possibly carcinogenic to humans."

Two cholesterol-lowering drugs, clofibrate and gemfibrozil, were also found to be "not classifiable" as to their carcinogenicity to humans. Phenytoin, which is used to treat epilepsy and certain cardiac arrhythmias, was found to be "possibly carcinogenic to humans."

IARC has evaluated the carcinogenicity of more than 800 agents. Of these, 70 have been deemed human carcinogens and about a half dozen of these are still in use.

panel, says Mary White, an epidemiologist with the CDC. "Both reports clearly state that the information available on the additives is very limited." White emphasized the need for additional research. "There are some troubling and unanswered questions about the acute health effects [of the oxygenates]. These are a real concern, which no one dismisses, but we're talking about acute headaches, not acute mortality," White said.

Representatives of the oil industry who support the use of oxygenates were satisfied with the report's conclusions. "HEI did a good job of reviewing the information. I found the report to be favorable, although it overemphasized the carcinogenesis [of the oxygenates]," said Robert Drew, director of health and environmental research for the American Petroleum Institute. "Even though the results highlighted the carcinogenic and neurotoxic endpoints, [HEI] was not concerned enough for the materials to be taken off the market in the short term," he said.

However, the HEI report findings have angered opponents of oxygenate use, including Myron Mehlman, a staff scientist at the Environmental and Occupational Health Sciences Institute at Rutgers University. Mehlman, who feels that the additives should be immediately removed from the market, says the HEI report does not accurately address the acute effects of the oxygenates, and he criticized the studies cited in the report. "I don't know of any studies that have been conducted [on oxygenates] that are adequate," he said. Mehlman also says there are not sufficient data to show that the additives reduce carbon monoxide emissions; therefore, using the oxygenates is causing unnecessary health risks. Drew counters, "MTBE at this point is a thoroughly studied chemical. We concur with the conclusion that MTBE is certainly no more harmful than gasoline itself."

Although HEI acknowledges that it is not possible to have complete information about a substance before it is used, the report said that, in the future, more research—including a comprehensive testing program, rigorous exposure assessment, and epidemiologic studies—should be conducted before introducing a substance into widespread use.

Centered on Molecules

The Vanderbilt University Center in Molecular Toxicology is one of nineteen NIEHS-supported Environmental Health Sciences Centers throughout the United States. Its primary purpose is to enhance individual and collaborative toxicology programs at Vanderbilt and nationwide. As scientists uncover how chemicals can cause damage to people at the molecular level, they will be able to develop techniques for screening for individual genetic susceptibility to environmental toxins and for effective preventive measures to protect human health. A hallmark of the Vanderbilt center is its interdisciplinary approach to understanding toxicological problems from the biochemical perspective and investigating questions related to toxicity at the molecular level.

CENTER IN MOLECULAR TOXICOLOGY
Vanderbilt University
School of Medicine

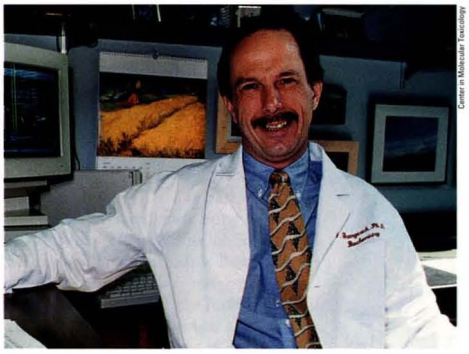
Center in Molecular Toxicology

Center of the center. Most of the Center in Molecular Toxicology's core groups are located in the Medical Research Building I, on the campus of Vanderbilt University.

The Vanderbilt School of Medicine has been in the vanguard of research on cancer-causing substances since the Division of Toxicology was founded in 1967 in the Department of Biochemistry under the direction of the late Frank R. Blood. At its inception, the mission of the Division of Toxicology was the coordination of research and teaching related to harmful substances in foods such as chemical additives, naturally occurring toxins, and residues from agricultural pesticides and packaging materials. The division's success led to the establishment of the Vanderbilt Center in Environmental Toxicology under the auspices of an NIH Environmental Health Sciences grant in 1969. Over the years, the program has become increasingly interdisciplinary, with faculty in the departments of biochemistry, cell biology, chemistry, medicine, pathology, and pharmacology. Research emphasis has broadened to encompass endogenous chemicals and synergistic deleterious effects resulting from combinations of different chemicals in the body, all of which may give rise to mutations and play a role in activating oncogenes and inactivating tumor suppressor genes. The name was changed to the Center in Molecular Toxicology in 1984 to reflect increasing specialization in areas of toxicology that use molecular methodologies to elucidate physiological and genetic changes due to environmental and biogenic toxins.

Structure

The Center in Molecular Toxicology is organized into administrative cores, service cores, research cores, and an outreach program. The administrative cores are responsible for a variety of tasks including coordinating the toxicology seminar series and an annual visit by the External Advisory Group, processing applications, writing manuscripts and grants, and managing computer databases. The editorial office of the American Chemical Society's journal, *Chemical Research in Toxicology,* is also located at the center.



Center in Molecular Toxicology

F. Peter Guengerich

The service cores provide a full range of technological support and facilities necessary for molecular experimentation including NMR spectrometry, mass spectrometry, molecular biology, cell biology, and protein chemistry. The NMR and mass spectrometry facilities include state-of-the-art instrumentation for high-resolution analysis of biological macromolecules, which are critical in analytical chemistry for characterization of oligonucleotides and natural and synthetic molecules. The cell biology core provides human tissue and cell cultures, as well as monoclonal antibodies for biological studies. The protein chemistry core provides amino acid analysis and sequence determination of peptides, and the molecular biology core provides recombinant DNA technologies such as automated DNA synthesis and amplification.

There are seven core areas of research: enzymatic oxidation and conjugation, oxidative damage, DNA damage and mutagenesis, regulation of gene expression, analytic method development, neurotoxicology, and clinical toxicology.

Enzyme Bioactivation of Toxic Chemicals

The proteins, lipids, and sugars that make up a major part of foods, as well as other chemicals entering the body such as drugs or xenobiotics, are broken down by enzymes into smaller molecules before cells either use or eliminate them. Cells oxidize molecules through a series of enzyme-mediated reactions. Different environmental chemicals interact with enzymes in the body in various ways to alter normal biological processes. Certain enzymes in the liver catalyze detoxication reactions in which water-insoluble drugs and xenobi-

otics, which would otherwise cause cellular damage by becoming permanently attached to cell membranes or DNA, are made water-soluble and removed from the body by urine.

Center Director F. Peter Guengerich has pioneered investigation of the enzyme cytochrome P450, a primary catalyst involved in the oxidation of drugs, steroid hormones, and carcinogens. According to Guengerich, "There are over forty different kinds of P450 in the human body. The ones I am most interested in are the ones that act on chemicals that are not normally found in the human body (xenobiotics). We are trying to understand how enzymes detoxify drugs and carcinogens and how these same enzymes can also make them more active."

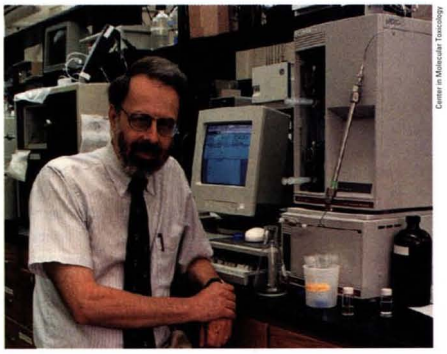
Center in Molecular Toxicology

Michael R. Waterman

A major contribution to this understanding was Guengerich's characterization of how P450 converts aflatoxin—a natural chemical produced by a fungus that grows on rice, peanuts, and corn—into a potent carcinogen when ingested. Human populations in Africa and China, countries that lack adequate inspection of agricultural products, have unusually high rates of liver cancer caused by aflatoxin. Guengerich and colleagues at Vanderbilt identified a particular P450 enzyme involved in aflatoxin bioactivation. Thomas Harris, associate director of the center, synthesized the active form of aflatoxin, a potent epoxide that sticks to DNA, and has done most of the center's chemical experimentation on this molecule.

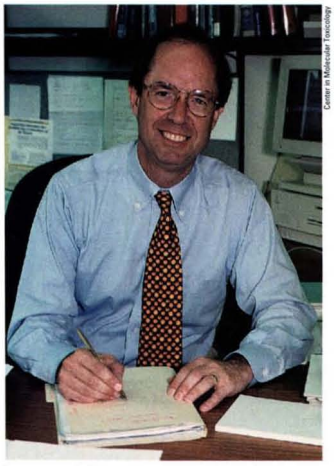
Guengerich is currently collaborating with scientists at Johns Hopkins University to investigate the role of a newly discovered cytochrome that is found in high concentrations in the breast, uterus, ovaries, and prostate gland, and that may play a role in cancers in the human reproductive system. An important objective of this research is to delineate the steps in initiation of P450 carcinogenic activity in order to design effective drug therapies that can be used to prevent enzyme-mediated cancers.

Working with Michael R. Waterman, professor and chairman of biochemistry, Guengerich has genetically engineered *E. coli* bacteria with human P450 genes that express high levels of the newly discovered enzymes. Human cytochrome P450s have also been engineered directly into the Ames *Salmonella* tester strain for genotoxicity, which has practical application for screening mutagenic effects of chemicals activated by human enzymes. The bacterial expression system will also be a valuable tool in site-directed mutagenesis studies.



Center in Molecular Toxicology

Thomas Harris



Center in Molecular Toxicology

Lawrence J. Marnett

DNA Damage from Endogenous Chemicals

Research in the A.B. Hancock, Jr., Memorial Laboratory for Cancer Research, directed by Lawrence J. Marnett, has shown considerable DNA damage may be caused by endogenous chemicals produced during the body's normal metabolic processes. Marnett says, "The scientific community originally thought that it was only chemicals in the environment [that caused damage]. We now know that our own bodies damage our DNA through normal processes." Marnett is particularly interested in enzymes that catalyze the conversion of polyunsaturated fatty acids to bioactive lipids such as prostaglandin. These fatty-acid derivatives bind to different cell-surface receptors and have various biological effects including smooth muscle contraction and inflammation. DNA adducts can be formed from lipid oxidation products. Marnett is investigating the biological consequences of the adherence of endogenous chemicals produced by lipid biosynthesis to DNA, and how such chemicals act as internal mediators to initiate mutations and tumors in human tissues. Certain byproducts of lipid oxidation are of particular concern because they have been implicated in tumor metastasis. Approaches to studying the causes and effects of DNA damage being used in Marnett's lab include measuring the amount of damage to DNA, and creating synthetic DNA molecules that have been damaged in various ways to use as models. "We try to introduce the damage to a specific site and then observe the effects of the damage," Marnett says. The findings of basic research promise to have practical benefits to health care by improving the ability to assess people at risk for different types of cancer. "The biggest impact our work can have is in human risk assessment," Marnett predicts. "We will have a better concept of what is causing cancer in people, and, therefore, will soon be better able to determine who is at risk for certain types of cancer."

Aspirin-Enzyme Link

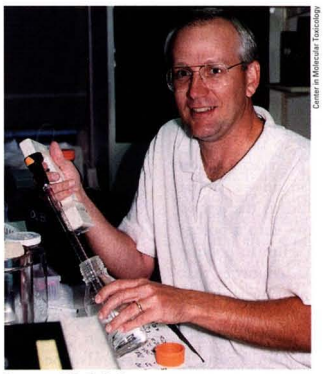
Many cancers are caused by oxidative stress. A recent discovery revealed that aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) function by blocking enzymes involved in the lipid oxidation process to prevent formation of harmful, biologically active intermediates. Comparing cancerous tissue and healthy tissue from 28 patients who underwent surgery for colon cancer, Raymond N.

Timothy Meredith

DuBois, an associate professor of medicine and cell biology, found unusually high levels of one such enzyme, cyclooxygenase-2 (COX-2), in cancerous colon tissue, whereas levels in healthy tissue were very low or undetectable. COX-2 leads to production of prostaglandin and other biogenic chemicals linked to cancer. It has been shown that arthritis patients who take NSAIDs regularly have a 50% reduction in risk for developing colon cancer. Colorectal cancer is one of the leading causes of cancer mortality; approximately 150,000 people are diagnosed with colon cancer and over 50,000 die from the disease each year. DuBois is investigating how aspirin inhibits COX-2 activity and thereby reduces cancer risk. Understanding the enzyme blocking mechanism can lead to better diagnostic and screening strategies, as well as development of highly specific, targeted therapies for the prevention of intestinal tumors.

Outreach

An important part of the center's outreach program is the Clinical Toxicology Center, which includes the Middle Tennessee Poison Center (MTPC). The MTPC comprises a clinical toxicology admitting service and outpatient clinic service, an occupational and environmental medicine section, and analytical toxicology laboratories. The clinical toxicology admitting and outpatient clinic is staffed by faculty members with backgrounds in internal, occupational, and emergency medicine. It is the only such service in Tennessee and one of three nationwide. "The addition of clinical toxicology at Vanderbilt is a tremendous opportunity," said Guengerich of the MTPC. "It should be one of the premier programs of its type in the nation and should complement our existing strengths in molecular programs."



Raymond N. DuBois

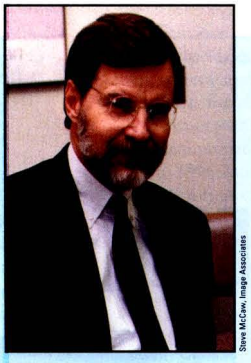
The MTPC program, directed by the Center in Molecular Toxicology's Timothy Meredith, serves 57 counties in Tennessee, covering a population of approximately 3 million. The MTPC is now extending the original primary service of prevention and management of poisoning due to household chemicals and drugs to include exposure to environmental chemicals. The MTPC is establishing an environmental chemicals "hotline" to answer questions about adverse health effects from exposure to environmental chemicals, and is developing an interactive database focused on environmental chemicals and health-related problems. The database will be accessible on-line via the World Wide Web at http://www.toxicology.mc.vanderbilt.edu.

Training in toxicology at the center extends from undergraduate to graduate, postdoctoral, and medical students. In addition to formal courses and seminars on toxicology, students are offered the opportunity to participate in ongoing research projects to gain skills essential to investigations in molecular toxicology and clinical applications. Two monthly seminars are conducted by center faculty and by outside scientists of national renown.

Reflecting on its past history and looking toward the future of the Center in Molecular Toxicology, Guengerich says, "Over the years, our center has been able to develop by emphasizing high quality science, and we've been able to assemble an outstanding group of individuals. There is considerable strength in chemistry and carcinogenesis, and some of the new directions such as neurotoxicology and gene regulation should enhance our overall scientific program." As scientists like those at the center carry on their work, they are charting new courses for delivering improved health care, enhancing human risk assessment, and solving modern environmental health problems.

Mary Eubanks

Wilson Named Deputy Director


Steve McCaw, Image Associates

On September 1, Samuel H. Wilson, an expert in the fields of environmental toxicology and genetic enzymology, will join the NIEHS as its new deputy director. Wilson will be responsible for helping Director Kenneth Olden administer the NIEHS, which employs over 850 people and has an annual budget and pass-through funds of approximately $365 million. Wilson said that he sees the position as an opportunity to "stimulate and foster outstanding research nationally in environmental health science and to make enhanced use of the research strengths [at the institute]."

Wilson emphasized that increased partnership and interaction between the academic community and industry would be necessary to achieve these goals. Increased cooperation will allow research to progress more efficiently, Wilson said, and will be beneficial to both sectors. "These relationships can be facilitated by NIEHS," he said.

Wilson also said he would like to see the NIEHS place more emphasis on the development of new research technologies. "We need to take advantage of new advances in molecular genetics to help us better understand the impact of environmental exposure on health," Wilson said. As an example of how such technologies could advance the field of environmental health, Wilson cited the use of genetically engineered bacteria and animals as sentinels for detecting toxicants.

A world-recognized authority on DNA polymerases, Wilson brings with him extensive knowledge and experience in genetics and toxicology. Currently at the University of Texas Medical Branch at Galveston, Wilson is the founding director of the Sealy Center for Molecular Science and holds the Mary Gibbs Jones Distinguished Chair in Environmental Toxicology. He also serves as director of the Centennial Center for Environmental Toxicology and is a professor in the Department of Human Biological Chemistry and Genetics.

Before his University of Texas appointment, Wilson was chief of the Nucleic Acid Enzymology Section at the National Cancer Institute's Laboratory of Biochemistry in Bethesda, Maryland. His career with NCI, a sister institute of the NIEHS, began in 1970. Since that time, Wilson's pioneering research in the field of genetic enzymes, which are responsible for replication and repair of genomic DNA, has led to a much greater understanding of how these enzymes function. His work could eventually enable scientists to design drugs that control replication of cancer cells or viral replication within HIV-infected cells.

Wilson received his M.D. from Harvard Medical School in 1968. He received postdoctoral training at Dartmouth Medical School and the National Institutes of Health. "With the appointment of Dr. Wilson, the institute is gaining the benefit of an outstanding researcher and science administrator," Olden said.

The Specter of Infection

In science fiction movies and tabloid magazines, new diseases "suddenly drop in from outer space or come up from the depths of the ocean," notes Stephen Ostroff, associate director of epidemiology for the National Centers for Infectious Disease, part of the Centers for Disease Control and Prevention (CDC). In the real world, Ostroff says, truly new diseases are rare, if they exist at all. Most newly identified diseases—from deadly Ebola in Africa to the hantavirus that swept through the southwestern United States in 1993—are relatives of preexisting bacteria or viruses. Almost always, these disease-causing agents emerge within a new host population or geographic region because of environmental changes resulting from human activities.

Urbanization, for example, may have played a role in the emergence of human immunodeficiency virus (HIV), says Stephen Morse, an assistant professor of virology at The Rockefeller University. Though the precise origin of HIV remains uncertain, Morse says, a rural resident of Liberia may have eaten a monkey afflicted with a simian version of the disease. HIV emerged only after the man moved to the city, where he infected others. "With rare exceptions, most of the disease emergence or reemergence that we've seen in recent years has had a human fingerprint on it," says Ostroff.

Diseases can flourish and spread to new geographic regions because of global warming, land-use changes, technological changes, drug resistance, pesticide resistance among disease-bearing insects, and contamination of air, soil, or water. Since high-speed, long-range transportation now makes it possible to circle the globe in days, diseases can easily hitchhike from region to region.

When it comes to preventing emerging diseases, the stakes are high in both human and economic terms. Costs associated with treating sexually transmitted diseases (excluding Acquired Immune Deficiency Syndrome or AIDS) may reach $5 billion annually, according to a 1994 CDC report, *Addressing Emerging Infectious Disease Threats.* Intestinal infections generate another $30 billion in direct costs and lost productivity every year, the report says, and hospital-acquired infections racked up $4.5 billion worth of bills in 1992 alone. "Infectious diseases remain the leading cause of death worldwide," the report adds, and an increasing number of cases are linked to environmental changes.

Global Climate Change

Of the environmental changes linked to disease, the risks associated with global warming are particularly troubling, says Paul R. Epstein, a faculty member at the Harvard School of Public Health. Warming estimates vary, but according to climatologist Thomas R. Karl, a senior scientist at the National Oceanic and Atmospheric Administration's (NOAA) National Climatic Data Center, surface temperatures increased between 0.3°C and 0.6°C (0.5°F and 1.1°F) during the last century. Warming has been strongest at night in the mid-to-high latitudes of the Northern Hemisphere, says Karl.

Epstein fears that continued global warming could cause widespread outbreaks of malaria and other mosquito-borne diseases. A mosquito's range, reproduction, and biting rates can be enhanced by warmer weather, Epstein reports in a 17 January 1996 article in the *Journal of the American Medical Association.* At the same time, he says, warmer waters would support increased growth of fish-killing algae, and refugees from flooded coastal regions might overwhelm city water and sanitation systems.

Malaria—a potentially fatal mosquito-borne ailment resulting in fever, headaches, nausea, vomiting, diarrhea, and fatigue—now claims an estimated 2 million lives annually. If global warming continues, the disease could kill another 1 million people every year as parasite-bearing mosquitoes spread to new geographic regions, says Rita R. Colwell, a microbiology professor and president of the University of Maryland's Biotechnology Institute. Already, "malaria is making a comeback," Colwell warned when she lectured at the 1996 American Association for the Advancement of Science meeting as outgoing president of the organization.

A doubling of atmospheric carbon dioxide levels over the next 100 years could boost temperatures by 1.5°C to 4.5°C (about 3°F to 9°F), Karl says. Sea level is expected to rise by 15–90 cm during the same period, according to *Human Health and Global Climate Change,* a 1996 report by the National Science and Technology Council and the Institute of Medicine of the National Academy of Sciences.

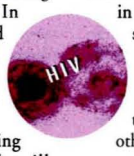
Such dramatic environmental changes would be devastating to human health, Epstein says. If surface temperatures jump by 2°C over the next century, he adds, malaria, dengue, yellow fever, encephalitis, and cholera will run rampant.

Shellfish poisoning—and resulting neurological illness in humans who eat the seafood—can occur with alarming frequency when waters are warm enough to support increased toxic algal blooms, notes

JoAnn M. Burkholder, an associate professor of aquatic ecology at North Carolina State University. Burkholder's recent studies have focused on a dinoflagellate named *Pfiesteria piscicida*, which is known as an "ambush-predator" because it targets certain fish. Attracted by fish secretions, the *Pfiesteria piscicida* puts fish into a narcotic stupor, strips off and eats their skin, then suffocates its victims by paralyzing them. These newly identified dinoflagellates are so toxic, in fact, that they've been known to cause skin lesions and short-term memory loss in fishermen and laboratory workers.

Pfiesteria piscicida may flourish when waters are loaded with nutrients such as nitrogen and phosphorus from farm runoff, industrial waste streams, or natural sources. And the dinoflagellates proliferate most readily when water temperatures range from 79°F to 91°F. "Increased warming trends would tend to encourage their growth," Burkholder says. Likewise, waters between 69°F and 82°F are ideally suited for the growth of the cholera *Vibrio*, says Martin Hugh-Jones, a professor at Louisiana State University's School of Veterinary Medicine.

Of course, if it gets too hot in certain areas, Morse points out, disease-bearing pests might simply perish there. In that event, "we may see malaria and dengue in New York City and Chicago again," he says. "But we may lose it in all other places where we currently worry about it."



Global warming will cause rising sea levels and flooding, which will undoubtedly result in crop losses. Food distribution systems could also be disrupted by civil war as feuding factions are forced to share increasingly limited resources. Research by Melinda A. Beck, an assistant professor at the Frank Porter Graham Child-Development Center at the University of North Carolina–Chapel Hill, suggests that nutritional deficiencies might actually prompt viruses to mutate and become more virulent among malnourished populations. Prompted by the observation that people in parts of China who are deficient in selenium are particularly susceptible to cardiac infections, Beck fed mice a diet deficient in either selenium or vitamin E, then injected them with coxsackie B, an RNA virus known to be harmless. Unlike a control group of well-fed mice, the malnourished rodents quickly showed signs of heart disease, says Beck. By comparing the genetic structure of benign coxsackie B with virus samples extracted from the sick mice, Beck determined that "what came out . . . is not what went into them." Though it's not yet clear exactly how dietary deficiencies could cause viral mutations, Beck speculates that a malnourished immune system may help RNA viruses replicate faster, thereby increasing the risk of genetic errors that cause mutations. Her study of selenium-deficient mice, published in the May 1995 issue of *Nature Medicine*, may offer the first clear evidence of a link between dietary deficiencies and disease evolution.

Between 1979 and 1996, atmospheric ozone over the Northern Hemisphere was depleted by 10–25%, NOAA announced on 24 April 1996. Some repletion should begin by the year 2000, thanks in part to an international ban on the production of ozone-destroying chlorofluorocarbons. But continued depletion of the earth's gaseous shield could make people more susceptible to disease, Epstein says, because increased exposure to ultraviolet radiation suppresses immune system responses.

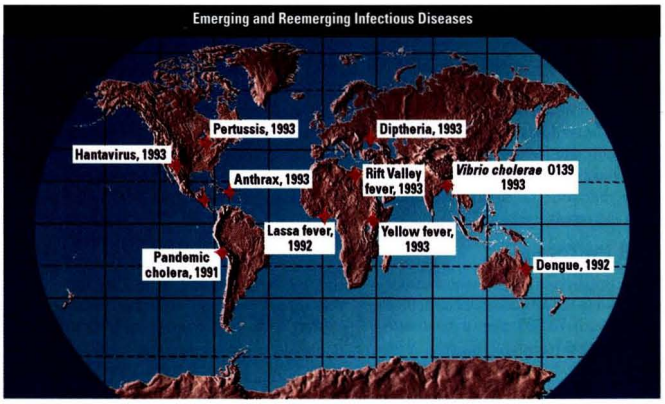
Even if global warming doesn't pose the suspected threat, Colwell says, communities around the world are already experiencing public health problems associated with El Niño, a periodic warming of waters in the tropical Pacific that causes wild fluctuations in precipitation, resulting in flooding and droughts. By understanding the link between climate change and disease, Epstein says, communities could respond more effectively to toxic algal blooms triggered by warming as well as other weather-related diseases such as a recent malaria outbreak in Sri Lanka, where drought-starved rivers turned into shallow pools perfect for breeding mosquitoes. Someday, he adds, improved disease monitoring and weather surveillance systems may prevent a recurrence of pneumonic plague, which struck India three years ago when temperatures soared to 124°F, killing livestock and generating clouds of fleas.

The 1993 emergence of hantavirus pulmonary syndrome (HPS) in the southwestern United States may be another example of El Niño's effects on public health. More than 130 people suffered sudden cardiac arrest after breathing air contaminated by feces from infected rodents, reports Robert R. Parmenter, a biologist at the University of New Mexico in Albuquerque. Roughly half the victims died. Though Parmenter's data are still preliminary, he says El Niño may have prompted an explosion of the rodent population, because unusually abundant spring rains boosted the growth of the vegetation that feeds the animals.

Urban Breeding Grounds

Cities have provided breeding grounds for disease ever since 5 B.C., when Rome was home to 1 million residents, writes author Laurie Garrett in The Coming Plague. Back then, she reports, only one in every three city dwellers lived to be 30, compared with 70% of rural residents. Whenever rural populations migrate to cities, Morse notes, they carry a diverse array of microbial luggage. In fact, many zoonotic diseases—those originating in animals—"may well go unnoticed so long as the recipients remain isolated," Morse wrote in the January–March 1995 issue of Emerging Infectious Diseases. "But with increasing movement from rural areas to cities, such isolation is increasingly rare."

Worldwide, the population in 425



Source: *Addressing Emerging Infectious Disease Threats: A Prevention Strategy for the United States*, Department of Health and Human Services, Centers for Disease Control and Prevention, (1994).

cities will reach 1 million or more by the year 2000, according to a 1992 report by the Institute of Medicine (IOM) entitled *Emerging Infections: Microbial Threats to Health in the United States.* Global warming could exacerbate urban overcrowding, Epstein says, because coastal residents would flock to inland cities as ice caps melt and flood shore communities. With or without global warming, demographers say, Mexico City's population is expected to reach 30 million people in the near future. As the populations in cities such as Seoul and Calcutta approach 20 million people in the next century, overcrowding could promote outbreaks of cholera, which is frequently transported by water systems. "In many parts of the world," the IOM report notes, "urban population growth has been accompanied by overcrowding, poor hygiene, inadequate sanitation (including wastewater disposal), and insufficient supplies of clean water." Already, Cairo city officials cannot provide enough clean water for the city's 188 million residents. (see *EHP*, March 1996, p.263)

Today's urban residents are more susceptible to diseases carried by mosquitoes and rodents, particularly in developing countries. The global incidence of dengue—a mosquito-borne illness signaled by a sudden fever, headaches, achy joints and muscles, nausea, hemorrhagic bleeding, and even vascular collapse—has skyrocketed since 1956, with an average of 29,803 hemorrhagic-type cases reported each year. Though the disease is rare in the United States, the hemorrhagic strain is a leading cause of death among children in Southeast Asia. Often, the report says, "increased urbanization, densely populated areas, and poor sanitation play a significant role" in the spread of dengue.

The prevalence of water containers and discarded junk can boost mosquito populations in cities. In the United States alone, a quarter-billion tires are discarded every year, and several million more are imported for retreading. Mosquitoes responsible for dengue, yellow fever (endemic to Africa and South and Central America), and viral encephalitis "prefer to lay their eggs in water that collects in [such] containers," the IOM report notes.

Land-Use Changes

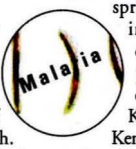
Although Lyme disease was virtually unknown in the United States before the 1960s, nearly 70,000 cases of the disease were reported between 1982 and 1994, according to David T. Dennis, chief of the CDC's Bacterial Zoonoses Branch. Affecting the skin, nervous system, heart, and joints, Lyme disease is transmitted to humans by deer ticks infected with the Lyme spirochete, a microbe that incubates inside mice before latching onto deer. Symptoms include a red "bull's-eye" lesion around the tick bite, followed by fatigue, headaches, stiff joints, and sometimes neurologic and cardiac abnormalities. Lyme disease is the direct result of cleared land, "which then returned to deciduous forest, providing the right circumstances for deer invasion," Dennis says. In the 1800s, the IOM report explains, the eastern United States was left virtually treeless after most land was cleared for farming and firewood. As farmers moved west, abandoned fields were retaken by forest. Because large predators had fled most areas, however, the deer population exploded. The proliferation of housing developments near wooded areas has exposed countless suburbanites to infected ticks.

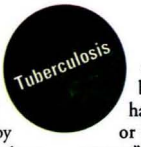
Dam construction and new farming practices can also promote disease emergence. In 1977, for example, construction of Egypt's Aswan Dam precipitated an outbreak of Rift Valley fever, which causes hemorrhagic fever and hepatitis. Almost 600 people died, and 200,000 others fell seriously ill. The dam stabilized the Nile River, but it also established ideal breeding pools for mosquitoes. In Argentina, the conversion of grassland for growing maize prompted the proliferation of a rodent known to carry the Junin virus, similar to hantaviruses. An increased number of Junin virus cases were reported during the harvest season. A change from single-species farming to pig and duck farming in China may allow pigs to serve as "mixing vessels" for new types of influenza originating in ducks, Morse says.

Air, Water, and Soil Contamination

In the spring of 1993, an estimated 403,000 people in the Milwaukee, Wisconsin, area suffered acute watery diarrhea, painful abdominal cramping, nausea, vomiting, and fever. For most victims, the agony dragged on about nine days. The cause, researcher William R. Mac Kenzie says, was *Cryptosporidium* (commonly referred to as "crypto"), a gastrointestinal parasite that spreads from animal or human feces to fingers, food, and water.

Mac Kenzie, a CDC epidemiologist, was quickly summoned to Milwaukee that spring. His study of the outbreak included a search for crypto oocysts in treated water, a telephone survey of Milwaukee residents, and stool-sample analyses. Key to the investigation was Mac Kenzie's examination of crypto-contaminated ice frozen between 25 March 1993 and 9 April 1993.





Even now, he says, the exact source of Milwaukee's crypto outbreak remains a mystery. "It could have been from agricultural runoff, or it could have been from human sewage," he says. "It may have to do with currents within Lake Michigan and water temperature or wind velocity. This is all speculative."

Whatever the source, the parasites clearly made their way into Milwaukee's drinking water; Mac Kenzie discovered high crypto levels in treatment plant samples. Because it's resistant to chlorine, crypto isn't easily scrubbed from drinking water supplies. The pores in standard water-treatment filters are about 70 times too large to capture the micron-sized parasites, he notes.

Water-treatment regulations haven't changed since the 1993 epidemic in Milwaukee, Mac Kenzie says. But the CDC has teamed up with the EPA to study the problem. Meanwhile, outbreaks continue. In 1994, crypto struck again in Las Vegas, Nevada.

Around the world, large ships draw water for ballast and later release it, sometimes contaminating drinking water. According to a 2 July 1993 article in *Science*, ballast contamination of water is a serious and widespread problem. Authors James T. Carlton and Jonathan B. Geller mapped the journey of plankton samples from 25 Japanese ports to ballast water released by 159 cargo ships in Coos Bay, Oregon. "The ecological roles and impacts of invading species can only be partially predicted from knowledge of their biology and ecology in donor regions," they warned.

Ballast water may transport bacteria and viruses, along with algae, from port to port, Colwell says. In the 1970s, Colwell made a startling discovery: the cholera *Vibrio* can remain dormant inside algae, biding its time until warm water temperatures spur it back into action. A Chinese freighter may have triggered a massive cholera epidemic in 1991 by releasing contaminated water near Lima, Peru. Because drinking water wasn't chlorinated, the disease ultimately killed 3,538 people and made 336,554 others sick throughout the Western Hemisphere, Garrett reports.

E. coli is perhaps most notorious in the United States for its role in killing four children and causing severe bloody diarrhea in 500 other Washington State victims who ate contaminated hamburgers in 1993. But *E. coli* can also cause illness when it contaminates the environment. A 1991 outbreak resulted from people swimming in a lake fouled by the bacterial pathogen, according to the CDC's report,

Addressing Emerging Infectious Disease Threats. Outbreaks of *E. coli* are a growing public health problem worldwide because of increasing resistance to antibiotics among these bacteria, Garrett says.

From Animals to Humans

Zoonotic diseases are "an important and potentially rich source of emerging diseases," says Morse. These diseases can be transferred to human populations through urbanization, ecological changes, or research activities. For example, a group of nonhuman primates from Uganda, slated for use in vaccine research, caused a deadly viral illness among humans in Marburg, Germany, in the late 1960s, according to the 1992 IOM report. A similar Filoviridae virus, Ebola, killed hundreds in Africa in the mid-1990s. In 1989, when a Filoviridae virus was discovered among nonhuman primates shipped from the Philippines to a U.S. research facility in Reston, Virginia, scientists feared the worst. However, although the virus infected some humans, no one got sick. In April of this year, 50 monkeys were euthanized at a research facility in Alice, Texas, after scientists discovered that the animals carried the same Reston virus.

The origin of HIV is unclear, but nonhuman primates displaced by land-use changes may have played a part in the emergence of the disease among humans. Shrinking primate habitats and other factors "opened the possibility of cross-species transmission among three or more monkey/chimp species," Garrett says.

Creutzfeldt-Jakob disease (CJD) is a devastating illness that causes severe brain damage, but it usually only strikes the elderly. When a number of British children were stricken by CJD recently, the cases triggered panic among the beef-eating public. "Mad cow disease," or bovine spongiform encephalopathy (BSE), was fingered by some as the likely cause of CJD in children.

Scientists haven't shown how BSE from cows might mutate to cause CJD in humans, and some say such species-to-species transmission is highly unlikely. "BSE appears to be a purely bovine disease," says Hugh-Jones. "There's no obvious connection [to CJD] at all."

Nevertheless, a handful of researchers wonder whether abnormal protein folding might allow BSE to circumvent the so-called species barrier. Infectious particles called prions are proteins that may be capable of reproducing without genetic code material, the researchers say. When prions from hamsters are exposed to abnormal proteins from other species, cross-species mutations occur spontaneously, according to research by a team directed by Richard A. Bessen of the National Institute of Allergy and Infectious Diseases, published in the 22 June 1995 issue of *Nature.* Despite this intriguing finding, however, the link between BSE and CJD remains speculative.

The Antibiotic Paradox

Professor Stuart B. Levy of the Tufts School of Medicine points out that antibiotics are so widely used among human and animal populations that they've become pervasive throughout the environment. Many diseases, including some forms of tuberculosis and malaria, are now impervious to antibiotics. In fact, "more than 90% of strains of *Staphylococcus aureus* (one of the most common disease-producing organisms in humans) are resistant to penicillin and other beta-lactam antibiotics," according to the 1995 *Report of the American Society for Microbiology Task Force on Antibiotic Resistance.*

According to the CDC, tuberculosis kills more people around the world than any other infectious disease. Until 1985, tuberculosis had been in decline for more than three decades, particularly in the United States. Currently, 8 million new cases are diagnosed each year worldwide, with 2.9 million fatalities each year. Although increased antibiotic resistance may be one factor in the resurgence, increased poverty, homelessness, substance abuse, and lack of access to health care are also cited by the CDC as causes.

Enterococci, the most common bacterial source of hospital-acquired infections, are rapidly learning to resist antibiotic treatment. Between January 1989 and March 1993, the American Society for Microbiology report says, U.S. hospitals reported a 20-fold increase in *Enterococci* resistant to the antibiotic vancomycin. The report notes that "vancomycin is often the last weapon available against these deadly microbes."

Some observers now fear that resistant *Enterococci* could share their resistance genes with staph and strep bacteria. "Such a bacterial strain, if it did emerge, would be virtually incurable and extremely dangerous," Garrett explains, "for it would possess not only special drug-resistant genes but also those for heightened virulence."

Pneumococci, a source of potentially fatal pneumonia, meningitis, bloodstream infections in the elderly, and middle-ear infections in children, are also gaining antibiotic resistance. The same holds true for certain strains of tuberculosis and malaria. At one time, chloroquine was the most commonly prescribed antimalarial drug. "Today," the 1992 IOM report says, "there are only a few areas in the world where chloroquine is effective." Chloroquine's successor, mefloquine, is also rapidly losing its efficacy.

Bacteria are built to survive and adapt to change, according to Levy, also author of the book *The Antibiotic Paradox.* Any bacteria that survive antibiotic treatment are more likely to flourish. Resistance can also be transferred from bacteria to bacteria via plasmids (genetic material), thanks to a "bacterial mating" process known as conjugation. Levy says that too many antibiotics now contaminate our food and water because of drugs given to livestock. Eighty percent of these antibiotics are used because farmers

Factors in Emergence of Diseases

Categories	Specific examples
Societal events	Economic impoverishment; war or civil conflict; population growth and migration; urban decay
Health care	New medical devices; organ or tissue transplantation; drugs causing immunosuppression; widespread use of antibiotics
Food production	Globalization of food supplies; changes in food processing and packaging
Human behavior	Sexual behavior; drug use; travel; diet; outdoor recreation; use of child care facilities
Environmental changes	Deforestation/reforestation; changes in water ecosystems; flood/drought; famine; global warming
Public health infrastructure	Curtailment or reduction in prevention programs; inadequate communicable disease surveillance; lack of trained personnel (epidemiologists, laboratory scientists, vector and rodent control specialists)
Microbial adaptation and change	Changes in virulence and toxin production; development of drug resistance; microbes as cofactors in chronic diseases

Source: *Addressing Emerging Infectious Disease Threats: A Prevention Strategy for the United States,* Department of Health and Human Services, Centers for Disease Control and Prevention, (1994).

believe they may promote growth, not because animals are sick, he says. In a 4 March 1976 article in *Nature*, Levy showed how antibiotic-resistant plasmids in chickens could spread to farm workers.

It remains to be seen if the pharmaceutical industry will discover new antibiotics in time to combat resistant bacteria. Levy isn't optimistic, but he admits it may be possible. Daniel Bonner, executive director of microbiology at Bristol-Myers Squibb, points out that oxazolidinones—discovered by DuPont and now being developed by Upjohn Co.—represent a fundamentally new class of antibiotics. In the search for new antibiotics, Levy says, researchers are now investigating chemicals in insects, compounds in frog skin, and components of soil mined from deep beneath the earth's surface. But like existing antibiotics, he cautions, new treatments will soon become obsolete without safeguards to prevent resistance.

Antibiotic resistance is costly; the average cost of treating nonresistant tuberculosis (including drugs, procedures, and hospitalizations) is about $12,000, according to Mitchell L. Cohen, director of the CDC's Division of Bacterial and Mycotic Diseases in an article in the 21 August 1992 issue of *Science*. For multidrug-resistant tuberculosis cases, he says, costs can soar to $180,000. To help prevent resistance, the American Society for Microbiology states that doctors must be trained to distribute antibiotics appropriately, and patients should be educated about the dangers of "sublethal" doses that fail to kill all of the bacteria and thereby promote resistance.

Pesticides and Disease

Using pesticides to control public health risks "can cause resistance in the very insects they are intended to kill," the IOM report says. The report adds that a "growing array" of pesticides no longer kill mosquitoes that transmit malaria.

According to the report, in 1987 farmers used 407,000 tons of pesticides in the United States. Worldwide, only 10% of all pesticides are used to control public health problems. In 1992, the IOM urged the EPA to develop faster procedures for approving pesticide use in the event of an emergency. The committee also recommended stockpiling designated pesticides to ensure a fast response to outbreaks of diseases such as malaria. Strategies for managing pesticide resistance include the rotation of chemicals, avoidance of sublethal doses, and the use of biodegradable materials.

Despite such efforts, pesticide resistance is a growing problem. A 1986 report published by the National Research Council entitled *Pesticide Resistance: Strategies & Tactics for Management*, pointed out that only seven species of insects and mites were resistant to insecticides before World War II. By 1984, the report says, at least 447 species were immune to one or more insecticides. Several agricultural pests, including the Colorado potato beetle, were impervious to all known pesticides in 1986.

Crops engineered to resist pests may allow farmers to use fewer chemical pesticides, but some observers say genetically engineered plants might also transfer undesirable traits to neighboring plants. In the 7 March 1996 issue of *Nature*, researcher Thomas R. Mikkelsen and colleagues from the Riso National Laboratory in Denmark reported the transfer of herbicide-resistance genes from a genetically manipulated rape plant to a weedy relative, *Brassica campestris*. In response to Mikkelsen's report, a large German environmental organization, BUND, called for a ban on field experiments with bioengineered plants. Meanwhile, some consumer advocates have criticized the Flavr Savr tomato, which is genetically engineered by Calgene, Inc. of Davis, California, to resist rotting. An antirotting gene is added to the Flavr Savr's genome using an antibiotic-resistant marker gene. Critics fear the marker gene could increase antibiotic resistance in humans. Levy says, however, that the kanamycin-resistant gene used in processing the Flavr Savr tomato is "one that we don't use much anymore," and it is "millions of times more evident in our own gut flora, compared with its presence in the tomato."

Preparing for the Future

"Humans are not powerless against this relentless march of microbes," wrote Morse in his article in *Emerging Infectious Diseases*. Improved surveillance and a better understanding of disease can go a long way toward reducing human suffering and health care costs, he added.

A variety of initiatives are under way to prevent epidemics. The CDC's 1994 report calls for improved disease surveillance, applied research, better prevention and control, and a stronger public health

Some Pathogenic Microbes and Infectious Diseases Recognized since 1973

Year	Microbe	Type	Disease
1973	Rotavirus	Virus	Major cause of infantile diarrhea worldwide
1976	*Cryptosporidium*	Parasite	Acute and chronic diarrhea
1977	Ebola	Virus	Ebola hemorrhagic fever
1977	Hantanvirus	Virus	Hemorrhagic fever with renal syndrome (HRFS)
1981	Toxic producing strains of *Staphylococcus aureus*	Bacteria	Toxic shock syndrome (tampon use)
1982	*Escherichia coli* 0157:H7	Bacteria	Hemorrhagic colitis; hemolytic uremic syndrome
1983	Human immunodeficiency virus (HIV)	Virus	Acquired immunodeficiency syndrome (AIDS)
1988	Human herpesvirus-6 (HHV-6)	Virus	Roseola subitum
1992	*Vibrio cholerae* 0139	Bacteria	New strain associated with epidemic cholera
1995	HHV-8	Virus	Associated with Kaposi sarcoma in AIDS patients

Reemerging Infections in the Last Two Decades

Disease or agent	Type	Factors in reemergence
Rabies	Virus	Breakdown in public health measures; changes in land use; travel
Dengue	Virus	Transportation, travel, and migration; urbanization
Malaria	Parasite	Drug and insecticide resistance; civil strife; lack of economic resources
Toxoplasmosis	Parasite	Increase in immunocompromised human hosts
Giardiasis	Parasite	Increased use of child-care facilities
Tuberculosis	Bacteria	Human demographics and behavior; industry and technology; international commerce and travel; breakdown of public health measures; microbial adaptation
Cholera	Bacteria	Travel: a new strain (0139) was introduced to South America from Asia by ship,and spread by reduced water chlorination and food

Source: *Addressing Emerging Infectious Disease Threats: A Prevention Strategy for the United States*, Department of Health and Human Services, Centers for Disease Control and Prevention, (1994).

infrastructure. Thus far, Ostroff reports, a multiagency task force directed by the CDC has strengthened surveillance by setting up emerging-infections programs to track "high priority diseases" and to analyze health risks in specific communities. The CDC is also providing additional funds to help state health departments respond more effectively to emerging diseases. New electronic networks, for example, will allow state officials to make use of laboratory diagnoses in real time. For example, ProMED—the international Program for Monitoring Emerging Diseases—will provide grassroots surveillance of pubic health problems. (See sidebar article.)

In addition, Ostroff says, "sentinel networks" composed of hospital emergency departments have been formed to identify emerging diseases more promptly. Public health microbiologists receive training through a new fellowship program, he says, and the CDC is "beefing up collaborating World Health Organization [disease] surveillance centers."

In May 1995, WHO identified emerging-disease surveillance as a top priority, reports Lindsay Martinez, a senior program manager in the newly formed Division of Emerging and Other Communicable Diseases Surveillance and Control. The division's key activity is directing a network of laboratories to study antibiotic resistance patterns. "Resistance is an extremely critical problem in the developing world," Martinez says. "When a routine antibiotic for common infections is no longer effective, it's necessary to look to second-line antibiotics, which are usually more expensive and may not be available at all."

The National Aeronautics and Space Administration (NASA), meanwhile, has launched the Global Monitoring and Disease Prediction Program. Using data from satellites and other remote-sensing equipment, NASA is analyzing land-use changes and population demographics to predict outbreaks of Lyme disease, malaria, and other illnesses, says the program's manager, Maurice Averner. NASA is even analyzing the African landscape in search of a nonprimate reservoir, such as a mosquito or tick, that may carry the Ebola virus. Since Ebola victims often lived near charcoal-making pits, Averner explains, surrounding geographic features might help NASA identify the most likely source of the disease.

When antibiotics allowed doctors to triumph temporarily over microbes, "we acted as though we had won the war on infectious disease," says Professor Joshua Lederberg of The Rockefeller University. But, Lederberg notes, "the fact is, infectious microbes have been around all along and will continue to pose threats to public health."

Ginger Pinholster

Microbe photo credits: Ebola, Frederick A. Murphy; HIV, C. D. Fermin and R. F. Garry; Rotavirus, M. Stewart McNulty; Malaria, Blackwell Scientific Publications; Tuberculosis, Edward C. Klatt; Rabies, Frederick A. Murphy; Giardia, James A. Sullivan.

ProMED: A Global Early Warning System for Disease

"Until we knew there was such a thing as AIDS, there was no [monitoring] system that would recognize it. And if AIDS were to come along today as a new disease, we would still be in the same situation," said Stephen S. Morse, assistant professor of virology at The Rockefeller University in New York City.

Currently, an international group of some 35 senior scientists and health experts who make up the steering committee of the Program for Monitoring Emerging Diseases (ProMED), are working to see that this situation soon changes. ProMED's goal is to provide an effective global system of infectious disease surveillance that could give early warning to public health officials and others of new diseases, as well as of outbreaks of familiar ones.

A project of the nongovernmental policy group Federation of American Scientists (FAS), ProMED had its inception when the Institute of Medicine Committee on Emerging Microbial Threats to Health of the National Academy of Sciences issued its *Emerging Infections* report in 1992.

According to Morse, who was a member of that committee, "Many people were very concerned that all over the world, the ability to spot even known diseases and identify them properly, and in time to take appropriate action, is very variable." Hence, one of the report's primary recommendations was that steps be taken to fill that void. In response, a meeting, cosponsored by FAS and the World Health Organization, was held in late 1993 and ProMED was born.

To date, the most visible manifestation of ProMED is its e-mail network. Accessible at majordomo@usa.healthnet.org, the network has some 4,500 subscribers from over 100 countries, says Morse, who chairs the ProMED steering committee. ProMED selected e-mail, the most basic Internet technology, as its primary means of communication so that those in developing countries, where the World Wide Web may not yet be available, can participate fully. However, the group also maintains a WWW site, http://www.fas.org/promed, through which its files can be accessed.

Established only two years ago, the e-mail network "has taken off in a way that we are very gratified to see," says Morse. Already it has carried the first report of Japanese encephalitis in Australia. Subscribers have also reported the 1994 deaths of racehorses and their trainer in Australia from the newly identified morbillivirus, the 1995 Ebola outbreak in Zaire, and the 1995 outbreak of avian influenza in Mexico.

But e-mail is only one tool available. A formal international surveillance system must exist on one end of the communications network, with an assessment and response apparatus at the other end for the system to be effective. ProMED is actively working with various organizations to realize these goals.

Victor Chase

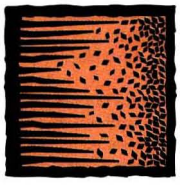
Never have technologies developed at a faster rate. Hardly a day passes that doesn't see the introduction of some breakthrough, with rapid advances occurring in such wide-ranging fields as electronics, composite materials, and genetic engineering. And, as has been the case since humans first toyed with fire, with new technologies come risks to human health, some obvious and some difficult to predict.

To cope with these risks, some people have started to think of workplaces as environmental niches in which humans are a natural species and are made vulnerable by the technology around them, says Carroll Pursell, a technology historian at Case Western University. "We're like spotted owls," he says. "We're not sure how these things are going to affect us." Such heightened environmental awareness has developed throughout the twentieth century. "But it's not a steady curve of increased concern. It comes and goes in more-or-less 30-year cycles," Pursell explains. Public and governmental attention to the hazards associated with emerging technologies started with the Progressive movement at the turn of century, flared up again with the New Deal in the 1930s and 1940s, and peaked for a third time with the radical baby boomer movements of the 1960s and 1970s.

These peaks, says William Burgess, a former Harvard professor of industrial hygiene, represent reactions to leaps in technological advances. "Right after the Second World War, in the late 1940s and early 1950s, there was a very dynamic shift in manufacturing technology, but there were also few regulatory boundaries in place," he says. This shift was followed by increases in both governmental and industrial attempts to predict the impacts of burgeoning technologies. But after each period of increased concern, Pursell says, the resulting government institutions have been eroded by backlash from vested interests. The current round of governmental budget slashing represents such a reaction, he says.

In spite of recent governmental downsizing, Burgess says, a century of experience with predicting the impacts of emerging technologies has left a robust regulatory infrastructure and a cadre of environmental health professionals. "My prognosis is that in the '90s and in the future—especially because of EPA and OSHA—we have a better shot at looking at what we're introducing and anticipating problems from it." And, he says, although new technologies often result in health impacts that are difficult to predict, "now, the technologies that are coming in have gone through some review because [manufacturers] have to be sensitive to regulations."

Fibers and Fine Particles



Perhaps no material in the last 20 years has been examined more extensively for health impacts than fibers, both natural and man-made. Much of that attention can be attributed to the risks now known to be associated with asbestos, once considered a miracle material. Asbestosis—a blanket term for lung diseases related to asbestos including mesothelioma (cancer of the peritoneal cavity), lung cancer, and lung fibrosis—first drew serious study in the 1930s, although it had been identified decades earlier. But it was the burst of asbestosis starting in the 1960s—a result of wide-spread wartime use—that led to research that has identified the properties of asbestos that make it, and perhaps similar materials, hazardous.

Often appearing where asbestos would have been used, glass and ceramic fibers are found in such applications as insulators, friction materials (such as automobile brake pads), and structural components. Many of the new fibers share some or all of asbestos's characteristics. Exactly which of these characteristics could spell trouble for people that come into contact with the fibers is still the subject of debate.

Seeking an elusive combination of high strength and light weight has driven engineers to develop a staggering variety of new fibers and particles. Typically composed of various combinations of ceramics, polymers, and metals, these composites can pose a health risk to workers who inhale fibers and particulates, and may present health hazards as serious as those of asbestos. "We're introducing new materials, and it's hard to predict what their toxicology might be," says Vincent Castranova, chief of the Pathology and Physiology Branch of the National Institute for Occupational Safety and Health (NIOSH). "In some situations, they have developed fibers that seem to be less toxic, but in other situations there are fibers that are not necessarily less toxic, and so are not necessarily better," he said.

Predicting the impacts of fibers and dusts can be tricky, Castranova says, because biological effects take a long time to appear. "So we won't have a read on what the occupation hazards might be from the worker population for maybe 20 years or so after the introduction of a new fiber,"

he explains. Working from animal models, however, researchers have identified many of the characteristics that appear to affect a material's fiber toxicity. "What we've learned from asbestos is that the fiber geometry and the fiber size and its durability are important in health effects," says Brooke Mossman, a University of Vermont cell and molecular biologist. "If you want a . . . safe fiber, you need to make one that is not durable, that will dissolve in lung tissue and not persist and cause disease," she said, although such fibers would probably have limited applications. Most important, she says, is whether the fibers can be inhaled at all. Fibers larger than 10 microns in diameter won't penetrate deep enough into the lungs to cause disease, she says.

Unfortunately, many of the most desirable manmade fibers have many of the least desirable health-related characteristics. High-performance ceramic fibers—which are made from raw materials such as silicon carbide, boron, carbon, zirconia, and alumina—combine the high melting points (greater than 1400°C) and durability needed for such applications as high-temperature insulation, reinforced structural materials, and high-wear components such as bearings, piston rings, and cutting tool inserts. In 1987 the Office of Technology Assessment projected that, although currently limited, these applications would grow to a $1–$5 billion dollar per year business by the turn of the century. Much of this demand would be generated from the automotive industry's drive to decrease the overall weight of vehicles. Many of these materials, however, may also pose serious health risks. Unlike glass fibers, which are usually designed to be soluble in tissue, ceramic fibers are durable, persisting as an irritant in lungs. Like asbestos, ceramic fibers also tend to be very rigid. This rigidity may allow them to penetrate the peritoneal cavity, possibly leading to mesothelioma, Castranova says. Fibers that are long and thin exacerbate these problems. The hypothesis, he says, is that because the lung can't engulf long fibers, its tissues secrete damaging enzymes and reactive oxygen radicals. And even fibers that are soluble enough for lung tissue to absorb may persist in the peritoneal cavity.

Where possible, Castranova says, the fibers industry, which provides much of the funding for fiber toxicity research, tries to avoid combinations of characteristics that result in potentially toxic materials, but "sometimes the constraints of the application don't allow that." Fibers for high-temperature insulations, for example, must be durable to withstand heat and convoluted to trap air. But their twisted shape also helps them to be trapped in the lungs.

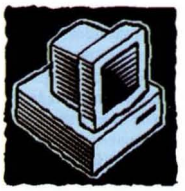
Manufacturers also prefer the least toxic fibers possible, says toxicologist Candice Wheeler, a General Motors staff research scientist. "All materials, before they get into the product or into the process, have to be reviewed for both their health and their environmental impact," she says. "There are several engineering controls you can implement to minimize exposure from the very beginning." For example, GM installs high-powered fans and electrostatic filters, and, when practical, asks that raw materials be delivered as pellets rather than loose fibers. "Our standard so far is that we've been very conservative and we treated almost everything as asbestos," she explains. "That way we know we will protect our workers to the best of our ability."

Although the risks of toxic airborne fibers have been well accepted for several decades, ultrafine articles—those smaller than 0.1 microns—are just beginning to be investigated as a potential health concern. Ceramic powders are finding their way into many types of high-tech composites. For example, particles of such materials as silicon carbide or graphite are added to lightweight metals—typically aluminum or magnesium—to significantly stiffen and strengthen them. Some researchers, however, now suspect that ultrafine particles may cause diseases similar to those of toxic fibers. "There has been a renewed interest or renewed realization that maybe the fine particles are the ones that have an important role that has been overlooked," says George Guthrie, a mineralogist and geochemist at Los Alamos National Laboratories.

Even normally inert materials, such as titanium dioxide, silica, and aluminum dioxide, may become biologically active when broken into very small particles, explains University of Rochester toxicologist Gunter Oberdörster. As particles get smaller, their surface area increases in relation to their mass. Like chemical catalysts, ultrafine particles may be more reactive because of their greater surface area. And, adds Wheeler, smaller particles reach deeper into the lungs.

In fact, she says, researchers don't have a good understanding of the mechanisms that may contribute to the toxicity of ultrafine materials. Studying these materials presents special problems. Epidemiological studies, she says, are inconclusive because the particles are rarely found in pure form outside the laboratory. Instead, they are often contaminated with other materials that have been absorbed onto their surfaces. Animal tests are also inconclusive, according to Robert McCunney, director of environmental medical services at the Massachusetts Institute of Technology (MIT). When exposed to high levels of ultrafine particles, rats develop tumors, but hamsters and mice don't. "This whole business of lung overload in the rat model has thrown a proverbial monkey wrench into the risk assessment [for ultrafine particles]," McCunney says. "Many reputable authorities are of the opinion that the rat model may not be appropriate for predicting human risk when conditions of lung overload occur."

Computer-related Technologies



Since the explosion of workplace computing started in the 1970s, a wide array of physical ailments has been linked to long hours spent staring at cathode ray tubes and pounding on keyboards. In some professions, such as data entry, more than 50% of workers report repetitive stress injuries to their hands or wrists. "Here we have an office technology that has completely changed that particular population," says industrial hygienist William Burgess. Additional physical symptoms that have been reported include back and neck pain, spontaneous abortions, and gastrointestinal ailments, although a cause-and-effect relationship has not always been substantiated.

"There is no one solution for these problems," says Louis DiBerardinis, industrial hygiene officer for MIT. Some repetitive stress injuries, such as carpal tunnel syndrome, may be prevented by using innovative ergonomic keyboards with nontraditional key arrangements that guide the hands into more relaxed positions. Keyboards with audible "clicks" can help workers prevent the fingers from bottoming out at the end of keystokes, which sends damaging vibrations through the hands. And flexible workstations, with adjustable keyboard trays and monitor stands, help people find the perfect computing posture and vary their orientation during the course of a workday. Often, DiBerardinis says, avoiding injury is as simple as taking frequent breaks or learning new typing techniques.

But for some people, none of these approaches seem to work. Recently, the idea has emerged that many of these physical symptoms have their origins in psychological stress. "Up until about five years ago, most of the concentration [was] on the physical aspects of work and how to change, say, workstation design, equipment design, and so on to relieve some of the

problems people were having," says NIOSH psychologist Naomi Swanson. "The design of the physical work environment for computer users has rapidly changed and is much better than it used to be, but people still keep having problems, and the problems seem to be increasing." The source of many of these problems, she says, may be workplace stress associated with new computer technologies.

In the short term, stress can lead to depression, tension, and anxiety. Over extended periods it may result in physical ailments such as high blood pressure and migraines, as well as increased vulnerability to physical injuries and infections. Stress-related physical ailments may result from increases in muscle tension and changes in the autonomic nervous system, Swanson says. "For anyone, it is stressful when they're asked to learn new technologies," say Lawrence Rosen, a California State University–Dominguez Hills psychology professor who specializes in the psychosocial effects of computer use. A survey of federal workers, for example, found that 84% felt undertrained on the computers they use, and 80% complained that they didn't have adequate time to learn how to use their computers.

Workplace stress-related ailments can increase dramatically when workers' performance on the computer is remotely monitored. Supervisors for data entry workers, for example, often remotely track the number of keystrokes completed each hour. And telemarketers and reservations clerks are monitored to make sure that the number and average length of calls they handle fall within acceptable limits. Such monitoring methods are counterproductive, says Janet Cuhill, a psychology professor at Rowan College of New Jersey. "The technology should do no harm at the very least and should improve the work environment at the best." In a project to find the best ways to incorporate technologies into the workplace, Cuhill is helping to introduce computers to an agency that deals with child abuse. "When we introduce the computers," she explains, "we don't just say 'this is going to make you work faster or work differently.' We expressly measure what changes occur as a result in the work environment itself." It's important, she says, to provide adequate training, to allow workers to control their own pace, and to have tasks away from the computer. "We also avoid the obvious hazards, which are monitoring keystrokes and . . . breaks, and those kinds of things," she said.

For many people, the revolution in personal computing has not only changed the nature of the workplace, it has changed its location. "The computers allow telecommuting, which allows a reduced level of stress," says Wendall Joyce, a psychologist for the U.S. Office of Personnel Management, who recently completed a review of the federal telecommuting program. Most telecommuters in both government and private sectors report that working at home significantly lowers their stress levels. Anyone who must look at a computer monitor constantly will develop psychosomatic stress and tension, he says. "But in a standard workplace, most don't feel as though they can take breaks because it looks like they're loafing. In their own work environment, they can probably take those breaks and still keep up the productivity without worrying about that."

Yet, others worry about the effects of isolation from the actual workplace and lack of socialization with coworkers on the emotional and ultimately physical well-being of workers. Like women and children who toiled at home on piecework more than a century ago, many workers are now isolated from their fellow workers. Where once the product might have been matchbooks or artificial flowers, now computers allow home-based workers to produce items from completed insurance forms to sophisticated computer programs. Although the potential health effects of the contrasting freedoms and isolation of the home workplace are not well understood, some experts predict that within 10 years some 25% of Americans will work outside of traditional venues.

Transgenics

Genetic engineering, in which genes that code for desirable traits are transplanted from one organism to another, is a rapidly expanding field with applications in research, agriculture, pharmaceuticals, and bioremediation. It is also a battlefield on which prodevelopment industry and academic professionals face off against environmentalists and public health advocates over the safety of transgenic techniques and organisms. People on both sides disagree on the magnitude of the potential risks genetic engineering poses, whether it is the technology of transgenics or genetically engineered organisms themselves that deserve special scrutiny, and whether current federal regulations are adequate to patrol transgenic products.

An example of the debate concerns the risk of introducing allergens to the food supply. Recently, for example, Pioneer Hi-Bred International decided not to market a strain of soybean that was found to trigger a reaction in people who are allergic to Brazil nuts. Normal soybeans lack two of the 20 amino acids that combine to make complete proteins. To round out the set, researchers inserted a Brazil nut gene that encodes for the missing proteins, methionine and cysteine. With the protein, however, came the allergen. "That demonstrated something that those of us in the environmental community had said for years: that sooner or later someone is accidentally going to transfer an allergen into a crop plant with genetic engineering," says biologist and Environmental Defense Fund Senior Scientist Rebecca Goldburg.

Genetic engineering proponents say that they, too, could have predicted that borrowing genetic sequences from common allergenic foods would eventually result in such problems. But, they maintain, the allergen was identified and the company voluntarily withdrew the product. "To me, that says the system worked," argues Peggy Lemaux, a microbiologist at the University of California–Berkeley. Food and Drug Administration regulations require that companies test genetically altered plants if they suspect that the plant will cause allergic reactions. From the FDA's viewpoint, such suspicions are reasonable if the genetic material is borrowed from any of a group of foods—such as crustaceans, milk, eggs, legumes, fish, and nuts—to which many people are allergic.

"That policy is far too narrowly focused," says Goldburg. "There is no scientific distinction between commonly allergenic foods and uncommonly allergenic foods." Under the current policy, she says, people with less common allergies—say, to bananas—will become increasingly at risk as more foods with borrowed sequences enter the marketplace. "The people who will have absolutely no protection are people who may at some time in the future find that they are allergic to a protein from a nonfood source."

If genetically engineered foods are to be sold, Goldburg and other transgenics conservatives say, they should be labeled with the source of the added genes. Additionally, industries should notify the FDA of all new genetically engineered foods they release. That would permit a sort of "food recall" if consumers began reporting new allergies.

Neither of these suggestions pleases pro-transgenics groups. The food industry resists the added expense of such comprehensive labeling, and they may be reluctant to dull consumers' appetites with labels

listing genes from nonfood sources such as bacteria, or odd combinations such as flounder genes in tomatoes. And tracking foods just because they were genetically engineered simply doesn't make sense, says Martina McGloughlin, associate director of the biotechnology program at the University of California–Davis. "We've been modifying our food supply since the beginning of time. And although people would like to think when they go into the supermarket that the fruits and vegetables that they see there have been like that forever, even if you look 50 or 100 years ago, the produce was very much different than it is today. That's because human beings have been involved in changing them."

The trick, says Thomas Zinner, a biologist with the University of Wisconsin–Madison's biotechnology center, is to distinguish between the tool itself and the products it produces. "Do you regulate based on what was done or how it was done?" he asks. Each product should be evaluated on its merits rather than on the technology that was used to produce it, he says. "To imply that there are no risks of introducing allergens through selective breeding isn't accurate. There are risks based on your gene pool. What recombinant DNA technology does is expand your gene pool."

But that expansion is the core of the problem, Goldburg says. "If you are, say, breeding soybeans with each other, the chances are that all of the soybeans that you are crossing contain the same suite of allergens. Transgenic plants can contain genes, at least in theory, from any other organism. There's a whole new spectrum of proteins we can put into the food supply. I would argue the odds of putting an allergen into an unexpected place are much higher with genetic engineering."

Just as intense as the debate over direct human health impacts from transgenics has been the disagreement over the indirect impacts through potential environmental damage. One such area includes plants that have been engineered to carry the genes for natural insecticides. Monsanto, for example, is in the process of adding the bacterium *Bacillus thuringiensis*, or Bt, to potatoes. As unappetizing as it may sound, there are no apparent risks from eating such plants. The insecticidal proteins are not toxic until they are broken down into toxins in the insects' highly alkaline stomachs. And humans don't have receptors for the insect-specific toxins. But, some environmentalists argue, engineering bacterial insecticides into plants could drive insects to evolve resistance to the common, environmentally benign insecticide.

"When you starting putting Bt into crops like corn and planting them on a large scale, so that the Bt is present in the plant all the time, insects will be affected by it every time they feed on the plant and the selection pressure for Bt resistance is going to skyrocket," Goldburg says. "There is virtual unanimity among the entomology community that if we put Bt out on the market without any plan for managing the evolution of resistance, we can kiss it goodbye." Such a situation would leave organic farmers, who depend on Bt, without one of their only acceptable insecticides.

The answer, McGloughlin says, is to both closely regulate the application of Bt-containing plants and to develop many versions of the plants, each with a different strain of the insecticide. Like any new technology, people have to adjust to its strengths and weaknesses. "Biotechnology is a tool and people will use the tools that are available to them," she says. "Gradually there will be greater and greater use of this tool as people get used to it."

We are in the midst of a second industrial revolution, says Tai Chan, program manager of occupational health and safety research for General Motors, one in which new high-tech materials are entering the workplace at an almost overwhelming rate, and the nature of the workplace itself is steadily changing. Although some new technologies may present new hazards, Chan says, as researchers are better able to predict environmental and health hazards, they will develop other new technologies to mitigate such risks. "Technology should be about the exercise of prudence," says Pursell. "But economic considerations usually push new developments forward."

Scott Fields

LMOs: TREASURE CHEST OR PANDORA'S BOX?

Biotechnology is beginning to transform agriculture across the globe. After thousands of years of traditional plant and animal breeding, and centuries of mechanization and chemical application, genetic research has opened a Pandora's box of living modified organisms (LMOs) designed to improve the productivity and efficiency of commercial agriculture. A multitude of transgenic crops and animals is now being introduced into commerce by biotechnology companies, and nations are puzzling out how to appropriate the benefits and manage the risks.

American biotechnology companies and agencies are the leading proponents of using LMOs. They claim that two decades of costly and careful research and several years of field testing in the United States prove that the LMOs being offered are a safe and effective means of improving productivity, reducing dependency on toxic chemicals, preventing environmental and health hazards, enhancing nutrition, achieving a reliable food supply for burgeoning populations in poorer countries, and promoting sustainable growth.

In their new book, *Agricultural Biotechnology and the Environment,* professors Sheldon Krimsky and Roger Wrubel of Tufts University assess the emerging universe of LMOs and divide it into several broad categories including:

- **herbicide-resistant crops** (such as new strains of corn, soybeans, and potatoes) in which new genes are introduced to diminish plant sensitivity to chemical herbicides, or to detoxify the herbicides;
- **insect-resistant crops** (such as new strains of corn, soybeans, and potatoes) in which the introduced genes, most commonly from *Bacillus thuringiensis* (Bt), kill specific insect pests that ingest the Bt without causing toxic or other harmful effects on nontarget species;
- **disease-resistant crops** (such as alfalfa, squash, corn, and potatoes) in which the new genes provide antifungal properties and other defenses against plant pathogens;
- **product-producing crops** in which the new genes enable plants to produce more nutritious or attractive foods (Calgene's Flavr Savr tomato), serums and vaccines (W.R. Grace's modified potato and tobacco plants), and oils for industrial use (new strains of rapeseed);
- **biopesticides,** which thus far involve release-modified forms of Bt in field applications to kill targeted pest species such as the European corn borer and the Colorado potato beetle, and which may soon include virulent strains of baculoviruses;
- **productivity-enhancing bacteria** such as nitrogen-fixing bacteria (to improve soil fertility) and frost-inhibiting bacteria;
- **animal growth hormones** to produce leaner meat for health-conscious consumers, or more productive animals (for example, using bovine somatotropin to increase milk production); and
- **transgenic animals,** created by introducing foreign DNA into fertilized eggs, to provide leaner meat, carry human disease for health research, or function as bioreactors in "pharming" for therapeutic products (such as Genzyme Transgenics' goat capable of producing BR-96, an experimental anticancer drug).

To develop such LMOs and introduce them into commerce, American companies must comply with a detailed framework of federal biosafety requirements that use risk assessment and risk management methods. The biosafety requirements range from containment of certain LMOs for research in secure laboratories to subsequent procedures for the assessment, approval, and monitoring of field releases. Since most LMOs intended for agricultural use ultimately require their release into the environment outside the lab, field test requirements are the most critical feature of the biosafety review process. Depending on the type of biotechnological product involved, regulatory authority of the United States Department of Agriculture, the Environmental Protection Agency, or the Food and Drug Administration applies to and governs company testing and introduction of agricultural LMOs into commerce.

In the past five years, over 2,000 field tests of LMOs at over 8,000 carefully selected sites in the United States have been approved and conducted without a single reported adverse or even unpleasant impact on the environment or public health, according to officials at the USDA. This far surpasses the total experience of all other nations, according to biotech industry and agency officials, and should provide considerable assurance to other nations that LMOs offered by American firms can be safely managed.

Concerns About LMOs

Genetically modified organisms that are not intended for agriculture but are used for research or development purposes, such as transgenic mice or bacteria, are subject to "contained use" requirements in the United States and the European Union countries. Such requirements are intended to prevent accidental release of LMOs, which could present an immediate or delayed hazard to health or the environment, and are risk-based and somewhat variable. Some containment measures used include physical and chemical barriers, engineering controls, negative air pressure, spill containment equipment, and treatment of wastes prior to off-site disposal. These measures are reinforced by requirements for employee training, protective equipment and decontamination facilities, emergency plans and practice codes, record-keeping and reporting, and biosafety oversight and accountability. By far the larger controversy deals with genetically modified organisms and agriculture.

Despite experience and claims about LMO benefits and safety, American companies and policymakers face dogged opposition as they promote the introduction of LMOs into commercial agriculture at home and abroad. Opponents challenge the scientific adequacy of American field testing for biosafety and company claims that LMOs ensure greater productivity. Many also contend that commercial use of LMOs will cause environmental and socioeconomic disruptions and undermine the self-reliance of developing nations.

For example, Jane Rissler and Margaret Mellon of the Union of Concerned Scientists, in their recent book, *The Ecological Risks of Engineered Crops*, raise questions about environmental risks. Although finding that "most genetically engineered organisms will not be harmful," they oppose unregulated commercial use on the grounds that American field testing for biosafety has been short-term and small-scale, and is even waived as a requirement for certain transgenic plants. Although the small field test sites have been carefully selected and monitored to minimize transgenic interbreeding with wild plant relatives and other undesirable consequences, these precautionary measures are not likely to be followed in developing nations at much larger commercial sites covering millions of acres. In addition, they say, the American test sites do not match likely commercial sites abroad in terms of ecological conditions, biodiversity, plant relatives with interbreeding potential, and natural events such as floods which can transport seeds to more vulnerable off-site regions.

Thus, Rissler and Mellon contend that American field testing offers little, if any, assurance of biosafety to other nations and, drawing on ecological principles, point to several risk scenarios:

- **gene flow,** in which new genes for insect, disease, or herbicide resistance flow to wild plant relatives and weeds, causing agricultural and ecological havoc unless effective controls are available and affordable;
- **harms to nontarget species** arising, for example, from new gene products with toxic qualities being ingested by birds and other feeders in the regions where such LMOs are cultivated;
- **cascading effects** on an ecosystem triggered by the introduction of LMOs, such as pests developing resistance over time to Bt in transgenic plants, or being deflected to other food sources; and
- **loss of biological diversity,** arising from LMO displacement of other species, with particular regard for those developing nations that possess great concentrations of crop diversity but lack infrastructure and expertise for preventing the loss.

Biotechnology companies and agencies draw on biosafety experience to dispute such contentions and argue that any residual risks are manageable. Conflicts over certain LMOs have also become quite intense, with both sides using various tactics to win over public perception and trigger market forces.

The newest conflict involves evidence of gene flow from an herbicide-resistant rapeseed that was engineered by Germany's AgroEvo GmbH for enhanced production of canola oil. According to a report by Danish researcher Thomas Mikkelsen and colleagues at the Riso National Laboratory in Roskilde, published in the 7 March 1996 issue of *Nature*, the herbicide-resistant genes quickly flowed to a wild plant relative through cross-breeding, and produced fertile offspring that are now herbicide-resistant as well. According to environmental and consumer groups, this study demonstrates that LMOs pose gene flow risks when plant relatives are nearby, that current agency oversight for biosafety is scientifically and legally inadequate to prevent such occurrences, and that the biotechnology mantra that "hybrids don't survive, so don't worry" is wrong.

However, Richard Godown of the Biotechnology Industry Association (BIO), which includes over 500 companies, government centers, and academic institutions in over 20 countries, describes the Danish study as "a propagation deliberately done in the laboratory which was then theoretically extrapolated to natural conditions," and believes that such gene flows in nature can be prevented by careful site selection for commercial agriculture, buffer zones, and other risk management methods.

Val Geddings, international team leader for the USDA's Animal and Plant Health Inspector Service, adds that the gene flow finding has been improperly described as an "unpleasant surprise," when in fact, it was well-known beforehand that rapeseed genes flow to certain weedy relatives without serious environmental consequences, whether the plant was traditionally bred or recombinant, as is the case with broccoli and cauliflower genes. Nor was it an adverse outcome according to Geddings, because the gene flow from the recombinant plant posed no greater environmental risk than the gene flow from its traditionally bred counterpart. As for the consequence of herbicide-resistant weeds, "one answer is to switch to another herbicide," he said.

In addition to environmental opposition, commercial use of LMOs is also resisted on grounds that it will cause major socioeconomic dislocations, particularly in developing nations where the initial impact would involve displacement of small family farms by more efficient, high-tech corporate agriculture. Some say a subsequent domino effect could include the erosion of traditional village culture, unemployment for the unskilled, and growing dependence on foreign interests and experts for food supply. Concurrently, native populations and their environments would become the subjects of further research to fine-tune the safer or more productive use of LMOs without adequate safeguards. Eventually, opponents worry that multinational firms from the United States or other developed nations could capture other local enterprises in the food production system, such as seed suppliers, shippers, distributors, brokers, processors, and retailers through acquisition, joint venture, or various forms of strategic alliance.

Although these concerns may seem to be merely another variation on the familiar tension between developed and developing nations, there is evidence in various regions of the United States that at least some of

these socioeconomic impacts can and do flow from corporate entry into local agriculture.

Environmental and consumer organizations in developed nations share these socioeconomic concerns, and many developing nations share their ecological concerns regarding biodiversity protection. An international coalition formed by non-governmental organizations such as Greenpeace, certain Scandinavian countries, and the G-77 nations (an informal coalition of developing countries that plays a major role in many U.N. decisions and policy processes), has called for the United Nations to establish means of assuring biosafety for the protection of biodiversity, as well as improve the capabilities of developing nations for managing the biotechnological transformation of agriculture so that it is consistent with their agricultural, socioeconomic, and environmental goals and sustainable development. Authority for U.N. enactment of such a legally-binding framework is provided by the 1992 Convention on Biological Diversity.

Convention on Biological Diversity

Biological diversity was raised at the U.N. Conference on the Human Environment held in Stockholm in 1972 and prioritized by the newly formed United Nations Environmental Program (UNEP) in 1973. In 1988, a joint resolution of the U.S. Congress signed by President Reagan lent support to U.S. initiatives for an international agreement, and UNEP convened the first of several expert groups to evaluate the need for (and later, to draft) an international legal instrument, or convention, for the conservation and sustainable use of biological diversity.

In June 1992, the convention was "opened for signature" at the U.N. Conference on Environment and Development in Rio de Janeiro, Brazil, despite displeasure by the United States and France over irregularities in the hasty negotiation process, and legal ambiguities and substantive deficiencies in the final text. By June 1993, 168 nations had signed on, and in late December 1993, the Convention on Biological Diversity (CBD) came into force. Almost 150 of the signatory nations have since ratified the CBD and are thereby subject to it as "parties." The United States, a late and reluctant signatory, has never ratified the CBD, but has been allowed to participate as an observer at the subsequent conferences of the parties and working group meetings. In 1996, the CBD secretariat was installed in Montreal, Canada, with Calestous Juma of Kenya as its executive secretary.

Initially conceived as a framework for preventing "loss of species and equitable sharing of genetic resources," the enacted CBD now stands for more—namely, for ensuring sustainable development, for ensuring that all parties have biosafety procedures, and for building the capabilities of developing nations in biotechnology, biodiversity protection, and associated matters such as biosafety. The CBD is generally regarded as one of the most significant developments in international law for environmental protection and national development.

The CBD is laden with principles, suggestions, and legally ambiguous but potentially obligatory mandates for the party nations. However, to the extent that it obligates party nations, it will also affect their relationships with nonparty nations such as the United States, and thereby have indirect effect on nonparties.

Many provisions call for, in a mixture of obligatory and aspirational terms, actions by parties that will build the biotechnical and biodiversity capabilities of developing nations, such as providing research, training, and financial assistance; engaging in technology transfer; using impact assessment and information sharing; and making equitable arrangements for sharing the fruits of research and commerce involving biodiversity. Several matters that were hotly disputed in drafting the CBD are left unresolved in the final text, which designates them for future resolution. These include intellectual property rights, liability and sanctions, and biosafety.

Two provisions directly address biosafety. Article 19(3) provides that: "The parties shall consider the need for and modalities of a protocol setting out appropriate procedures, including, in particular, advance informed agreement, in the field of the safe transfer, handling, and use of any living modified organism resulting from biotechnology that may have an adverse effect on the conservation and sustainable use of biological diversity."

Article 8(g) calls upon each contracting party, "as far as possible and as appropriate [to] . . . establish or maintain means to regulate, manage, or control the risks associated with the use and release of living modified organisms resulting from biotechnology, which are likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity, taking into account the risks to human health."

These provisions are amplified by Article 19(4), which requires that each party share any available information about its use of and safety regulations for handling LMOs (including potential risks) with any other party.

But other provisions, such as development of a future protocol on biosafety, are substantively related to Article 19(3), and can influence its implementation. For example, Article 16 calls for a cooperative approach to patent rights and technology transfer among the parties. It also calls on the parties to have their private sectors (such as biotechnology companies) similarly cooperate on intellectual property matters and engage in technology transfer. Article 16 further defines the technologies to be transferred as those "that are relevant to the conservation and sustainable use of biological diversity or make use of genetic resources" without causing "significant harm to the environment." Significantly, it also expansively provides that technology includes biotechnology.

Thus, if parties comply with Article 16, developing nations would be able to develop their own capabilities for both biotechnology and biosafety, gaining far more than what would be gained from a prescriptive protocol developed under Article 19. Nevertheless, development of an Article 19 protocol on biosafety now preoccupies the CBD parties, probably because of immediate concerns about environmental risks, the desire to slow down the onslaught of LMOs made in the United States, and the need for time to develop economic and political strategies.

Biosafety Protocol

Parties to the CBD meet annually at a Conference of the Parties (COP), with the United States and numerous nongovernmental organizations permitted to attend as observers. Discussion of a biosafety protocol dominated the first two COPs in 1994 and 1995, and led to the creation of an open-ended ad hoc working group in November 1995 with the mandate to develop a protocol on biosafety. The working group will meet in Arhus, Denmark, in July to take first steps such as defining terms, selecting issues to be addressed, setting the boundaries of the protocol, and reviewing existing biosafety policies.

According to Juma, dates for completion of the protocol have not been fixed, although it is hoped that a draft will be available so that negotiation of a final text can begin in 1998. A consensus decision on the protocol will then be sought at the next COP, with disputed provisions to be negotiated to resolution, following traditional U.N. practice. Thus, a final biosafety protocol could be enacted by 1999.

The working group's mandate is provided by *Decision Document II/5*, approved at the COP-2 meeting held in Jakarta, Indonesia, in November 1995. The mandate sets forth reasons for a protocol including: gaps in knowledge regarding the "inter-

action between LMOs resulting from modern biotechnology and the environment, taking into account the relatively short period of experience with the releases of such organisms, the relatively small number of species and traits used, and the lack of experience in the range of environment . . .;" that existing international agreements do not specifically address the issue of transboundary movements of LMOs; that international action "should offer an effective and efficient framework for . . . insuring biosafety through effective risk assessment and risk management . . .;" and a large majority of the parties favor the development of a protocol on biosafety.

The mandate directs the working group to evaluate existing LMO biosafety policies in developing the protocol, such as guidelines enacted by the United Nations' Food and Agriculture Organization, the Organization for Economic Cooperation and Development (OECD), and most importantly, the International Technical Guidelines on Safety in Biotechnology now being finalized by UNEP.

The UNEP guidelines, based on work done by a U.K.–Dutch team, represent a more flexible, nonbinding approach, which is preferred by the United States and several parties to the CBD. Instead of being fitted with a rigid protocol, each nation would have the opportunity to consider guidelines and ultimately craft its own approach to biosafety.

The mandate provides that finalization of the UNEP guidelines "does not prejudice the development and conclusion of such . . . protocol," and suggests that the guidelines may serve as an interim mechanism during protocol development, as well as a complementary system after completion and adoption of the protocol in order "to facilitate development of national capacities to assess and manage risks, establish adequate information systems, and develop expert human resources." Thus, the working group is directed to consider a parallel biosafety development within the United Nations, one that can accomplish "capacity building" as envisioned by Article 16 of the CBD.

The mandate concludes with the COP-2 decision that the working group is "to seek solution to the abovementioned concerns through a negotiation process to develop. . . a protocol on biosafety, specifically focusing on transboundary movement of any living modified organism resulting from modern biotechnology that may have an adverse effect on the conservation and sustainable use of biological diversity, setting out for consideration, in particular, appropriate procedure for advance informed agreement."

The annex to the decision document contains terms of reference to give more detailed guidance to the working group. According to the annex, priority is to be given to the form and scope of advance informed agreement, the relevant categories of LMOs, the "precautionary principle" contained in UNEP's Rio meeting declaration, completion of the group's work by 1998, and ratification of the protocol by the largest number of parties. The working group is further directed to ensure that the protocol will minimize unnecessary negative impacts on biotechnology research, development, and transfer.

At the July meeting in Arhus, the working group will begin to grapple with protocol development under this complex mandate. A task force of agencies has been formed at the State Department to represent U.S. interests, although the U.S. role will be limited by its observer status. Germany, Japan, the United Kingdom, and the Netherlands, nascent biotechnological powers who are parties to the CBD, also favor the more flexible, nonbinding guidelines approach advocated by the United States.

According to Simon Best, CEO of Zeneca, a biotechnology firm, and chairman of BIO's Committee on Agricultural Biotechnology, "The widely held view of developed nations and many developing countries is to follow a guidelines approach to assure that the most appropriate system can be used in each developing nation, including advance informed agreement and capacity-building procedures, rather than the more inflexible binding protocol approach. Guidelines would allow for the customized adoption of best practices for each nation, and compatibility with its existing laws and infrastructure. Most countries believe this approach would assure a more fully protective approach to be taken in each nation. The protocol effort also has [the] unfortunate potential for diverting COP attention and resources from major needs like sustainable development and preventing deforestation."

Lisa Zannoni of the OECD points out that "many nations use guidelines as the United States uses regulations," and that "the UNEP, FAO, OECD, and other guidelines do not leave many gaps. UNEP is now developing guideline implementation procedures [that] will fill remaining gaps, such as how to define and implement advance informed agreement for commercial transboundary shipments." The OECD has started developing a series of expert consensus documents, state-of-the-art reviews on the environmental biosafety of transgenic plants. Like UNEP and the CBD secretariat, the OECD is providing biosafety information through the Internet for informing the general public in all nations.

Despite these views and guideline enhancement efforts, strong pressure for a legally binding protocol with stringent features, a virtual biosafety regulation template for each nation, is anticipated from the G-77 group of nations, along with China, Sweden, and Denmark, with support from environmental organization observers.

As a result, the working group must grapple with many technical, legal, and policy issues in a volatile political context, such as whether the protocol should be legally binding and require national adoption, or be advisory; if binding, whether it should provide for enforcement, sanctions, and liability; and whether binding or advisory, to what extent it should adopt, or defer to, the biosafety features of UNEP and other guidelines, and the biosafety experience gained in other developed nations.

On the issue of consent, the group must also consider whether the advance informed agreement process should be a simple notification procedure like the prior informed consent procedure used for pesticide exports under various laws and treaties, or more expansive and require, for example, that LMOs intended for shipment meet special testing requirements, be evaluated by a particular method of risk assessment, or be used subject to a particular method of risk management; whether advance informed agreement should be carried out by public officials in each nation or by the private parties arranging for transboundary shipment and, if the latter, how the proprietary information should be safeguarded; and whether the public should be provided with information about the shipment.

In addition, the issue remains whether the protocol should contain features that minimize conflicts with the General Agreement on Tariffs and Trade (GATT) and World Trade Organization, or leave such conflicts to subsequent case-by-case resolution.

The stage is thus set for reaching accord on a biosafety protocol for LMOs. Depending on its features, such a protocol could play a major role in shaping the future use of biotechnology in agriculture, sustainable development in developing nations, and the growth of the biotechnology industry around the world.

Michael Baram

Dissolving Medical Waste

As environmental regulations stiffen and the costs of waste disposal continue to rise, industries are searching for alternatives to the traditional methods of waste disposal. American hospitals, which produce an average of 2 million tons of waste per year, according to the American Hospital Association (AHA), are among those reevaluating their waste disposal methods.

Concerns about the disposal process of infectious waste have increased with the spread of viruses such as HIV and hepatitis B. "When the public became aware of AIDS, it happened to be at the same time that medical waste began washing up on beaches," said Gary Urbanowicz, vice president of Doucet & Mainka, an environmental consulting and engineering firm in Peekskill, New York. This, along with impending regulations to reduce air pollution released by medical waste incineration, has prompted hospitals to explore alternative waste disposal methods.

According to the AHA, about 15% of hospital waste is classified as infectious and its disposal is regulated. Traditional methods of medical waste disposal include incineration and autoclaving, which involves sterilizing the waste at high temperatures before it is taken to a landfill. Infectious waste is regulated by the states because there are no federal regulations that specifically govern disposal of medical waste other than hazardous waste. Officials do not anticipate any federal involvement in medical waste regulation in the near future, other than medical waste incineration reform. "Medical waste regulations are already addressed on a state level adequately," Urbanowicz said.

Mounting Waste Regulations

However, waste regulations for all industries are continuing to rise at state and federal levels as waste disposal options diminish and environmental standards are strengthened. As the AHA pointed out in a book entitled *An Ounce of Prevention: Waste Reduction Strategies for Health Care Facilities*, "In short, end disposal options (landfills, incinerators, etc.) are becoming increasingly narrow. The pipeline of 'products in, wastes out' has now become a funnel with an ever narrowing outlet for waste."

As part of the 1990 Clean Air Act, the EPA has been working to draft emissions standards for all types of incinerators. Medical waste incinerator emissions standards will be released in July 1997, and will set limits for emissions of particulate matter, carbon monoxide, dioxins and furans, hydrogen chloride, sulfur dioxide, nitrogen oxides, lead, cadmium, and mercury, said Rick Copland, an environmental engineer in the EPA's Office of Air and Radiation in Research Triangle Park, North Carolina. Officials in the medical waste industry speculate that the new regulations will result in the shutdown of anywhere from 80% to 95% of the approximately 2,400 onsite hospital medical waste incinerators because the cost of adjusting the incinerators to meet the standards will be too high. Copland said that if the final standards are released as they have been proposed, the cost of medical waste incinerators could double or triple. "Once [the regulation] finally happens, you will see a large segment of onsite hospital medical waste incinerators shut down," said Rich Moskowitz, director of the Medical Waste Institute, an industry trade group that represents companies that manufacture waste treatment technologies and transport medical waste. "As hospitals choose new treatment methods, some will choose alternative technologies," he said.

Many states are setting goals and regulations mandating reductions in solid waste generation by the turn of the century. California, which has some of the strictest environmental regulations in the country, mandated a 25% reduction in landfill waste by the end of 1995 and a 50% reduction by the year 2000. Monetary penalties are being enforced for noncompliance. As a result, California hospitals have been among the first in the country to employ alternative methods of waste disposal.

Reducing the amount of products used by hospitals is not a likely option, as most hospitals prefer to use disposable products. About 90% of hospitals now use one-use disposable gowns and sterile drapes because of their potential to be infectious after use. "The disposables trend started in the 1950s, blossomed in the 1960s, and it's been growing ever since," Urbanowicz said. "There's been some interest in going back to reusable products, but for now [hospitals] continue to rely heavily on disposables." Therefore, focusing on alterna-

tives to the disposal of waste is the key to reducing costs and environmental impact.

Disposable Alternatives

In the wake of this search by hospitals for alternative waste disposal methods, the Isolyser Corporation has introduced new disposable healthcare products that it claims are more cost-effective, safer, and more environmentally friendly than traditional products.

Isolyser, based in Norcross, Georgia, has developed what it calls a "Bio-Cycle" approach to manufacturing healthcare products, which involves creating products from natural compounds that can be degraded back into natural compounds after use. The goal of the company is to provide a way for hospitals to minimize waste, which benefits the hospitals by reducing disposal costs and benefits the environment. To do this, the company has developed a material called OREX, which is used to make products that perform like traditional disposables, but can be dissolved in water after use, rather than being deposited in a landfill or incinerated.

OREX is made from hot-water-soluble polyvinyl alcohol (PVA), a nontoxic synthetic polymer. PVA is currently used as a component of many commercial products including adhesives, binding agents, paper, ceramics, emulsifiers, fabric, and pharmaceuticals. OREX is used to make such products as surgical gowns, towels, patient drapes, sponges, bowls, basins, and diapers.

After the OREX products are used, they are placed into an onsite processing unit, similar to a commercial washing machine, where they are disinfected and dissolved in water heated to about 200°F for approximately 45 minutes. A large-sized machine can hold up to 100 pounds of materials and requires between 50 and 100 gallons of water to dissolve a large load. An Isolyser study, entitled *Disinfection Using the OREX Degradables Processor*, found that a temperature of 190°F disinfects OREX products contaminated with vegetative forms of bacteria, fungi, mycobacteria, and viruses. The leftover liquid residue is safe enough to be sent directly into the sewage system, where the polymer is further degraded by microorganisms.

PVA has been found to degrade rapidly, usually within the first 24 hours in a sewage treatment facility using acclimated sludge organisms, according to an Isolyser report, *Biodegradation of Polyvinyl Alcohol in Sewage Treatment Facilities*. Twenty different genera of bacteria and several molds and yeasts have been found to degrade PVA. The report concluded that PVA is totally biodegradable in sewage treatment facilities and the final products of degraded PVA are carbon dioxide and water. The leftover sludge containing OREX by-products is chemical-free and safe for agricultural use.

According to Isolyser, PVA is environmentally safe and has been found to be nontoxic and nonhazardous to humans and aquatic organisms in studies conducted by the company on rabbits, rats, mice, bluegill, fathead minnows, water fleas, and bacteria.

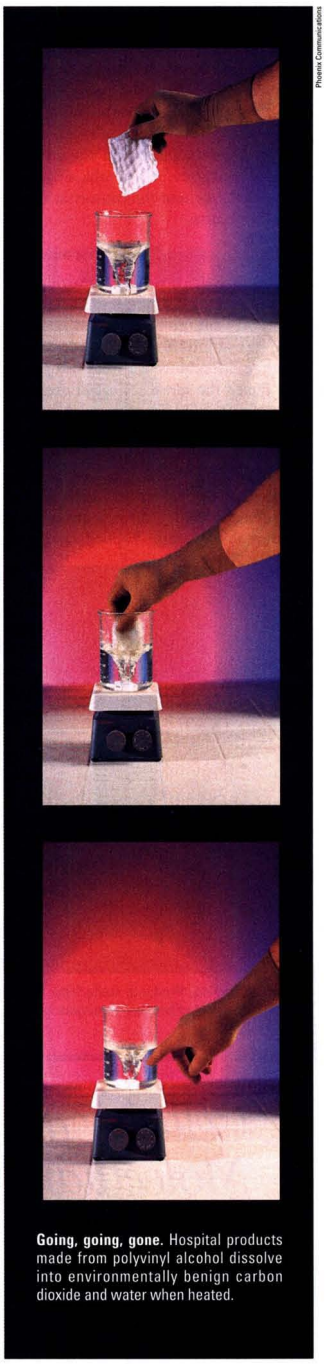
Customer Satisfaction

So far, reaction to the products has been positive. Public health officials in California tested the products because of concerns with water recycling, but after thorough testing, OREX was approved for use in California. "We feel that the OREX method is safe and offers another alternative to the treatment of medical waste," said Vernon Reichard, supervisor of the medical waste management program for the Environmental Management Branch of the California Department of Health Services. "This is a unique method; it's the only one that we've reviewed and approved that actually dissolves into a liquid and can be sewered."

Several hospitals in California are currently using OREX. "So far, the wastewater treatment plants are very satisfied that it's not creating a problem," said Jack McGurk, chief of the Environmental Management Branch of the California Department of Health Services. McGurk said California public health officials are impressed with OREX, particularly the benefits of quality control and quality assurance. "If the water is not hot enough, [the material] doesn't dissolve. OREX provides good visual quality assurance that it's working correctly," he said.

Queen of the Valley Hospital (QVH) in Napa Valley, California, has been using OREX products since March of this year. The hospital is currently using all the products that are available, including gowns, nurses' caps, physicians' hoods, drapes, and towels. The hospital will use other products, such as scrub clothes and basins, as soon as they are available. "I've seen the basins and I'm very impressed with them," said Mary Fiddler, manager of surgical services, the post-anesthesia care unit, anesthesia, and central processing for QVH. "We plan on using everything they have out." So far, the products have proven to be durable and the staff at QVH has been very satisfied with them, Fiddler said.

Anaheim Memorial Hospital, in Anaheim, California, is conducting a trial



Phoenix Communications

Going, going, gone. Hospital products made from polyvinyl alcohol dissolve into environmentally benign carbon dioxide and water when heated.

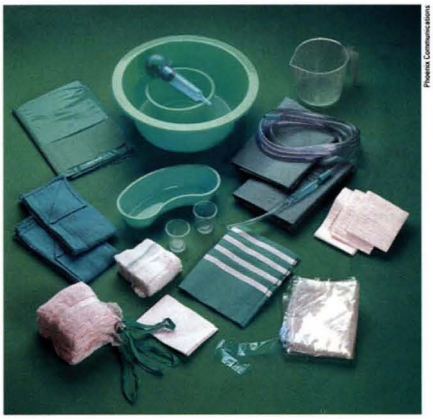
period with the products, says Tracy Balen, a public relations specialist for the hospital. The operating room staff is using the products and giving feedback to Isolyser, which has been cooperative in modifying the products and sending them back, Balen said. "We won't implement the products until they are equal to our current disposables." Currently, about 400 hospitals throughout the country are using OREX products.

Saving Money and the Environment

The use of OREX products provides the double incentive for hospitals of reducing both costs and environmental impacts. "The cost of OREX is equal to or cheaper than the disposables we were using, and then there's the added savings of decreased disposal costs," Fiddler said.

According to the Medical Waste Institute, hospitals spend an average of $.19 to $.30 per pound to dispose of infectious waste, depending on factors such as the volume of waste, the amount of competition among waste disposal services in the area, and energy costs. The disposal process for OREX products costs about $.03 per pound, including water and electricity costs, according to Isolyser. Isolyser estimates that hospitals can save 5–15% of total spending on disposables by using OREX.

Because the products are safe for sewage disposal, they pose no water quality threat, and subsequently divert waste away from landfills and incinerators, thus reducing pollution. "OREX products present a cutting edge opportunity to help decrease the volume of medical waste," Urbanowicz said. According to Travis Honeycutt, executive vice president of Isolyser and inventor of OREX, approximately 10–15% of all hospital waste could be eliminated by the use of OREX, including virtually all infectious waste. "The biggest advantage is that we don't have mountains of solid waste [with OREX]," Honeycutt said.



Phoenix Communications

Truly disposables. Products made from OREX may significantly reduce the amount of hospital hazardous waste.

Honeycutt says he was driven to develop the products by environmental concerns. "My partner and I (Robert Taylor) recognized that what we call disposables are really discardables; they lay around and won't go away," he said. "Everything is biodegradable; it just matters where you put it."

So far, the products have received little criticism. However, they are still new to the market and lack thorough reviews. "We want to review the process after it's been in use for a while to see if it holds up to the expectations surrounding it. We feel it probably will, but you never know," Reichard said.

Isolyser is optimistic about the future of OREX. The new EPA emissions standards to be released next year could positively affect OREX sales as hospitals search for alternatives to incineration, Honeycutt said. "When fully utilized by hospitals, 50 to 70% of all regulated medical waste from disposable products coming out of the hospitals could ultimately be made out of OREX," said Ted DuBose, president of SafeWaste Corporation, a subsidiary of Isolyser.

In the immediate future, Isolyser plans to continue developing other hospital supplies. According to Honeycutt, OREX also has the potential for use beyond infectious and hazardous waste, and Isolyser plans to eventually market to industrial, consumer, and international corporations. "We are very excited about the potential for OREX. It's a product and an idea whose time has come," Honeycutt said.

Brandy E. Fisher

Suggested Reading

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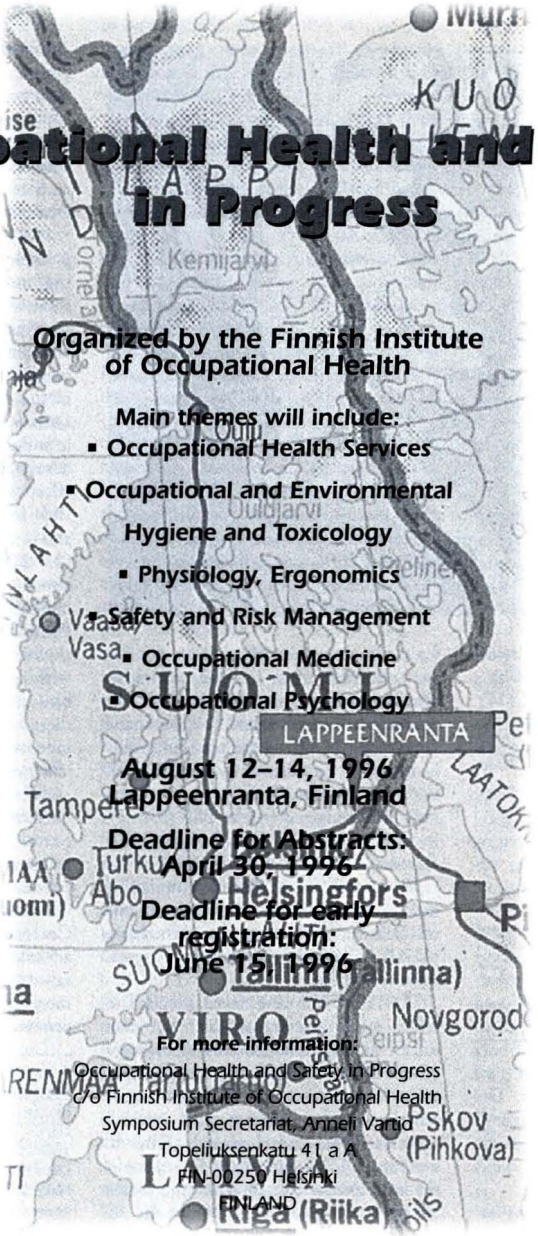
Occupational Health and Safety in Progress



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Research

Enzyme Induction and Acute Endocrine Effects in Prepubertal Female Rats Receiving Environmental PCB/PCDF/PCDD Mixtures

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Air, subsurface soil, and superficial dust from a National Priorities List landfill located in southern Illinois were sampled to determine their potential toxicities. The major components of these landfill extracts were polychlorinated biphenyls (PCBs), with significant amounts of polychlorinated dibenzofurans (PCDFs) and small amounts of polychlorinated dibenzodioxins (PCDDs). The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalency factor approach has been proposed to estimate the toxic potency of complex mixtures of chlorinated aromatics for environmental risk assessment. However, most components of environmental residues are nonplanar and do not act as aryl hydrocarbon (Ah) receptor agonists, so there is a great risk of not identifying adverse responses that are not dioxinlike. We used a 2-day prepubertal female rat bioassay to examine multiple biological responses, including both dioxinlike and nondioxinlike effects from these landfill extracts. As expected, both types of effects were detected. The soil and dust extracts produced similar dose–response relationships for 7-ethoxyresorufin *O*-deethylase, 7-pentoxyresorufin *O*-dependentylase, 7-benzyloxyresorufin *O*-debenzylase, and 4-nitrophenol UDP-glucuronyltransferase induction; the dose response for the air extract deviated from the other two extracts. Soil, dust, and air extracts effectively reduced serum total thyroxine (T_4) with similar dose–response relationships, despite the significantly different TCDD toxic equivalent (TEQ) values of these three extracts. Both soil (346 mg PCB/kg) and air (175 mg PCB/kg) extracts caused a greater than 30% increase in uterine wet weight. This study suggests that a more comprehensive approach is required to improve current risk assessment of environmental mixtures. TCDD TEQs reflect only a portion of effects and may especially underpredict effects on T_4. *Key words*: cytochrome P450 induction, environmental mixtures, polychlorinated biphenyls, polychlorinated dibenzodioxins, polychlorinated dibenzofurans, thyroxine, toxic equivalency factor, UDP-glucuronyltransferase, uterotropic responses. *Environ Health Perspect* 104:712–722 (1996)

Polychlorinated biphenyls (PCBs) are widespread environmental contaminants with a broad range of biological activities (*1–4*). Worldwide commercial production declined dramatically in the 1970s and essentially ceased by 1990. However, the level of PCB residues has decreased slowly in various environmental and biological samples since the 1980s (*5–8*). Currently, used electrical equipment and leaking disposal sites continue to be anthropogenic sources for PCBs in the environment. In addition, dispersion of PCBs from point sources to global distribution can occur through atmospheric transport and subsequent deposition (*9–12*). Wildlife and humans still continue to be exposed to PCBs through the environment, and only limited data are available on the biological effects of environmental mixtures. The accurate assessment of the potential hazards from environmental PCB exposures remains a challenge for regulatory agencies and environmental toxicologists.

The most accepted approach for assessing the toxicity of environmental PCDD/PCDF/PCB mixtures uses toxic equivalency factors (TEFs). This approach is based on the fact that TCDD and related TCDD-like compounds elicit their toxicity via a common mechanism of action, i.e., the aryl hydrocarbon (Ah) receptor-mediated mechanism. Using this approach, the potencies of mixtures and environmental samples for TCDD-like effects can readily be estimated by calculating their TEF values from the sums of the product of each individual congener and its TEF (*3,4,13*). In developing TEFs, one of the most responsive parameters is induction of the *CYP1A1* gene, most often determined by measuring ethoxyresorufin-*O*-deethylase (EROD) or aryl hydrocarbon hydroxylase (AHH) activity in cultured cells or hepatic microsomes (*3*).

TCDD TEFs were never intended to reflect other toxic actions (*13*), but their use frequently excluded consideration of other actions (*2*). However, most components of environmental PCB residues are nonplanar and do not act as Ah receptor agonists. These chlorobiphenyls (CBs) do not cause TCDD-like effects. For example, the less chlorinated and *ortho*-chlorinated congeners have low affinities for the Ah receptor and are poor inducers of CYP1A1; but these congeners can induce CYP2B and have a profile of hormone and neurotransmitter disruption distinct from the coplanar Ah receptor agonists (*14–17*). In spite of their lower potencies, these congeners are present in far greater amounts than are coplanar CBs (*1,2,18*). Therefore, it is important to evaluate both Ah receptor-dependent and Ah receptor-independent effects of environmental PCB mixtures to better predict the potential hazards of environmental PCB mixtures.

Chemical analysis of all possible compounds in a mixture is costly, time consuming, and usually incomplete. Therefore, short-term bioassays have been suggested to serve as alternative screening methods in assessing the toxicity of complex environmental mixtures (*19,20*). Currently, most of the short-term bioassays proposed for assessing the toxicity of chlorinated aromatic mixtures are *in vitro* assays and only detect Ah receptor-dependent biological effects, such as the induction of cytochrome P4501A in rat H-4-II-E hepatoma cell lines and in chicken embryo primary hepatocytes (*19–21*). Indeed, these *in vitro* bioassays also show a good correlation with dioxin-inducible effects, i.e., *in vivo* cytochrome P4501A1 induction, body weight loss, and thymic atrophy in mammals (*22,23*) and with embryolethality and deformities in bird embryos and chicks (*24*). However, these bioassays cannot detect Ah receptor-independent effects and ignore possible toxicokinetic interactions *in vivo*.

We developed a short-term *in vivo* bioassay in this laboratory by using prepubertal female rats to examine both Ah receptor-dependent and Ah receptor-independent effects of some *ortho*-chlorinated CBs and Aroclor mixtures (*15,17,25,26*). Compared to short-term *in vitro* bioassays, advantages of this short-term *in vivo* bioassay include: 1) examination of both Ah receptor-dependent and Ah receptor-independent effects simultaneously; 2) account-

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ing for possible early toxicokinetic interactions in the whole animal; 3) evaluation of the acute endocrine-disrupting effects (such as depression of thyroid hormone levels), which usually cannot be measured in an *in vitro* bioassay. The intention is to develop a bioassay, such as this female rat integrated endocrine disruption assay (FRIEDA), which can be used as a rapid screening method for potential biological effects of mixtures before deciding whether more intensive and long-term studies are needed. Therefore, similar but distinct environmental mixtures were sought to further test the FRIEDA.

Air, subsurface soil, and superficial dust and debris from the small but highly contaminated Sangamo landfill in southern Illinois (*27*) were sampled to determine potential toxicities of environmental mixtures. The chemical composition of these extracts has been determined, and the chemical profiles of these extracts have been described elsewhere (*28,29*). In a preliminary study (*29*), the extract of this landfill soil caused both Ah receptor-dependent and Ah receptor-independent responses in prepubertal female rats. Therefore, the present study used the short-term bioassay mentioned above, with slight modifications, to examine enzyme induction, thyroid hormone depletion, and uterotropic effects of the Sangamo landfill soil, dust, and air extracts in an attempt to define the net potency of these environmental extracts with different PCB/PCDF/PCDD profiles.

Materials and Methods

Sample collection. The Sangamo landfill has been inactive since 1964, and access was limited when it was placed on the National Priorities List in 1984 (*27*). For the air samples, 18 separate 24-hr high-volume (34 m^3/hr) samples were collected 15 cm above the surface of the landfill between 16 September and 20 October 1992. The vaporized compounds were collected on 45 g XAD-2 resin downstream from a glass fiber particle filter (*30*). Then dust and surface debris were collected from the site by whisk broom; the next 5–10 mm of soil were removed by scraping with trowels, and finally subsurface soil beneath the dust and debris was collected and sieved (no. 10) into a precleaned stainless-steel bucket. In addition, a reference soil sample was collected from a control site located several kilometers south–southeast of the Sangamo landfill.

Extraction and chemical analysis. Detailed procedures of the extraction, cleanup, and chemical analysis have been described elsewhere (*28*). In brief, for air samples, XAD-2 resins were Soxhlet extracted with 300 ml of acetone:hexane (1:1, v:v) for 24 hr and then with 300 ml of dichloromethane for another 24 hr. The individual extracts of each sample were concentrated by rotary evaporation and then combined and solvent was exchanged to hexane (*30*). Each 100-g soil or dust sample was extracted with 200 ml of acetone:hexane (A:H, 1:1). The pooled extracts were dried over sodium sulfate and vacuum-concentrated at 57°C and were exchanged to hexane. Soil and dust extracts were cleaned of oil by Florisil slurry, and all extracts were subsequently cleaned by alumina (3% deactivated) column chromatography. The refined extracts were subdivided and separate aliquots were transferred to other laboratories for chemical analysis.

Specific PCB congener analyses were conducted by two independent laboratories. At the New York State Department of Health Wadsworth Laboratories (NYSDH), the aliquots were analyzed by gas–liquid chromatography (GLC) with an electron capture detector (ECD) (*31*). At the Illinois Hazardous Waste Research and Information Center, Hazardous Materials Laboratory, the samples were diluted and analyzed directly by GLC, but the effluent was split between the ECD and an ion trap MS. The average PCB concentrations from three methods were 43, 21, and 4 mg/ml for soil, dust, and air extracts, respectively, and the variation was only 20%. However, the compositions in Tables 1 and 2 were based on the GLC-MS results that could identify individual PCB congeners not resolved by GLC-ECD. In addition, PCDFs and PCDDs in these three extracts were analyzed by capillary GLC/low-resolution MS at the NYSDH (*28*). The concentrations of PCDDs were 47.3 µg/ml in the soil extract and 11.4 µg/ml in the dust extract, whereas PCDDs were not detected in the air extract. The concentrations of PCDFs were 761.5 µg/ml in the soil extract, 250.3 µg/ml in the dust extract, and 74.1 µg/ml in the air extract. The TCDD toxic equivalence (TEQ) for TCDD-like actions of the extracts were calculated using TCDD TEFs suggested by Ahlborg et al. (*13*) for PCBs and by Safe (*4*) for PCDDs and PCDFs. In the air extract, quantitation of CB 126 (3,3′,4,4′,5-pentachlorobiphenyl) may be unreliable due to its low concentration; therefore, a conservative TEQ estimation was adopted by using the possibly maximal CB 126 concentration (1 µg/ml) in the air extract for the TEQ estimation.

Even though the PCDF content was high relative to most environmental mixtures (*28*), total PCB still accounts for more than 98.3–99.5% of the chlorinated aromatics in

Table 1. Contents of PCDDs, PCDFs, and PCBs in landfill extracts and summations of toxic equivalencies to 2,3,7,8-TCDD

	Concentration (µg/ml)[a]		
Compounds	Soil	Dust	Air
2,3,7,8-TCDD	0	0	0
Tetra-CDDs	2.9	0.8	0
Penta-CDDs	6.3	0.7	0
Hexa-CDDs	10.9	3.3	0
Hepta-CDDs	14.9	3.3	0
Octa-CDD	12.2	3.4	0
Total PCDDs	47.2	11.5	0
Sum PCDD TEQ (µg TCDD/ml extract)	0.52	0.06	0
2,3,7,8-TCDF	46.1	19.3	3.9
Tetra-CDFs	329.9	109.5	18.3
Penta-CDFs	194.8	68.9	0.3
Hexa-CDFs	104.4	35.5	0
Hepta-CDFs	58.8	12.2	0
Octa-CDF	27.6	5.0	0.6
Total PCDFs	761.6	250.4	23.1
Sum PCDF TEQ (µg TCDD/ml extract)	27	8.96	0.39
Mono-CBs	0	0	<1
Di-CBs	407	41	56
Tri-CBs	17820	4010	2095
Tetra-CBs	19361	8069	1799
Penta-CBs	5682	5323	550
Hexa-CBs	2551	2650	102
Hepta-CBs	940	509	3
Octa-CBs	110	52	0
Nona-CBs	8	5	0
Deca-CB	1	1	0
Total PCBs	46888	20660	4606
Sum PCB TEQ (µg TCDD/ml extract)[a]	1.50	1.55	0.11
TEQ/ml of extract (µg TCDD/ml extract)	29.02	10.57	0.50
TEQ/unit of matrix (µg TCDD/g)	14.51	5.29	0.0001
TEQ concentration (µg TCDD/mg PCBs)	0.62	0.51	0.11

Abbreviations: CDD, chlorinated dibenzodioxins; CDF, chlorinated dibenzofurans; TEQ, toxic equivalents.
[a]See Table 2.

Table 2. PCB congeners in the landfill extracts used for calculating TCDD toxic equivalents

IUPAC no.	Chlorine substitution	TEF[a]	Concentration (µg/ml) Soil	Dust	Air
77	3,3′,4,4′	0.0005	212	110	10.5
105	2,3,3′,4,4′	0.0001	369	370	8.4
114	2,3,4,4′,5	0.0005	18	15	0.6
118	2,3,4,4′,5	0.0001	651	726	27
123	2′,3,4,4′,5	0.0001	26	1	0
126	3,3′,4,4′,5	0.1	12	13	1
156	2,3,3′,4,4′,5	0.0005	95	102	0
157	2,3,3′,4,4′,5′	0.0005	25	22	0
169	3,3′,4,4′,5,5′	0.01	<0.001	0	0
170	2,2′,3,3′,4,4′,5	0.0001	140	90	0
180	2,2′,3,4,4′,5,5′	0.00001	312	146	0
189	2,3,3′,4,4′,5,5′	0.0001	4	3	0

[a]Toxic equivalency factors (TEF). TEF values suggested by WHO (*13*).

Table 3. Dose administered and body weight gains in prepubertal female rats treated with landfill-associated extracts containing PCBs, PCDFs, and PCDDs

Group	Dose[a] (g/kg)	*n*	Actual dose[b] (mg PCB/kg)	TEQ dose (µg TCDD/kg)	% Body weight gain or loss[c]
Control site	1.5	4	0.004 ± 0.001		7.85 ± 1.84
Soil	0	10	0	0	3.55 ± 0.78
	0.1	5	2.44 ± 0.19	1.51 ± 0.12	4.29 ± 1.68
	0.4	5	9.57 ± 0.69	5.93 ± 0.43	5.91 ± 1.32
	1.5	5	32.44 ± 2.59	20.11 ± 1.61	3.88 ± 0.90
	2.6	5	56.54 ± 3.03	35.05 ± 1.88	1.96 ± 1.05
	4.0	5	86.59 ± 6.16	53.69 ± 3.82	2.90 ± 0.56
	16.1	5	345.60 ± 7.71	214.27 ± 4.78	-3.24 ± 1.26*
Dust	0	6	0	0	4.96 ± 1.72
	1.2	5	12.84 ± 0.62	6.55 ± 0.32	6.27 ± 1.95
	3.7	5	38.44 ± 2.27	19.60 ± 1.16	4.54 ± 0.52
	7.5	5	78.33 ± 3.58	39.95 ± 1.83	3.77 ± 1.88
	36.4	5	382.20 ± 13.14	194.92 ± 6.70	1.40 ± 1.63
Air	0	10	0	0	3.67 ± 1.34
	0.005	5	6.19 ± 0.30	0.68 ± 0.03	5.75 ± 2.43
	0.010	6	12.37 ± 0.14	1.36 ± 0.02	7.97 ± 0.95
	0.016	5	19.39 ± 1.50	2.13 ± 0.16	2.67 ± 1.72
	0.032	5	37.95 ± 1.73	4.18 ± 0.19	4.31 ± 1.12
	0.071	3	83.85 ± 0.56	9.22 ± 0.06	6.48 ± 2.40
	0.148	5	175.43 ± 8.92	19.30 ± 0.98	4.18 ± 1.23

TEQ, toxic equivalents.
[a]Matrix equivalent dose expressed as g of matrix/kg of body weight. One ml of soil or dust extract is equal to 2 g of soil or dust and 1 ml of air extract is equal to 3.67 m^3 of air (4.74 kg of air).
[b]The actual dose is [the amount extract administered)/(individual body weight)], mean ± SE.
[c]Body weight gain = [(weight day 23 - weight day 21)/weight day 21] × 100%, mean ± SE.
*Significantly different from controls by Dunnett's *t*-test, $p \leq 0.01$.

Table 4. Uterotropic response in prepubertal female rats administered landfill-associated extracts containing PCBs, PCDFs, and PCDDs

			Uterotropic effect[a]		
Group	Dose (mg PCB/kg)	*n*	Uterine weight (mg)[b]	Uterine weight/ body weight (mg/g)	% of control
Control site		4	29.5 ± 1.7	0.47 ± 0.03	101.9 ± 5.9
17β-Estradiol (20 µg/kg)		4	70.5 ± 6.6**	1.13 ± 0.05**	245.7 ± 10.2
Soil	0	10	29.4 ± 1.1	0.49 ± 0.02	100.0 ± 3.6
	2	5	33.5 ± 1.4	0.56 ± 0.02	114.2 ± 3.5
	10	5	33.5 ± 2.0	0.54 ± 0.02	110.9 ± 3.8
	32	5	32.2 ± 1.5	0.60 ± 0.05*	123.1 ± 10.6
	57	5	33.2 ± 2.5	0.54 ± 0.03	110.7 ± 7.0
	87	5	29.8 ± 1.4	0.55 ± 0.02	113.4 ± 4.4
	346	5	39.3 ± 2.2**	0.64 ± 0.02**	131.2 ± 4.9
Dust	0	6	27.9 ± 0.8	0.43 ± 0.01	100.0 ± 2.2
	13	5	36.5 ± 4.4*	0.58 ± 0.07	136.1 ± 17.5
	38	5	32.6 ± 1.7	0.52 ± 0.04	122.5 ± 9.1
	78	5	35.9 ± 4.1*	0.59 ± 0.09	138.9 ± 2.0
	382	5	33.6 ± 1.3*	0.54 ± 0.03	127.6 ± 7.3
Air	0	10	29.9 ± 1.0	0.46 ± 0.01	100.0 ± 2.6
	6	5	36.0 ± 3.2	0.56 ± 0.03*	122.0 ± 6.0
	12	6	35.7 ± 1.8	0.56 ± 0.03*	120.9 ± 5.7
	19	5	34.6 ± 2.8	0.58 ± 0.07	125.9 ± 14.1
	38	5	33.2 ± 3.4	0.53 ± 0.03	115.2 ± 6.7
	84	3	37.7 ± 1.9	0.59 ± 0.02**	128.2 ± 5.2
	175	5	38.2 ± 2.2*	0.63 ± 0.05**	137.7 ± 11.7

[a]Mean ± SE; uterine weights are wet weights.
[b]Absolute wet weight.
*Significantly different from controls by Dunnett's *t*-test or Student's *t*-test, $p \leq 0.05$.
**Significantly different from controls by Dunnett's *t*-test or Student's *t*-test, $p \leq 0.01$.

the extract (Table 1). Because PCB content is the dominant and most often reported value for similar samples, it was considered useful to present total TEQs relative to the total PCB content. This also provides a useful comparison value for relative Ah receptor-dependent and independent effects compared to relative TEQs for the three extracts.

Animals and dosing. Appropriate dilutions of the extracts were semiquantitatively analyzed by GLC-ECD to permit initiation of toxicity studies before the completion of chemical analysis. Table 3 shows the actual doses of each extract used in this study. Although the intent was to formulate the doses of the three landfill extracts at similar PCB concentrations, the actual doses for each extract deviated from target concentrations because of the variation between initial estimation and the final chemical analysis. In addition, the amount of air extract available was limited.

Sprague-Dawley breeder rats were obtained from Harlan (Indianapolis, Indiana). Pups were culled to 8–10 animals per litter on the day of birth (day 0) and were weaned at 21 days of age. Female pups were injected intraperitoneally with landfill extracts dissolved in 0.1 ml corn oil or corn oil alone between 1300 and 1400 hr on day 21 and day 22. A negative control was included for each litter, along with as many representative dose groups as the number of females would permit. Positive controls were included intermittently and agreed with historical uterotropic responses to 17β-estradiol (*15,17,25,26*) (Table 4). PCB-induced mitogenic activity in the uterus had been confirmed to accompany the weight increase in a previous study (*25*), and increased uterine protein content was also confirmed (*17*).

Necropsy and tissue processing. Rats were decapitated between 0900 and 1100 hr on day 23 and blood was collected immediately after decapitation and allowed to clot. The uterus was excised, trimmed of fat, cut at the cervical os, and weighed to the nearest 0.01 mg. The uterotropic effects were determined by comparing ratios of uterine wet weight in milligrams to body weight in grams to control animals. As soon as the uteri were removed, livers were perfused *in situ* with ice-cold 0.05 M Tris-0.15M KCl (pH 7.4), excised, blotted on tissue paper, and weighed followed by homogenization in 12 ml of the same Tris-KCl buffer. Liver microsomes were then prepared as described in Li et al. (*15*). In addition, thymus and adrenal glands were removed and weighed.

Enzyme assays and thyroid hormone analysis. 7-Ethoxyresorufin (EROD) and 7-pentoxyresorufin (PROD) *O*-dealkylation and 7-benzyloxyresorufin (BROD) *O*-debenzylation were determined by a modifi-

cation of the method of Pohl and Fouts (*32*) as previously described in Li et al. (*15*). UDP glucuronyltransferase (UDPGT) activity in the microsomal suspension was measured using 4-nitrophenol (4-NP) and phenolphthalein (PP) as substrates by a modification method of Watanabe et al. (*33*) as described in Seo et al. (*34*). Microsomal protein was determined by the modification of the Lowry method reported by Guengerich (*35*) using bovine serum albumin as a standard. Serum total T_4 was measured by using a radioimmunoassay (RIA) kit (Coat-A-Count) purchased from Diagnostic Products Corporation (Los Angeles, California). The detection limit of the assay was 0.25 µg/dl. All samples were run in duplicate. T_4 assays were conducted at different times with different RIA kits, and comparison with archived serum samples revealed a significant variation between two sets of RIA kits; therefore, serum T_4 was compared to the control animals of each test replicate.

Data analysis. All data are expressed as means ± SE. Results for serum T_4 were calculated by comparing each T_4 value to its own control in the same litter to reduce the variance between two different sets of RIA kits. Bartlett's test was performed to test for variance homogeneity. In case of heterogeneity of variance ($p \leq 0.05$), rank transformations were used (*36*). A one-way analysis of variance (ANOVA) was performed on homogenous data or transformed data for all the endpoints measured for each extract in this study. If a significant result was found, the Dunnett's *t*-test was used to compare treatment groups versus a control group. The Pearson correlation coefficients (r) for enzyme activities and T_4 levels were calculated from each individual rat collectively for all three extracts. The dose–response relationship of T_4 for each landfill extract was evaluated by linear regression analysis and the difference among three extracts was also determined by ANOVA. In addition, all endpoints measured from control site treatment as well as uterotropic responses from 17β-estradiol treatment were compared to the control group from the soil extract treatment by Student's *t*-test.

Results

Chemical Composition of Extracts

The only pesticide detected was *p,p'*-bis-4-chlorophenyl-1,1 dichloroethene (DDE) at less than 100 µg/ml control soil, 130 µg/ml landfill soil, 170 µg/ml landfill dust, and 10 µg/ml landfill air extracts.

The soil extract contained 46,888 µg/ml total PCBs, 761.6 µg/ml total PCDFs, and 47.2 µg/ml PCDDs. The major PCB isomers present in this extract were tri-CBs (37%) and tetra-CBs (42%) (Fig. 1). The dominant congeners were CB 28 (2,4,4'-trichlorobiphenyl), CB 41 (2,2',3,4-tetrachlorobiphenyl), CB 16 (2,2',3-trichlorobiphenyl), CB 22 (2,3,4'-trichlorobiphenyl), CB 52 (2,2',5,5'-tetrachlorobiphenyl), and CB 18 (2,2',5-trichlorobiphenyl). These six congeners accounted for about 45% of the total PCBs in the soil extract. Both CB 77 (3,3',4,4'-tetrachlorobiphenyl) and CB 126 were present in the soil extract at 212 µg/ml (0.45%) and 12 µg/ml (0.03%), respectively (Table 2), but no 2,3,7,8-TCDD was detected. The major PCDDs in the soil extract were hexa-, hepta-, and octa-CDDs (Fig. 1) and 47% of the penta- to octa-CDDs contained the 2,3,7,8 substitution pattern (*28*). The major PCDFs included tetra-CDFs (49%) and penta-CDFs (26%) (Fig. 1); 2,3,7,8-TCDF was present at 46.37 µg/ml (6%) and other 2,3,7,8-substituted congeners accounted for 23% of total PCDFs (*28*). The relative TCDD TEQ concentra-

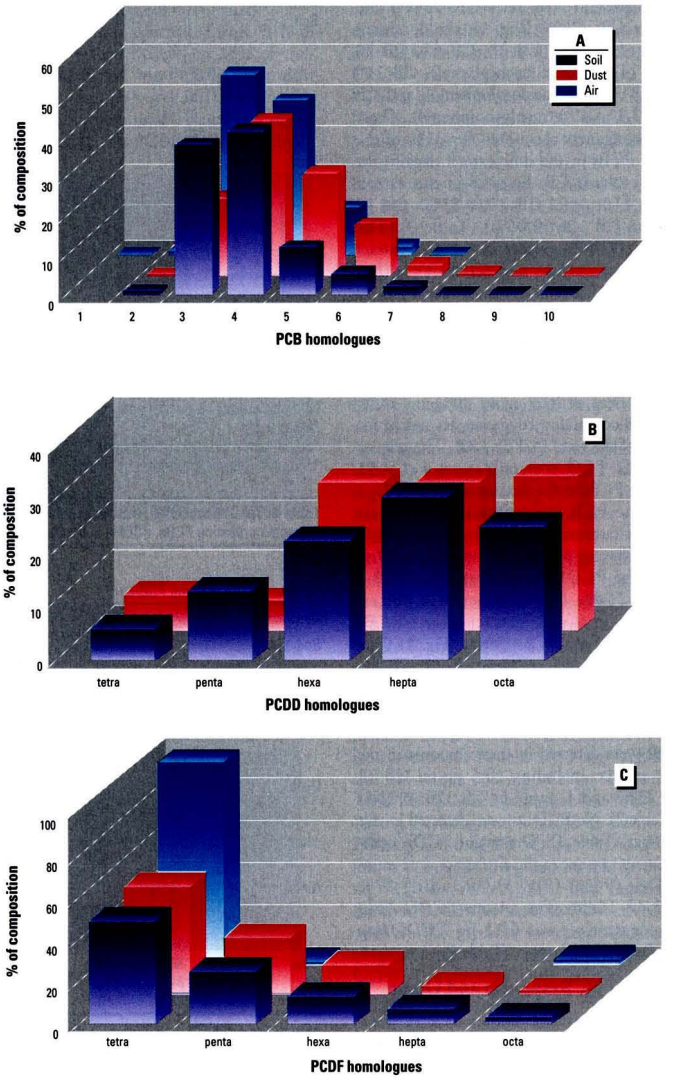

Figure 1. The percent composition of PCB, PCDD, and PCDF homologues in soil, dust, and air extracts.

tion was 0.62 µg TCDD/mg PCBs in the soil extract (Table 1).

The control soil extract contained 0.29 µg/ml total PCBs, 0.96 µg/ml octa-CDF, and no detectable PCDDs (*28*). Traces of polynuclear aromatic hydrocarbons were detected but were not present at concentrations adequate to induce EROD activity.

The PCB concentration of the dust extract was 20,660 µg/ml. The PCDF concentration of the dust extract was 250.4 µg/ml, and the PCDD concentration was 11.5 µg/ml (Table 1). The major PCB isomers present were tetra-CBs (39%) and penta-CBs (26%) (Fig. 1); the dominant congeners were CB 28, CB 41, CB 70 (2,3′,4′,5-tetrachlorobiphenyl), CB 110 (2,3,3′,4′,6-pentachlorobiphenyl), and CB 66 (2,3′,4,4′-tetrachlorobiphenyl). These five congeners accounted for 31% of the total PCBs in the dust extract. Again, the two coplanar CBs detected in this extract were 110 µg/ml CB 77 (0.53%) and 13 µg/ml CB 126 (0.06%) (Table 2). The major PCDDs present in the dust extract were hexa-, hepta-, and octa-PCDDs (Fig. 1); no 2,3,7,8-TCDD was detected but, as with soil, 47% contained the 2,3,7,8-substitution pattern (*28*). The major components of PCDFs included tetra-CDFs (51%) and penta-CDFs (28%) (Fig. 1); 2,3,7,8-TCDF was present at 19.3 µg/ml (8%) and other 2,3,7,8-containing congeners accounted for 19% of the total PCDFs (*28*). The relative TCDD TEQ concentration was 0.51 µg/mg PCBs in the dust extract (Table 1).

The PCB concentration of the air extract (airborne trapped by XAD-2 resin after filtering particulates) was 4606 µg/ml and the PCDF concentration was 23.1 µg/ml (Table 1). The major PCB isomers present in the air extract were tri-CBs (45%) and tetra-CBs (39%) (Fig. 1). The dominant congeners were CB 28, CB 16, CB 52, CB 18, and CB 22. These five congeners accounted for about 47% of the total PCBs in this extract. Two of three coplanar CBs were detected in trace amounts in the air extract, including 10.5 µg/ml CB 77 (0.23%) and 1 µg/ml of CB 126 (0.02%) (Table 2). No PCDDs were detected in this extract (Table 1). The major PCDF components present in the air extract were tetra-CDFs (96%) (Fig. 1), including 17% 2,3,7,8-TCDF. The relative TCDD TEQ concentration was 0.11 µg TCDD/mg PCBs in the air extract (Table 1).

Uterotropic Responses and Organ Weights

Soil extracts caused mild but significant uterotropic responses of 23% and 31% at 32 and 346 mg/kg, respectively (Table 4). Soil extracts also caused significant relative liver weight increases in a dose-dependent manner (Table 5). In addition, there was a decrease in body weight gain at the highest dose, 346 mg PCB/kg (Table 3). Due to the decrease of body weight in the highest dose group, the uterotropic response was also examined by absolute uterine wet weight. The absolute uterine weight in the 346 mg PCB/kg was also significantly higher than controls.

For dust extracts, there were no significant differences in body weight gains (Table

Table 5. Relative organ weights in prepubertal female rats administered landfill-associated extracts containing PCBs, PCDFs, and PCDDs

Group	Dose (mg PCB/kg)	*n*	Relative organ weight[a] Liver weight/ body weight (×100)	Adrenal weight/ body weight (mg/g)	Thymus weight/ body weight (mg/g)
Control site		4	4.36 ± 0.01	0.34 ± 0.01	3.99 ± 0.30
Soil	0	10	4.04 ± 0.08	ND	4.33 ± 0.14
	2	5	4.15 ± 0.10		4.15 ± 0.26
	10	5	4.48 ± 0.13*		4.23 ± 0.21
	32	5	4.75 ± 0.11**		4.37 ± 0.34
	57	5	5.06 ± 0.12**		4.43 ± 0.25
	87	5	5.11 ± 0.15**		4.17 ± 0.25
	346	5	6.51 ± 0.11**		4.27 ± 0.39
Dust	0	6	4.05 ± 0.14	0.33 ± 0.03	4.28 ± 0.17
	13	5	4.53 ± 0.16	0.32 ± 0.02	4.53 ± 0.16
	38	5	5.10 ± 0.18**	0.34 ± 0.02	4.45 ± 0.36
	78	5	5.40 ± 0.20**	0.36 ± 0.08	4.27 ± 0.42
	382	5	6.43 ± 0.20**	0.37 ± 0.02	3.40 ± 0.14
Air	0	10	3.91 ± 0.09	0.34 ± 0.01	4.27 ± 0.10
	6	5	4.33 ± 0.14	0.36 ± 0.02	4.82 ± 0.16
	12	6	4.35 ± 0.08	0.35 ± 0.01	3.92 ± 0.19
	19	5	4.45 ± 0.16	0.35 ± 0.01	4.13 ± 0.24
	38	5	4.68 ± 0.11**	0.37 ± 0.01	4.55 ± 0.33
	84	3	4.65 ± 0.14**	0.34 ± 0.02	4.63 ± 0.30
	175	5	5.57 ± 0.18**	0.40 ± 0.01	4.14 ± 0.23

ND, not determined (incomplete data).
[a]Mean ± SE.
*Significantly different from controls by Dunnett's *t*-test, $p \leq 0.05$.
**Significantly different from controls by Dunnett's *t*-test, $p < 0.01$.

Table 6. Total microsomal enzyme activities in prepubertal female rats administered landfill-associated extracts containing PCBs, PCDFs, and PCDDs

Group	Dose (mg PCB/kg)	*n*	Total microsomal P450 enzyme activities[a] (pmol/min/liver) EROD	PROD	BROD
Control site		4	651 ± 127	68 ± 8	142 ± 20
Soil	0	10	799 ± 181	55 ± 8	194 ± 42
	2	5	4146 ± 3551	239 ± 149	185 ± 101
	10	5	60895 ± 6487**	283 ± 14**	1119 ± 92**
	32	5	107895 ± 14772**	388 ± 43**	1712 ± 288**
	57	5	215685 ± 12429**	535 ± 26**	2310 ± 42**
	87	5	166133 ± 21080**	510 ± 44**	2674 ± 161**
	346	5	317009 ± 34381**	955 ± 55**	9281 ± 230**
Dust	0	6	1002 ± 244	57 ± 14	249 ± 49
	13	5	85542 ± 30263**	278 ± 17**	1488 ± 129**
	38	5	130024 ± 13327**	472 ± 13**	2178 ± 214**
	78	5	162340 ± 14725**	613 ± 45**	2327 ± 334**
	382	5	284322 ± 19266**	793 ± 39**	8484 ± 580**
Air	0	10	1114 ± 317	71 ± 14	260 ± 45
	6	5	7920 ± 2011**	134 ± 16	337 ± 71
	12	6	9874 ± 2407**	229 ± 37*	768 ± 140*
	19	5	27557 ± 3022**	292 ± 32**	881 ± 200**
	38	5	58489 ± 4269**	418 ± 52**	1591 ± 245**
	84	3	115883 ± 13339**	306 ± 22**	4821 ± 221**
	175	5	228313 ± 30069**	875 ± 10**	10497 ± 603**

Abbreviations: EROD, 7-ethoxyresorufin-*O*-deethylase; PROD, 7-pentoxyresorufin-*O*-depentylase; BROD, 7-benzyloxyresorufin-*O*-debenzylase.
[a]Mean ± SE.
*Significantly different from controls by Dunnett's *t*-test, $p \leq 0.05$.
**Significantly different from controls by Dunnett's *t*-test, $p \leq 0.01$.

3), thymus weights, or adrenal gland weights (Table 5). Although absolute wet uterine weights were significantly higher than controls, differences in relative weights were variable and not statistically significant (Table 4). Relative liver weights increased in a dose-dependent manner to 159% controls in the highest dose group (Table 5).

For air extracts, there were no differences in body weight gain during the 2-day treatment (Table 3). Mild but significant increases in relative uterine wet weights were observed at the lower doses (21–22%) and at the highest doses (28–38%) (Table 4). Relative liver weights increased significantly in a dose-dependent manner to 142% of controls at 175 mg PCB/kg (Table 5). On the other hand, there were no marked changes in relative thymus weights or adrenal gland weights at any dose (Table 5).

Serum Total T_4 Levels

Decreases in serum T_4 were plotted against both TCDD TEQs (Fig. 2A) and total PCB (Fig. 2B). The relative potency of the air extract was similar to soil and dust based on total, but greater than soil and dust when based on TCDD TEQs (Fig. 2).

At 2 mg PCB/kg, serum total T_4 was not affected by soil extracts. The serum total T_4 started to decline significantly from 62% of control values at 32 mg PCB/kg to less than 15% of control values at 346 mg PCB/kg ($r = 0.989$, $p<0.001$; Fig. 2).

Serum total T_4 decreased from 94% of control at 10 mg PCB/kg to 15% of control levels at 382 mg PCB/kg from dust extracts ($r = 0.985$, $p<0.001$; Fig. 2). For air extracts, serum total T_4 increased significantly at 12 mg PCB/kg, then declined precipitously in a dose-dependent manner to less than 25% of control values at the highest dose (175 mg PCB/kg; $r = 0.892$, $p<0.001$; Fig. 2). There were no significant differences among the slopes of the three extracts by ANOVA.

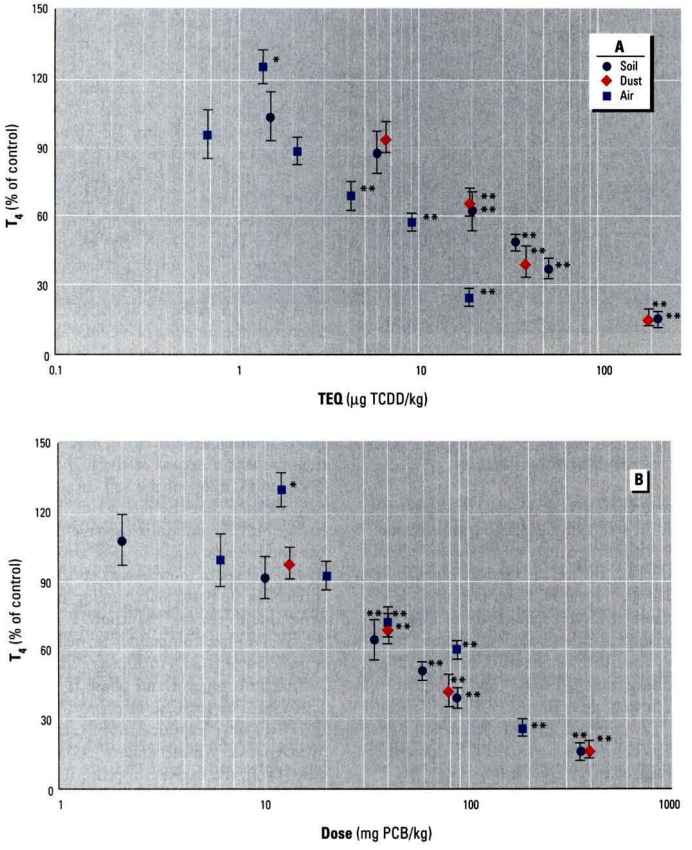

Figure 2. Relative serum total T_4 in prepubertal female rats dosed with landfill extracts. (A) Relative serum total T_4 versus TEQ (µg TCDD/kg; $r = 0.845$); and (B) relative serum total T_4 versus PCB (mg PCB/kg; $r = 0.854$). Each symbol represents the mean of a dose group, bars represent SE, and asterisks indicate a significant difference from control values. The Pearson correlation coefficient (r) was calculated from each individual rat collectively for all three extracts.

Microsomal Enzyme Activities

The total hepatic P450 enzyme activities versus PCB concentrations were calculated (Table 6), and the specific enzyme activities versus TEQ and PCB concentrations for EROD and PROD are shown in Figures 3 and 4, respectively. Sangamo landfill soil extracts induced all three P450 activities in a dose-dependent manner (Table 6). Specific EROD was increased 4-fold at 2 mg PCB/kg and 173-fold at 346 mg PCB/kg (Fig. 3), whereas total EROD was induced 5-fold at 2 mg PCB/kg and 397-fold at 346 mg PCB/kg (Table 6). Both PROD and BROD were significantly increased from 10 mg PCB/kg to 346 mg PCB/kg, but not at the lowest dose (2 mg PCB/kg) (Table 6). At 346 mg PCB/kg, total PROD and BROD were increased 17-fold and 48-fold, respectively (Table 6). The total UDPGT activity versus PCB concentration was calculated for Table 7. Total 4-nitrophenol UDPGT activities were significantly induced from 10 mg PCB/kg to 346 mg PCB/kg. Specific phenolphthalein UDPGT was slightly increased in all soil extract-dosed groups, whereas total phenolphthalein UDPGT was significantly induced from 10 mg PCB/kg to 346 mg PCB/kg due to increased liver weights in these dose groups (Table 7).

The control site soil extract from the non-PCB-containing landfill was only tested at a single dose with the landfill soils. It did not induce P450 enzyme activities (Table 6) or UDPGT activities (Table 7) compared to the landfill soil controls.

For dust extracts, all three P450 enzyme activities were induced in a dose-dependent manner (Table 6). Total EROD activity was increased about 86-fold at 13 mg PCB/kg and 284-fold at 382 mg PCB/kg (Table 6). Total PROD was increased about 5-fold at 13 mg PCB/kg and 14-fold at 382 mg PCB/kg, and total BROD was increased 6-fold at 13 mg PCB/kg and 34-fold at 382 mg PCB/kg (Table 6). The dose response for specific EROD and PROD activities were similar to those for the soil extract (Figs. 3 and 4). Total 4-nitrophenol UDPGT activities were significantly induced at 38, 78, and 382 mg PCB/kg (Table 7). Like the soil extract, specific phenolphthalein UDPGT was only slightly increased in all dust extract-dosed groups, but total phenolphthalein UDPGT was significantly increased at 38, 78, and 382 mg

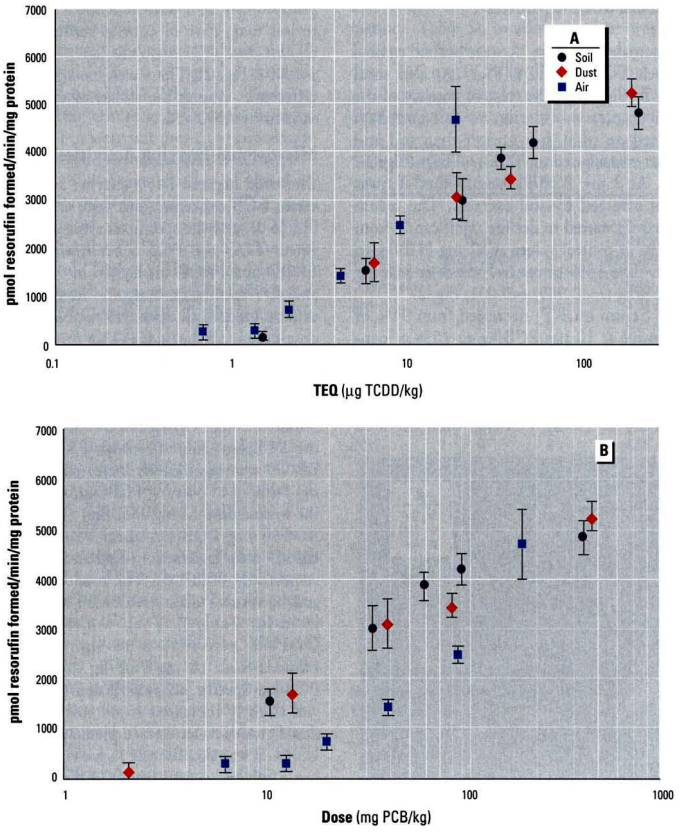

Figure 3. The specific EROD activities versus doses expressed as (A) TEQ concentrations (µg TCDD/kg; r = 0.902); and (B) as PCB concentrations (mg/kg; r = 0.867) in prepubertal female rats administered landfill extracts. Each symbol represents the mean of a dose group and bars represent SE. The Pearson correlation coefficient (r) was calculated from each individual rat collectively for all three extracts.

PCB/kg because of increased liver weights at these doses (Table 7).

Like the other two extracts, all three P450 enzyme activities were induced by air extracts. Total EROD activity was significantly increased about 7-fold at 6 mg PCB/kg and continued to increase to 205-fold at 175 mg PCB/kg (Table 6). Induction of EROD by the air extract was less than that by the soil and dust extracts at lower PCB concentrations; however, when normalized to TEQs for all three extracts, specific EROD activity was similar for all three extracts (Fig. 3). Nevertheless, the pattern of dose response for EROD induction by the air extract appears to be markedly different from induction by the soil extract: at the higher doses, induction by air has an upward inflection, whereas that by soil is trending toward a plateau (Fig. 3). Unfortunately, the amount of airborne extract available was inadequate to test the soil equivalent high concentration.

A similar pattern may exist for PROD induction by the air extract, but the low value for 84 mg PCB/kg, limited to n = 3 because of limited extract, distorts the relationship (Table 6; Fig. 4). Total PROD was induced between doses of 12 mg PCB/kg and 175 mg PCB/kg, but not at the lowest dose (6 mg PCB/kg; Table 6) and, as expected, the correlation was better when compared to total PCB than when compared to TEQs (Fig. 4).

BROD was induced in a dose-dependent manner by the air extract from 6 mg PCB/kg to 175 mg PCB/kg and to a greater extent than by the other two extracts (Table 6). Total 4-nitrophenol UDPGT activities were significantly induced at 12, 38, 84, and 175 mg PCB/kg. Like the other two extracts, specific phenophthalein UDPGT activities were not significantly induced at any dose, but total phenophthalein UDPGT was significantly increased at 38 and 84 mg PCB/kg (Table 7).

Discussion

Acute Endocrine Effects

The estrogenicity of polychlorinated aromatic hydrocarbons is not mediated via the Ah receptor and therefore is not a TCDD-like effect. Estrogenicity, as measured by the uterotropic response, is characteristic of some lower chlorinated CBs such as CB 18 (*26*) and of some nonplanar *ortho*-substituted CBs such as CB 47 (*17*), CB 52 (*25*), and CB 153 (*15*). Coplanar, dioxin-like compounds tend to be antiestrogenic (*37,38*) and can decrease the response to estrogens (*25*). Therefore, it is not surprising that the air extract with a low TEQ tended to be more effective in causing a uterotropic response in prepubertal female rats than the soil and dust extracts. More than 85% of the air extract was composed of lower-chlorinated CBs and *ortho*-substituted CBs and only limited amounts of TCDD-like compounds were present.

Even though the soil extract contained about 80% lower-chlorinated CBs, this extract also contained high levels of PCDFs as well as PCDDs compared to the other two extracts. The antiestrogenicity of polychlorinated aromatic hydrocarbons is associated with Ah receptor agonists (*37,38*). Therefore, the presence of TCDD-like compounds in the soil extract could have antagonized the weak estrogenic effects of those lower-chlorinated PCBs. This may explain the lack of estrogenicity in the soil extract. Nevertheless, a significant uterotropic response was observed at the highest dose (345 mg PCB/kg) of the soil extract. This may indicate that the interaction between estrogenic CBs and antiestrogenic TCDD-like compounds would be dose dependent; however, it seems more likely that the explanation can be based on changing toxicokinetics due to enzyme induction. For example, TCDD-like compounds as well as PB-type CBs (PROD-inducing CBs) can induce both phase I and phase II enzyme activities. The increase of phase I enzyme activities may produce more estrogenic hydroxylated PCB metabolites (*39*). On the other hand, the increase of phase II enzyme activities can enhance the elimination of these estrogenic hydroxylated PCB metabolites. Therefore, a balance between bioactivation and inactivation processes can influence the estrogenic activity and estrogenic/antiestrogenic balance of a mixture. However, total phase II UDPGT induction was about equal to PROD induc-

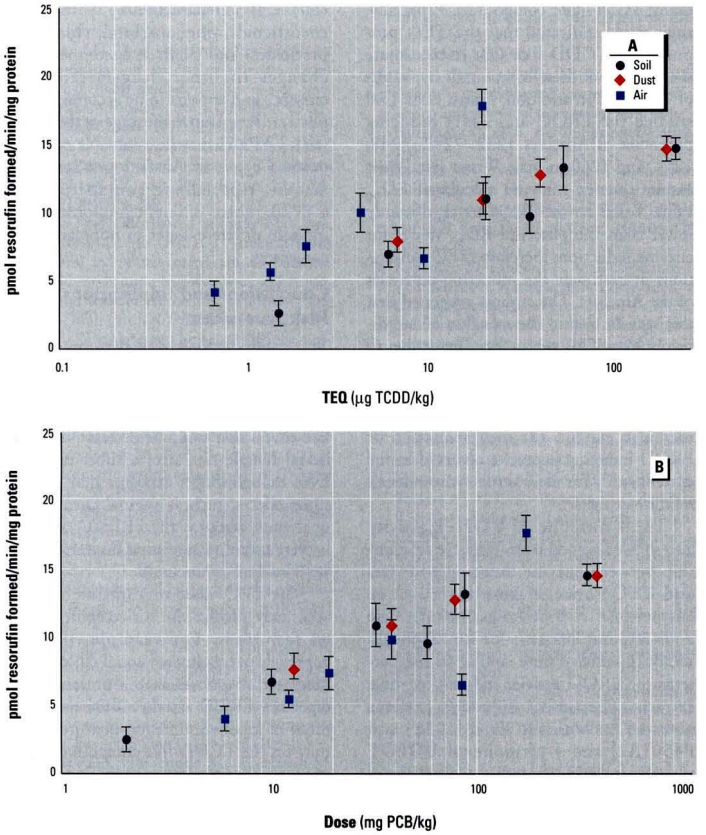

Figure 4. The specific PROD activities versus doses expressed as (A) TEQ concentrations (µg TCDD/kg; r = 0.744; and (B) as PCB concentrations (mg/kg; r = 0.806) in prepubertal female rats administered landfill extracts. Each symbol represents the mean of a dose group and bars represent SE. The Pearson correlation coefficient (r) was calculated from each individual rat collectively for all three extracts.

Table 7. Total microsomal UDPGT activities in prepubertal female rats administered landfill-associated extracts containing PCBs, PCDFs, and PCDDs

Group	Dose (mg PCB/kg)	n	Total microsomal UDPGT activities (nmol/min/liver)[a] 4-NP	PP
Control site		4	651 ± 80	427 ± 55
Soil	0	10	501 ± 74	242 ± 36
	2	5	687 ± 133	316 ± 25
	10	5	1827 ± 119**	486 ± 37
	32	5	2533 ± 286**	493 ± 46
	57	5	4459 ± 253**	584 ± 34**
	87	5	4162 ± 481**	524 ± 96
	346	5	7873 ± 355**	664 ± 59
Dust	0	6	757 ± 146	267 ± 85
	13	5	1819 ± 227	432 ± 56
	38	5	3641 ± 394**	609 ± 63*
	78	5	4186 ± 298**	659 ± 67**
	382	5	8139 ± 416**	543 ± 86*
Air	0	10	617 ± 95	312 ± 52
	6	5	959 ± 71*	375 ± 33
	12	6	1274 ± 84**	340 ± 36
	19	5	1344 ± 168**	513 ± 75*
	38	5	2305 ± 190**	591 ± 34**
	84	3	3029 ± 696**	577 ± 61*
	175	5	5152 ± 631**	464 ± 34

Abbreviations: UDPGT, UDP-glucuronyltransferase; 4-NP, 4-nitrophenol; PP, phenolphthalein.
[a]Mean ± SE.
*Significantly different from controls by Dunnett's t-test, $p \leq 0.05$.
**Significantly different from controls by Dunnett's t-test, $p \leq 0.01$.

tion, lower than BROD induction, and much less than the degree of induction of EROD activity.

A more likely explanation can be based on the fact that coplanar CB 77 and some mono-*ortho* CBs are substrates for the highly-induced EROD, while *ortho*-nonplanar CBs are substrates for the less profoundly induced PROD (*40*). At the higher doses of the soil extract, the disproportionate increase in EROD activity would be expected to reduce the effective *in vivo* levels of antiestrogenic non-*ortho* and mono-*ortho* coplanar compounds, permitting the expression of estrogenicity by noncoplanar compounds. In humans exposed to higher levels of PCBs (i.e., chloracne patients), the more responsive CYP1A induction results in lower residues of coplanar (CBs 37, 77, and 126) and mono-*ortho* (CBs 28, 70, 105, 118, and 156) congeners than in humans exposed to ambient PCBs (*41*). In the same comparison, weakly estrogenic CBs 18, 47, 52, and 153 are found at higher levels in the chloracne patients, as would be expected. In summary, balance between bioactivation and inactivation and more rapid metabolism of coplanar antiestrogens may explain the significant uterotropic response at the highest dose (346 mg PCB/kg) of the soil extract. The possible mechanisms involved need to be further investigated in order to predict the possible estrogenicity and/or antiestrogenicity of environmental mixtures during chronic exposure.

Most PCBs appear to depress serum total T_4 (*42–44*). Both TCDD-like CBs and non-TCDD-like CBs can depress rat serum T_4 (*34,44–46*). In fact, there are multiple mechanisms by which PCBs can affect thyroid hormone homeostasis, and both TCDD and PCB effects on T_4 are species, stage, and time dependent. PCBs could directly affect thyroid hormone synthesis or release in thyroid glands (*43,47*). In addition, PCBs can indirectly influence thyroid function via either enhanced thyroid hormone metabolism by UDPGT induction and increased bile flow (*48–50*) or decreased plasma T_4 levels through enhanced metabolism and excretion after displacement of T_4 from its carrier protein by hydroxylated PCB metabolites (*51,52*). In this study, all three extracts effectively depressed serum T_4 levels to similar extents in immature female rats, even though the PCB congener composition in these extracts were varied and there was a more than five-fold difference in their TEQ values based on µg TCDD/mg PCB. This indicates that both TCDD-like compounds (such as PCDFs and coplanar CBs) and *ortho*-substituted CBs (such as PROD-inducing CBs) can effectively depress serum T_4. A recent model suggests that Ah receptor-mediated T_4 depletion by TCDD is monodimensional, depending mainly on UDPGT induction (*53*). However, different combinations of mechanisms that influence thyroid hormone homeostasis must be considered for different PCB mixtures. If only the effect of

TCDD-like compounds in a mixture, such as the TEQ value of a mixture, is considered, the effect of a PCB mixture on thyroid hormone homeostasis may be underestimated (Fig. 2).

Thymic atrophy, an Ah receptor-mediated response, was not observed for any of the three extracts in this study. Because of the significant P4501A1 induction, a sensitive Ah receptor-mediated response observed for all three extracts, the lack of thymic atrophy in this study was probably due to the relatively short exposure time (44 hr). Nevertheless, Harris et al. (*54*) examined thymic atrophy in immature Wistar rats treated with Aroclors 1232, 1242, 1248, 1254, and 1260 (10, 40, 160, 480, and 2000 mg/kg) measured 14 days after treatment. Thymic atrophy was not observed in any of the dose groups. This may indicate that thymic atrophy is not a sensitive indicator in this short-term bioassay for exposure to Ah receptor agonists, at least when present in a mixture.

Enzyme Induction

EROD activity is one of the most sensitive indicators of exposures to Ah receptor agonists (*3,4*). All three extracts significantly induced EROD activities in prepubertal female rats in a dose-dependent manner. As shown in Figure 3, there was a good dose-response relationship for EROD-specific activity when concentrations of the three extracts were expressed as TEQ concentrations (r = 0.902, p<0.001), but the lower TEQ air extract was clearly less potent at lower concentrations when plotted against total PCB (Fig. 3). In a previous study using the same bioassay conditions (*55*), EROD activity was 1142 pmol/min/mg protein at 1.6 µg CB 126/kg (TEQ = 0.16 µg TCDD/kg) and 4315 pmol/min/mg protein at 65.5 µg CB 126/kg (TEQ = 6.55 µg TCDD/kg). In the present study, EROD activity was only 1500 pmol/min/ mg protein at 10 mg PCB/kg (TEQ = 6.35 µg TCDD/kg) in the soil extract-treated group and 1705 pmol/min/mg protein at 13 mg PCB/kg (TEQ = 7.45 µg TCDD/kg) for the dust extract-treated group. Thus, the EROD induction caused by these extracts was lower than expected based on their TEQ values; therefore, calculated TEQs for these extracts would overestimate their EROD inducing potencies. Conversely, EROD activity alone would underestimate the TEQs.

De Vito et al. (*56*) compared the ability of various PCBs, PCDFs, and TCDD to induce EROD activity in female B6C3F$_1$ mice after 4 weeks of treatment. Their results showed that the present TEFs do not reliably predict induction potency for many TCDD-like compounds. Especially, their study indicated that the TEFs proposed for TCDD-like CBs overestimate the potency of these compounds by factors of 10–1000. In addition, Harris et al. (*54*) studied the EROD and AHH inducing potencies of Aroclors 1232, 1242, 1248, 1254, and 1260 in male Wistar rats. Their results showed that the calculated ED_{50} values based on the TEQs are significantly lower than the observed ED_{50} values for enzyme induction; therefore, TEQ summations overestimate the induction potencies of the Aroclors. The authors suggested that this may be due to the selection of inordinately high TEF values for CBs or due to the possible antagonistic interactions between the coplanar and mono-*ortho* coplanar CBs and other CBs. These factors may also explain the overestimation of EROD-inducing potencies observed in the current study for these three environmental mixture extracts.

The 4-nitrophenol UDPGT activity also showed a good dose–response relationship between enzyme inducing potencies and TEQ concentration for all three extracts (r = 0.884, p<0.001). The dose–response patterns for 4-nitrophenol UDPGT activity were similar to the patterns for EROD activity induced by these extracts. The similar induction patterns observed for both EROD (P4501A1 and P4501A2) and 4-nitrophenol UDPGT activities may indicate that 4-nitrophenol UDPGT induction can also be used for an indicator of Ah receptor-mediated responses, even though 4-nitrophenol is a substrate for several UDPGTs. However, the degree of 4-nitrophenol UDPGT induction was much less than EROD induction in these extracts. The mild induction of phenolphthalein UDPGT was only apparent if total liver activity was considered.

Neither PROD nor BROD activities are induced by TCDD-like compounds in rats. The air extract was a more potent inducer of PROD and BROD activities than soil or dust extracts at the same TEQ dose level (Fig. 4; Table 6). Even though the patterns of PROD and BROD induction were more similar for the three extracts when expressed as PCB concentrations, BROD activity was still more highly induced by the air extract (Table 6). BROD activity can be regarded as a measurement of CYP2B and CYP3A induction (*57,58*), whereas PROD activity is more specific as a measurement of CYP2B induction. The prototype inducer for CYP3A1 is pregnenolone 16α-carbonitrile (*59*); however, nonplanar PCBs also induce CYP3A1, and the structure–activity relationships are different *in vivo* than *in vitro* (*60*). CYP3A1 induction by synthetic glucocorticoids, phenobarbital, chlorinated pesticides, and PCBs is accompanied by changes in other drug-metabolizing enzymes and appears to be accompanied by posttranscriptional message stabilization (*61*). CYP3A1 may be a valuable marker of certain types of Ah-independent PCB actions, especially in conjunction with CYP2B, where different proportions may indicate different types of nonplanar PCB congeners.

Conclusions and Implications for Risk Assessment

This study demonstrated that the environmental mixtures containing mainly PCBs with significant proportions of PCDFs caused both TCDD-like and non-TCDD-like effects that could be detected in prepubertal female rats after a short exposure. Even though longer exposure may enhance some effects, such as vaginal cornification or thymus atrophy, the FRIEDA assay can be very useful in screening mixtures as well as individual compounds.

The TEQ value of a mixture may not accurately predict the Ah receptor-mediated responses. For example, the TEF approach overestimated some Ah receptor-mediated responses in the present study, especially EROD activity. If the risk assessment of an environmental mixture focuses only on the TCDD-like compounds in the mixture, the important endocrine-disrupting effects of a mixture could be underestimated. For example, total serum T_4 was effectively depressed by the three extracts at similar PCB levels despite large differences (sixfold) among the relative TEQs of the three extracts when expressed as micrograms TCDD per milligram PCB in the present study. Therefore, the thyroid hormone depression by air extract would be underestimated based on its low TEQ compared to the other two extracts based on the TEF approach.

In summary, the different matrices from the same environmental source not only can vary widely in their congener compositions, but also differ significantly in their net biological effects. Furthermore, it is important to note that humans and wildlife are exposed to profiles of PCBs/PCDFs/PCDDs not reflected by profiles in food or human tissues. Transient exposure to airborne PCBs, for example, would superimpose a higher proportion of lower-chlorinated and readily metabolized congeners onto existing residues; thus, effects due to these congeners and/or their metabolites might be manifest at a later developmental stage when evidence of exposure (i.e., residues of parent CB) would no longer be apparent.

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International Society of Exposure Analysis (ISEA)

The ISEA was founded in 1989 in response to a growing need for a professional society devoted to the emerging science of exposure analysis related to environmental contaminants, human populations and activities, and ecosystems.

Since its founding, ISEA has always placed importance on international participation, in particular from individuals in lesser developed countries. The society is currently encouraging additional members to join, particularly those from Asia, South and Central America, and Africa. It is hoped that over the coming year several new international chapters will be formed.

Recently, a new international committee was formed to strengthen the international membership and the scope of the society. Among the plans of the committee are to institute a matching program between scientists, regulators, and policymakers in lesser developed countries with counterparts in North America and Europe. At an individual level, this matching program will facilitate technology and information transfer while forging new professional relationships between exposure analysts. For individuals from other disciplines who wish to increase their knowledge of exposure assessment, matching with an experienced exposure assessment expert would provide an excellent educational opportunity. The committee hopes to have this program in place by mid-1996.

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The Potential Effect of Global Warming on the Geographic and Seasonal Distribution of *Phlebotomus papatasi* in Southwest Asia

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The distribution of *Phlebotomus papatasi* in Southwest Asia is thought to be highly dependent on temperature and relative humidity. A discriminant analysis model based on weather data and reported vector surveys was developed to predict the seasonal and geographic distribution of *P. papatasi* in this region. To simulate global warming, temperature values for 115 weather stations were increased by 1°C, 3°C, and 5°C, and the outcome variable coded as unknown in the model. Probability of occurrence values were then predicted for each location with a weather station. Stations with positive probability of occurrence values for May, June, July, and August were considered locations where two or more life cycles of *P. papatasi* could occur and which could support endemic transmission of leishmaniasis and sandfly fever. Among 115 weather stations, 71 (62%) would be considered endemic with current temperature conditions; 14 (12%) additional stations could become endemic with an increase of 1°C; 17 (15%) more with a 3°C increase; and 12 (10%) more (all but one station) with a 5°C increase. In addition to increased geographic distribution, seasonality of disease transmission could be extended throughout 12 months of the year in 7 (6%) locations with at least a 3°C rise in temperature and in 29 (25%) locations with a 5°C rise. *Key words*: global warming, leishmaniasis, *Phlebotomus papatasi*, sandfly, sandfly fever, Southwest Asia. *Environ Health Perspect* 104:724–727 (1996)

Much of the impact of global warming on human activities has focused on physical consequences, such as more frequent violent storms and rising sea levels flooding low-lying areas. However, there are growing indications that the potential effects of global warming on human health are no less serious (*1*). For example, geographic and seasonal distribution of infectious diseases, particularly vectorborne diseases, could be markedly altered (*2,3*).

Vectorborne diseases are usually limited in their distribution, either by the range of the vector or by the range of a reservoir vertebrate host. The vector and host range are affected, directly or indirectly, by temperature and precipitation. According to Shope (*4*), global warming in North America could extend the distribution of the mosquito vectors *Aedes aegypti* and *A. albopictus*.

In addition to extending the geographic range of these vectors, the development of the mosquito larvae is faster in warmer climates, resulting in the mosquitoes becoming adults sooner. Also, the extrinsic incubation periods of yellow fever and dengue viruses in the mosquito vectors are dependent on temperature (*4*). With warmer temperatures, the incubation time required from when the mosquito first encounters an infected host until the mosquito is able to transmit an infectious virus may be shortened.

As a result of these factors, the mosquito vectors may be more widely distributed and metamorphose faster with global warming, and the extrinsic incubation period of viruses like dengue and yellow fever may be shortened. These altered factors could result in increased transmission of vectorborne disease in temperate climates where vectors already occur but where development of the parasite is limited by current temperature conditions (*4,5*).

The sandfly, *Phlebotomus papatasi*, is a vector throughout Southwest Asia of two important infectious diseases, sandfly fever and leishmaniasis. Sandfly fever is a viral infection characterized by rapid onset, with a 3- to 4-day course of high fever and severe debility, but mortality is low with this infectious disease. Cutaneous leishmaniasis, also known as Oriental sore or Baghdad boil in Southwest Asia, is caused by a protozoal infection, either *Leishmania tropica* or *Leishmania major*. The primary manifestation of this disease is nonfatal ulcerating skin lesions after an incubation period of several days to many months. Visceral leishmaniasis, or Kala azar, is a chronic systemic infection caused by *Leishmania donovani*. It has an incubation period from 2 to 4 months and can be fatal if untreated.

The distribution of *P. papatasi* is not well understood but is known to be highly dependent on environmental conditions. Both sandfly adults and larvae are sensitive to high temperatures and low humidities. In laboratory experiments, all adult sandflies died within 2 hr at temperatures above 40°C, and temperatures below 10°C are unfavorable for survival (*6*).

Besides temperature, laboratory studies have demonstrated that as the relative humidity increases, the number of sandfly survivors increases. Studies also have shown that the larvae, pupae and adult sandflies must have a habitat with a constant, relatively high humidity (*6*).

Rodent burrows, like those created by the gerbil, *Psammomys obesus*, provide the high humidity and cooler temperatures necessary for sandfly survival. Caves, deep cracks in walls, and dark corners in houses also provide favorable environmental conditions for the sandfly to survive and reproduce, even in areas of extreme temperature and aridity (*6*). With the onset of cold weather, however, sandfly larvae undergo diapause, permitting them to survive the winter and emerge as adults the following spring (*7*). *P. obesus* also serves as a reservoir of the parasite that causes cutaneous leishmanias (*8*).

Information on the seasonal distribution of *P. papatasi* indicates a definite seasonal occurrence which is consistent with environmental experiments: absent in all locations in the cold winter period of January and February, with a population increase beginning in most areas by April or May and declining in October (*9–11*). Also, most sandflies are nocturnal, probably due to environmental factors. The biting activity of *P. papatasi* starts immediately after sunset and increase afterwards, reaching a maximum around midnight when the temperature tends to be lower and the humidity higher (*12*). Biting activity has been observed in Central Iraq to decrease to 13% at 0300 hr and to almost stop after sunrise (*12*).

Using a computer model based on temperature, relative humidity, dew point, and previously reported vector surveys, this study explores the potential effect global warming could have on the seasonal and geographic distribution in Southwest Asia of the sandfly, *P. papatasi*.

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Initial Model

A model based on monthly weather and vector occurrence data was developed previously to predict the geographic and seasonal distribution of *P. papatasi* in Southwest Asia (*13*). For the model, information was compiled from 136 articles on the presence or absence of the vector, *P. papatasi*, as well as on the presence or absence of human cases of cutaneous and visceral leishmaniasis and sandfly fever. Weather data were obtained from the International Station Meteorological Climate Summary, version 2.0, June 1992 (Federal Climate Complex, Asheville, North Carolina). Data were available for 115 weather stations in 10 countries: Saudi Arabia, Kuwait, Yemen, Iraq, Iran, Syria, Jordan, Lebanon, Israel, and Egypt. These data encompassed measurements of mean high temperature, mean minimum temperature, mean temperature, extreme high temperature, extreme minimum temperature, relative humidity, and dew point.

Precipitation values were available for only 40% of the 115 weather stations. There were no precipitation values for any of the 21 stations in Saudi Arabia, the 6 weather stations in Jordan, or the 1 station in Kuwait. Mosquitoes require standing water for reproduction; however, sandflies lay their eggs in small batches in protected places with high humidity and a high content of organic matter (*14*). Pools of water are not necessary for reproduction, and therefore precipitation data were not considered critical to the development of a model for sandfly activity.

The major determinant of disease risk in this model is the dichotomy between environmental conditions where the vector is found and conditions where the vector does not occur. Therefore, for this exercise, negative data, the absence of the vector and disease, were given the same weight in the analysis as were positive data. Numerous articles which reported that *P. papatasi* is not found during January and February provided much of the negative data for the model.

The model was developed using the SAS stepwise discriminant analysis procedure to determine which variables were most useful in discriminating between vector presence and vector absence (SAS Institute, Cary, North Carolina). Six of the seven weather variables were selected by the stepwise analysis to be included in the model: mean minimum temperature ($F = 2.225$, Prob = 0.14), mean temperature ($F = 8.236$, Prob = 0.004), extreme high temperature ($F = 5.049$ Prob = 0.025), extreme minimum temperature ($F = 9.073$, Prob = 0.003), relative humidity ($F = 10.268$, Prob 0.001), and dew point ($F = 18.114$, Prob = 0.0001). Wilk's λ values ranged from 0.7553 to 0.7905.

Using the selected weather variables, the discriminant analysis function classified locations as vector present or absent and calculated probability of occurrence values for each location during each month of the year. The model classified the 1742 observations into presence or absence and probability of membership: 74% of the 416 observations indicating absence of the vector were classified as absent by the model; 67% of the 414 observations indicating presence of the vector were classified as present by the model.

Simulation of Global Warming

To simulate global warming, the temperature values for all weather stations were increased by 1°C, 3°C, and 5°C, and the dependent variable was listed as unknown. Dew point and relative humidity values were not changed because it was not possible to accurately predict these parameters in the isolated habitats of vectors and animal hosts. The model was then run, and probability of occurrence values were computed for higher temperatures at the 115 weather stations during the 12 months of the year.

Sandfly Survival and Disease Transmission

Laboratory studies of the sandfly have found that, depending on the temperature and relative humidity, *P. papatasi* eggs hatch in 4–14 days, larval development takes 3–27 days, and the pupal stage lasts 6–20 days. The entire life cycle requires 2–9 weeks (*12*). In Jerusalem, because of cooler temperatures, only one generation of sandflies reach the adult stage during the summer, with the larvae of this single generation going into hibernation and not hatching until May of the following year (*6,11*). Transmission of either leishmaniasis or sandfly fever rarely occurs in Jerusalem because the sandfly population apparently is insufficient to maintain endemicity. These infectious diseases appear to occur primarily in those areas where favorable temperatures and other environmental conditions allow at least two life cycles to be completed (4–18 weeks), creating larger sandfly populations and a greater probability of contact with both infected and noninfected host (*6,8,11,12*).

In those locations where the model predicts positive values for 1 or 2 months only in the summer, it is questionable whether *P. papatasi* would have sufficient time to complete more than one life cycle. Consequently, these areas were considered to have a low probability of transmission in this study and not endemic for sandfly-transmitted diseases. In contrast, weather stations with positive probability of occurrence values for at least 4 months (May, June, July, and August) were considered locations that could support endemic disease transmission.

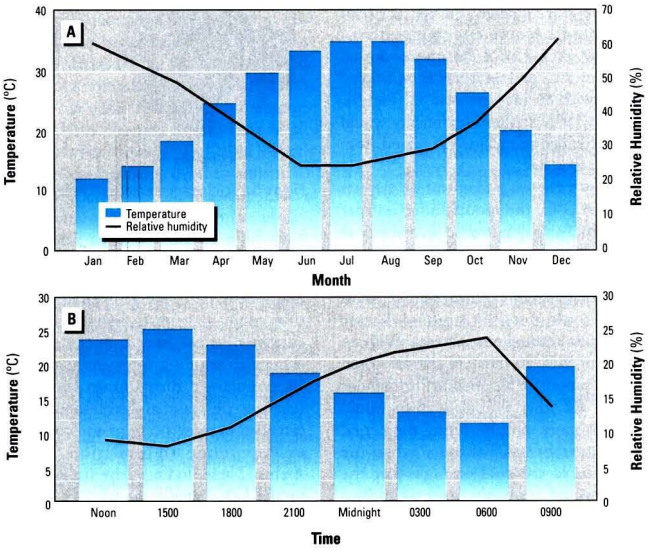

Figure 1. (A) The relationship of temperature and relative humidity by month for locations positive for sandfly activity in Southwest Asia. (B) The diurnal relationship between temperature and relative humidity at 3-hr intervals at Kuwait International Airport.

The temperature and relative humidity pattern of those locations considered to have a high probability of occurrence indicates that as monthly temperature rises, relative humidity declines (Fig. 1A). However, as the diurnal temperature declines, the relative humidity rises (Fig. 1B). This could explain the reason sandflies are nocturnal. During the day when the temperature is high and the relative humidity is low, the sandflies survive in isolated habitats, venturing outside to feed only during the evening and early morning hours when the temperature is lower and the relative humidity is higher (*11*).

Results

With current temperatures, 71 (61.7%) of the 115 weather stations would be considered by the model as endemic for disease transmission, permitting 2 or more life cycles of the sandfly. Fourteen (12.2%) could become endemic if the temperature was increased by 1°C, 17 (14.8%) more with a 3°C temperature rise, and 12 (10.4%) more, or all but one station, could become endemic with a 5°C rise in temperature. The one station that was not predicted to be warm enough, even with a 5°C increase in temperature, was Les Cedres in Lebanon at an altitude of 1916 m (Fig. 2).

Seasonality also could be markedly changed by global warming. Of the 115 stations, the model indicates that 17 (14.8%) would be endemic for the 8-month period from March to October with no temperature change. Fifteen (13.0%) more could become endemic with an increase of 1°C, 33 (28.7%) more with an increase of 3°C, and 34 (29.6%) additional stations with an increase of 5°C (Fig. 3). For 16 (13.9%) stations, seasonality would not be extended to include the period from March to October.

Unless there was a temperature increase of at least 3°C, the model indicates that disease transmission would not occur at any of the stations between November and February (Fig. 4). With an increase of 3°C, eight stations, seven of which were in Saudi Arabia, could possibly support disease transmission throughout the year. With an increase of 5°C, 30 (26.1%) stations could possibly support transmission throughout the year. All other weather stations would have 1 or more months during the period from November to February when the temperatures are cold enough that the sandfly would probably need to go into diapause to survive.

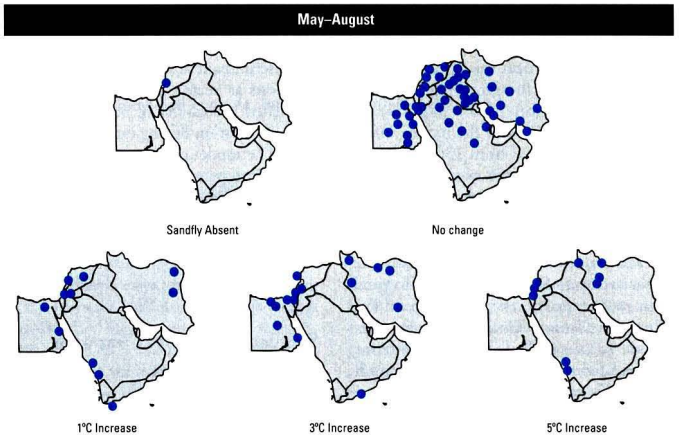

Figure 2. Locations of predicted distribution of *P. papatasi* between May and August.

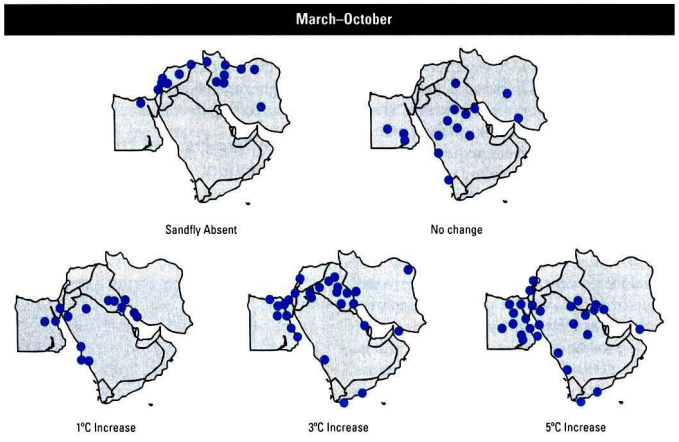

Figure 3. Locations of predicted distribution of *P. papatasi* between March and October.

Discussion

This predictive model indicates that higher temperatures due to global warming could greatly increase both the geographic and seasonal distribution of sandfly vectors in Southwest Asia. The geographic distribution could increase to include areas that currently do not have temperatures warm enough to permit a sufficiently large sandfly population to maintain endemicity. Likewise, the seasonal distribution could be extended in most locations and could result in year-round transmission in Saudi Arabia. These findings have to be qualified by the fact that the model only evaluated temperature increases and no other climatic factors, such as humidity and rainfall.

Unlike mosquitoes, sandflies do not need pools of water for breeding (*3*). The increased humidity in rodent burrows, deep cracks in walls and dark corners in houses, where sandflies usually reside during the day, provide not only lower temperatures but also increased humidity needed for survival even with higher daily outdoor temperatures. Nocturnal activities, however, could be shortened if temperatures remain high and humidity low during evening hours.

Sandfly fever is caused by viruses belonging to the phlebotomus fever serogroup, with serotypes Naples and Sicilian occurring in Southwest Asia. Temperature can affect the rapidity of the life cycle of arboviruses because as the temperature increases, the extrinsic incubation period decreases (*3*). Consequently, some

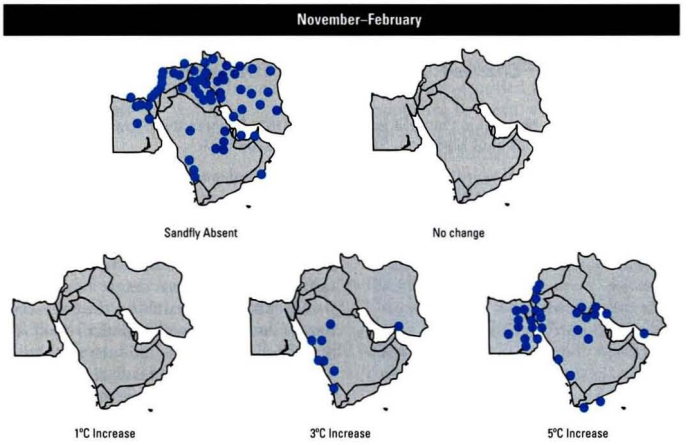

Figure 4. Locations of predicted distribution of *P. papatasi* between November and February.

viruses could replicate much more rapidly with global warming, which could also increase disease transmission. The effect of increased temperature on the phlebotomus serogroup of viruses, however, is unknown.

Leishmaniasis is caused by *Leishmania* spp., a protozoan infection. *Leishmania* spp. have acquired thermotolerance, experiencing temperatures of 22–28°C in their mammalian hosts, 31–35°C in skin lesions, and up to 37°C in visceral organs (*15*). Therefore, increased temperatures due to global warming probably would have little effect on these protozoans, and in those locations where sandflies can find habitats to survive the daytime heat, global warming should have little adverse affect on survival and on transmission of these infectious agents. Whether higher temperatures would adversely affect the rodent hosts of the sandfly vector has to be considered, but could not be evaluated in this analysis.

To determine the effects of global warming and validate this model, field collections of the vector and monitoring for increased disease distribution will have to be conducted. In locations that are on the periphery of current endemic areas, studies to ascertain if the vector has become established or if there is a marked rise in disease occurrence could indicate that global warming has increased temperatures sufficiently to permit the establishment of new endemic foci. Furthermore, detecting sandfly activity during the winter months in areas previously free of the vector during this period could provide strong evidence of the effect of global warming.

Malaria has been the disease most often studied in relation to the impact of global warming on human health. As with this model, the malaria mosquito models project an increase in the geographic distribution of the disease, particularly at the borders of current endemic malaria areas, at higher elevations within endemic malaria areas, and with current temperate climate zones (*5*).

Because *P. papatasi* is the vector for both sandfly fever and cutaneous and visceral leishmaniasis, several different diseases could be affected with an increase in this vector. In a nonimmune population, sandfly fever can easily reach epidemic proportions (*11*). Although usually not fatal, sandfly fever does cause high fever, headache, and general debility similar to influenza. Cutaneous leishmaniasis causes polymorphic skin lesions which can be disfiguring and a nidus for bacterial infection. Visceral leishmaniasis is a chronic systemic disease that can be fatal.

Sandfly fever, cutaneous leishmaniasis, and visceral leishmaniasis, are difficult to diagnose and to treat. Use of insecticide for vector control can be expensive and can be harmful to the population. If an increased geographic and seasonal distribution of *P. papatasi* occurs due to global warming, the impact of these diseases on human health could be substantial.

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Evaluation of Mortality and Cancer Incidence among Alachlor Manufacturing Workers

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Alachlor is the active ingredient in a family of preemergence herbicides. We assessed mortality rates from 1968 to 1993 and cancer incidence rates from 1969 to 1993 for manufacturing workers with potential alachlor exposure. For workers judged to have high alachlor exposure, mortality from all causes combined was lower than expected [23 observed, standardized mortality ratio (SMR) = 0.7, 95% CI, 0.4–1.0], cancer mortality was similar to expected (6 observed, SMR = 0.7, 95% CI, 0.3–1.6), and there were no cancer deaths among workers with 5 or more years high exposure and 15 or more years since first exposure (2.3 expected, SMR = 0, 95% CI, 0–1.6). Cancer incidence for workers with high exposure potential was similar to the state rate [18 observed, standardized incidence ratio (SIR) = 1.2, 95% CI, 0.7–2.0], especially for workers exposed for 5 or more years and with at least 15 years since first exposure (4 observed, SIR = 1.0, 95% CI, 0.3–2.7). The most common cancer for these latter workers was colorectal cancer (2 observed, SIR 3.9, 95% CI, 0.5–14.2 among workers). Despite the limitations of this study with respect to small size and exposure estimating, the findings are useful for evaluating potential alachlor-related health risks because past manufacturing exposures greatly exceeded those characteristic of agricultural operations. These findings suggest no appreciable effect of alachlor exposure on worker mortality or cancer incidence rates during the study period. *Key words*: agricultural chemicals, alachlor, cancer incidence, mortality. *Environ Health Perspect* 104:728–733 (1996)

There has been significant interest in recent years in the health experience of agricultural workers. Blair et al. (*1*) recently published a meta-analysis of epidemiologic studies of farmers and concluded that farmers have significantly elevated rates of lip cancer, Hodgkin's disease, melanoma, multiple myeloma, stomach cancer, prostate cancer, and leukemia. From these findings, they inferred a possible role for pesticides (i.e., herbicides or insecticides) and other work-related exposures. A major prospective study has been initiated to evaluate risk factors for cancers and nonmalignant diseases for farmers, their families, and for commercial pesticide applicators (*2*).

Fundamental characteristics of agricultural practice make it difficult to assess potential health effects of specific pesticides. The type, amount, and frequency of pesticide use depends on a number of factors including the crop, the level and severity of pest infestation, weather conditions, recommended usage, time of year, availability, and cost. Preemergent herbicides, for example, are used by farmers and pesticide applicators only in the days or weeks before planting. Such an occupational exposure scenario is different from the chronic exposure scenario typical of manufacturing environments. Another complicating factor is that farmers and pesticide applicators frequently use a large number of different pesticides each year, making it difficult to evaluate a single or predominant exposure scenario.

The primary advantage of studying pesticide users is the large number of subjects available to be studied. However, in light of the complications mentioned above, such studies should be supplemented by research on pesticide manufacturing populations. Although manufacturing populations tend to be relatively small, they frequently have chronic exposure to specific pesticides and have worked under conditions where exposures have been characterized or can be fairly well documented.

We initiated a study of mortality and cancer incidence among workers involved in the manufacture of alachlor [2-chloro-2′,6′-diethyl-*N*-(methoxymethyl)-acetanilide], the active ingredient in a family of preemergent herbicides. Monsanto has manufactured alachlor since March 1968 at a plant in Muscatine, Iowa. Registration and domestic use of alachlor began in the 1969 growing season. Since that time, alachlor has been used widely on corn, soybeans, and other crops.

Numerous experimental studies have been conducted to characterize alachlor metabolism and toxicology. Chronic feeding studies at high doses found increased frequencies of nasal, stomach, and thyroid tumors in laboratory rats (*3,4*). The lowest observed effect levels (LOELs) were 126 mg/kg daily for thyroid cancers and 42 mg/kg daily for thyroid tumors (exposure levels that were overtly toxic to rats) and 15 mg/kg daily for nasal cancers. Experimental evidence suggests that the alachlor-related stomach and thyroid tumors result from nongenotoxic mechanisms at exposures that exceeded tolerable doses (*4,5*). These mechanisms are not operative in rats at lower doses.

Mechanistic research on the rat nasal tumors points to a specific alachlor metabolite (2,6-diethylbenzoquinoneimine) that concentrates in rat nasal tissues (*6*). Enzymatic capabilities of rat nasal cells to produce this putative carcinogenic metabolite exceed the capabilities of human nasal cells by three to four orders of magnitude (*7*). Whole-body autoradiographic studies have shown accumulation of radiolabeled alachlor or its metabolites in nasal tissues of rats, but not in monkeys (*7*). It has been estimated that the nasal tumor LOEL exceeds manufacturing exposures in the early years of production by at least 40-fold and typical current agricultural exposures by 25,000-fold (*8*).

An alachlor chronic feeding study in mice found a statistically significant increase in lung tumors among females in the highest daily exposure group (260 mg/kg) (*5*). Lung tumor incidence for this exposure group, however, was within the range of historical control values. A second chronic study in mice did not show any dose-related increase in lung tumors (*9*).

Metabolic studies in monkeys, used to provide a surrogate model for human metabolism, show that oral and dermal alachlor exposures are metabolized and excreted largely through the urinary tract, with a small portion excreted through the large bowel (*10,11*). Alachlor and fecal and urinary metabolites of alachlor are negative in Ames tests (*12*).

Previous studies of alachlor workers from the Muscatine plant have considered ocular effects, mortality for the period 1968–1990, and cancer incidence for the

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period 1970–1990 (*8,13,14*). The present study updates mortality and cancer incidence analyses through 1993 and provides additional analyses of workplace and environmental exposures not addressed in the previous studies.

Methods

The total Muscatine plant population was enumerated from Social Security Administration (SSA) Quarterly Reports on Earnings (form 941A). Demographic and work history information was abstracted from company employment records. The mortality cohort was restricted to the 1199 workers employed at least 1 year from plant start up as an ammonia facility in 1961 through 31 December 1993.

The cancer incidence cohort was a subset of the mortality cohort, including 1169 workers who lived in Iowa for some time during the period 1969–1993. Thirty employees from the mortality cohort were excluded because they either lived nearby in Illinois or transferred to another Monsanto location before 1969. The criterion of Iowa residence reflects the catchment area for the State Health Registry of Iowa (SHRI), which was the source for identifying cancers in this study. The SHRI is a statewide, population-based cancer registry that has been operating since 1969 and has participated since 1973 in the National Cancer Institute's Surveillance Epidemiology and End Results Program.

Vital status was determined for the mortality cohort until the end of 1993 using a variety of sources, including company payroll, pension and mortality files, SSA (a submission prior to 1987 when SSA stopped doing mortality searches for researchers), the National Death Index, the Iowa state motor vehicle bureau, and a retail credit agency. In addition, direct tracing by mail and phone was conducted as part of the cancer incidence evaluation. As a result of these procedures, 1166 (97.3%) workers were found to be alive, 30 (2.5%) were deceased, and 2 (0.2%) were lost to follow-up. The proportions alive, deceased, and lost to follow-up were similar for the 1036 workers judged to have had alachlor exposure (see Table 1).

Death certificates were obtained for all known decedents. Two nosologists independently coded the underlying cause of death (UCOD) for each decedent according to the eighth revision of the International Classification of Diseases (*15*). The UCOD from each nosologist was compared for each decedent and the single disagreement, which involved the coding of an accidental death into different subcategories, was resolved by mutual agreement.

The incidence study cohort was matched against the SHRI master database to identify workers diagnosed with invasive cancer from 1969 through 1993. Cancer cases were identified based on the correspondence between workers' identifying information (social security numbers, full names, and birth dates) in company files and in SHRI's database. All inexact matches were verified by matching other data in the workers' personnel files and in SHRI's records. Incident cancer cases were coded according to the second edition of the International Classification of Diseases for Oncology (*16*). SHRI was also the source for general population Iowa cancer incidence rates, which were used as a basis of comparison for workers' cancer incidence rates.

The major methodologic issue for conducting a valid cancer incidence analysis was correctly enumerating person-years at risk within SHRI's catchment area. We used a number of data linkage and tracing procedures to address this issue. First, current addresses were obtained from company records for active workers and vested former employees (i.e., workers employed long enough to qualify for retirement benefits). Current Iowa residents who lived in Iowa or moved to Iowa when hired at the Muscatine plant were assumed to have lived in Iowa since their plant hire date. Former vested employees who had a current address outside of Iowa (mainly transferees within Monsanto) were assumed to have been Iowa residents from their start date at the Muscatine plant until their transfer or employment termination date. Present workers with a non-Iowa current address and workers who terminated employment before becoming vested were sent a letter to establish their dates of Iowa residency. We also matched these workers with the Iowa Department of Motor Vehicles, the company mortality database, and databases maintained by a credit search firm to establish possible Iowa residency after employment termination. Finally, we traced workers whose residence histories remained unknown through directory assistance and did a phone survey to identify as many residence histories as possible. As a result of these tracing procedures, residence history was determined for 98.4% of workers with potential alachlor exposure as of 31 December 1993 (Table 1). Follow-up improved with length of potential exposure: we determined residence history for all but 2 of 481 employees who had 5 or more years of alachlor exposure. These 2 employees became lost to follow-up 2 years and 1 month, respectively, before the end of study date.

Another methodologic issue concerns cancer incidence of workers who left the SHRI catchment area and were excluded from the cancer incidence analyses as of the date they left. Workers who remained in Iowa had more than twice as many years of alachlor exposure than workers who left Iowa, so migration is not likely to affect the validity of our results. Cancer risk was also assessed through national mortality analyses.

Exposure Assessment

There was insufficient information on plant conditions to estimate alachlor exposures quantitatively during the study period. Therefore, our exposure estimation was qualitative, based on work history information, judgment of an industrial hygienist, and, to a lesser extent, recent exposure monitoring data (*17*).

The first step in the exposure estimating process was the creation of a department/job title dictionary that included all work locations and job assignments in

Table 1. Distribution of workers by gender, alachlor exposure status, and follow-up status

Group	Men	Women	Total
Mortality cohort			
Total	954	245	1199
Nonwhites (excluded from analysis)	20	6	26
Nonalachlor (excluded from analysis)	93	44	137
Alachlor-exposed	841	195	1036
No. alive	817	191	1008 (97.3%)
No. dead	23	4	27 (2.6%)
No. lost to follow-up	1	0	1 (0.1%)
Incidence cohort			
Total	928	241	1169
Nonwhites (excluded from analysis)	20	6	26
Nonalachlor (excluded from analysis)	77	41	118
Alachlor-exposed	831	194	1025
No. in Iowa through 1993	573	142	715 (69.8%)
No. migrated from Iowa before 1993	228	43	271 (26.4%)
No. unknown migration date from Iowa	12	4	16 (1.6%)
Incident cancers	19	5	24[a]

[a]One worker had two incident cancers.

workers' personnel records. Jobs with similar exposure potential were consolidated by the plant industrial hygienist into occupational exposure categories (OEC). The plant hygienist then assigned each OEC a high, medium, low, or negligible qualitative exposure ranking for alachlor as well as for other specific chemicals. The exposure rankings considered changes in exposure potential over time resulting from changes in plant technology as documented by standard manufacturing process reports, industrial hygiene and safety reports, a 25-year chronology of the plant's history, and interviews with long-term employees.

The qualitative exposure rankings were based primarily on the opportunity for dermal contact with alachlor. Inhalation exposures were judged to be an extremely minor component of total exposure due to alachlor's extremely low vapor pressure (1.6×10^{-5} mm Hg at 25°C). Current and historical airborne measurements relative to alachlor vapor have averaged less than 10 ppb. The more recent granular and water dispersible alachlor formulations create the possibility of airborne exposure via dusts, but even in these operations airborne measurements have averaged much less than 100 parts per billion.

The qualitative exposure rankings did not discriminate between daily and intermittent exposures for workers with different jobs in the same department/location. Thus, for a given department/location, production and maintenance workers had the same exposure ranking. However, production workers, particularly those in formulation and packaging operations, had more frequent potential for (dermal) alachlor exposure than maintenance workers, except perhaps for the initial year(s) of the alachlor process when exposures were more similar for these two groups.

A source of exposure of uncertain magnitude and duration was contamination of the plant drinking water. The contamination was discovered incidentally in June 1975. While developing a method for measuring alachlor concentrations in water, a "control" sample from the plant's drinking water showed an alachlor concentration of 2 mg/l (2 ppm). Plant management immediately notified workers and brought in bottled drinking water to eliminate exposure. Soon thereafter the plant's water supply was switched to other wells at the plant. Subsequent alachlor measurements from the new wells averaged 8 µg/l (8 ppb) through 1980. At that time, installation of a carbon filtration system was completed, which reduced alachlor in the water supply to below the minimum detection level of 0.03 µg/l.

Workers' exposure to alachlor from drinking water would depend on the duration of the water contamination at the plant and the amount of water consumed on a daily basis. Both aspects of exposure were unknown to us. However, if we assume a constant well water concentration of 2 mg/l and that workers drank 1 l of plant water daily, we estimate that exposure from drinking water would equal that in high-exposure jobs.

In certain analyses we classified all workers employed from 1968 to 1975 in the high-exposure category (to allow for the maximum possible period of drinking water contamination), even if their jobs entailed no occupational exposure. We also conducted analyses based on various more restrictive periods of drinking water contamination to assess the potential impact of misclassification based on drinking water exposure.

We conducted analyses based only on occupational exposures. A relatively small number of workers had exposure only via drinking water, and excluding these workers from the analysis of alachlor-exposed workers did not appreciably affect the results.

Epidemiologic Analysis

The epidemiologic measures of effect for the mortality and incidence analyses were the standardized mortality and incidence ratios (SMR, SIR). These measures were expressed as the ratio of observed to expected events and are equivalent to the ratio of disease rates for workers and the general population adjusted for age, gender, and calendar period. The numbers of expected deaths or incident cases were calculated by summing the product of the number of employee person-years, stratified by age, calendar period, and gender, and rates for the corresponding groups in the Iowa general population. The Occupational Cohort Mortality Analysis Program was used to conduct the SMR and SIR analyses (*18*). The 95% CI was calculated as a measure of the statistical variability of the SMR or SIR. Approximate CI calculations were employed when the number of observed deaths exceeded five; Fisher exact CIs were calculated in the other instances (*19*).

Enumeration of person-years for the mortality and cancer incidence analyses began 1 year after first employment, in light of the 1 year employment eligibility criterion for cohort enumeration, or on the date of first alachlor exposure, if later.

For the mortality analysis, person-years were accumulated through the end of study date for employees found to be alive, until date lost to follow-up, or until date of death for deceased employees. For the cancer incidence analysis, person-years were accumulated until the end of the study period for employees who were alive and residing in Iowa at the end of study date, until date of death for employees who died in Iowa during the study period, until date of migration from Iowa, until date of last contact (usually employment termination date) for employees with unknown residence histories, or until cancer diagnosis date for incident cases.

SMRs and SIRs were evaluated for workers by the number of years of alachlor exposure and by time since first exposure. We dichotomized the analyses at 5 or more years of exposure and at 15 or more years since first exposure, which divided expected numbers approximately evenly on each dimension.

We excluded 26 non-whites from the analyses due to their small numbers. There were no incident cancer cases among these workers (0.1 expected) and all were alive as of the end of study date. We also excluded employees who did not work in alachlor departments and who were not employed at the plant during the 1968–1975 and 1976–1980 drinking water contamination periods. The major non-alachlor departments included ammonia production and storage before 1968, and acrylonitrile-butadiene-styrene plastics production after 1975.

Results

Mortality

A total of 1036 workers met the criteria for inclusion in the mortality analysis and had potential alachlor exposure in manufacturing jobs or via drinking water. Mortality from all causes combined for these workers was lower than Iowa rates for both the total cohort (27 observed, SMR = 0.7, 95% CI, 0.4–1.0) and for those with 5 or more years exposure and 15 years since first exposure (4 observed, SMR = 0.4, 95% CI, 0.1–0.9). Mortality from cancer was similar to Iowa rates (Table 2; 8 observed, SMR = 0.9, 95% CI, 0.4–1.7), and there were slight to moderate deficits of cancer mortality for workers with 5 or more years of exposure (3 observed, SMR = 0.6, 95% CI, 0.1–1.8) and 15 or more years since first exposure (1 observed, SMR = 0.2, 95% CI, 0–1.1). Results were similar for workers with high alachlor exposure (Table 2).

SMRs for specific cancers, heart disease, and accidents are given in Table 3 for workers with high alachlor exposure. There were no deaths due to stomach, thyroid, and nasal cancer (the three tumors observed in the chronic feeding studies of

laboratory rats) versus the small expected values. The six observed cancer deaths were distributed among six different cancer sites, and there were no noteworthy findings for specific cancers. Ischemic heart disease mortality was somewhat less than expected. Mortality from accidents was similar to Iowa rates for the total highly exposed subgroup and for those with 5 or more years of exposure and 15 or more years since first exposure.

Cancer Incidence

A total of 1025 white males and females met the criteria for the cancer incidence analyses and were estimated to have potential exposure to alachlor either in their jobs or via drinking water. Linkage with SHRI identified 37 cancers during the study period, 13 of which were *in situ* carcinomas, mostly cervical (n = 9) and skin (n = 2), and 24 were invasive cancers in 23 individuals. *In situ* cancers were not included in our analyses because SHRI incidence rates are routinely based on invasive cancers (with the exception of bladder cancer) and because population-based ascertainment of *in situ* cancers is questionable, especially for the cervix and skin melanoma.

Over the 1969–1993 study period, cancer incidence was slightly higher for alachlor workers than for the Iowa general population (24 observed, SIR = 1.4, 95% CI, 0.9–2.1; Table 4). SIRs were similarly elevated for workers during active employment (14 observed, 11.1 expected, SIR = 1.3, 95% CI, 0.7–2.1) and after employment termination (10 observed, 6.0 expected, SIR = 1.7, 95% CI, 0.8–3.0), suggesting that employment status was not a factor affecting cancer ascertainment. The cancer SIR varied by duration of exposure and time since first exposure (Table 4). The SIR was elevated for workers with less than 5 years of employment and less than 15 years since first exposure (10 observed, SIR = 1.9, 95% CI, 0.9-3.6). The 10 cancers were varied and included 1 salivary gland, 1 rectum, 1 female breast, 1 cervix, 1 uterus, 1 testis, 1 melanoma, 2 Hodgkin's disease, and 1 chronic myeloid leukemia (CML). Workers with 5 or more years of exposure (13 observed, SIR = 1.3, 95% CI, 0.7–2.2) and workers with 15 or more years since first exposure (9 observed, SIR = 1.2, 95% CI, 0.6–2.3) had cancer incidence similar to expected values. During the 1991–1993 update period, there were 6 observed and 5.3 expected cancers (SIR = 1.1, 95% CI, 0.4–2.5).

Of the 1025 alachlor workers, 701 (68%) were classified as having the potential for high exposures. These high exposures included occupational exposures and

Table 2. Standardized mortality ratios (SMRs) for all cancer for employees with potential alachlor exposure (workplace and drinking water)

Duration of exposure/ time since first exposure	No. of workers[a]	Person-years[a]	O/E deaths[b]	SMR	95% CI
All alachlor exposed workers					
<5 years/<15 years	1,036	8,774	4/2.6	1.5	0.4–3.9
<5 years/15+ years	336	1,687	1/1.8	0.6	0–3.1
5+ years/<15 years	485	4,452	3/1.9	1.6	0.3–4.7
5+ years/15+ years	434	2,488	0/3.0	0	0–1.3
Total	1,036	17,400	8/9.3	0.9	0.4–1.7
Workers with high alachlor exposure					
<5 years/<15 years	708	8,249	3/2.7	1.1	0.2–3.3
<5 years/15+ years	520	2,565	1/2.3	0.4	0–2.4
5+ years/< 15 years	159	1,553	2/0.8	2.5	0.3–9.0
5+ years/15+ years	160	1,445	0/2.3	0	0–1.6
Total	708	13,812	6/8.1	0.7	0.3–1.6

[a]Number of workers not mutually exclusive across groups, though person-years are.
[b]Observed number of deaths/expected number of deaths.

Table 3. Standardized mortality ratios (SMRs) for various causes of death for employees with potential high alachlor exposure (workplace and drinking water)[a]

	Total[b]			5+ years exposure; 15+ years since first exposure[c]		
Cause of death (ICD 8)	O/E[d]	SMR	95% CI	O/E	SMR	95% CI
All causes (0–999)	23/34.4	0.7	0.4–1.0	4/7.6	0.5	0.1–1.4
All cancers (140–209)	6/8.1	0.7	0.3–1.6	0/2.3	0	0–1.6
Stomach cancer (151)	0/0.2	—	—	0/0.1	—	—
Thyroid cancer (193)	0/0.04	—	—	0/0.01	—	—
Lung cancer (162)	1/2.3	0.4	0–2.4	0/0.8	—	—
Colorectal cancer (153,154)	0/0.8	—	—	0/0.3	—	—
Breast cancer (174)	0/0.3	—	—	0/0.1	—	—
Prostate cancer (185)	0/0.2	—	—	0/0.1	—	—
Kidney cancer (189)	0/0.2	—	—	0/0.1	—	—
Leukemia (204–207)	1/0.4	—	—	0/0.1	—	—
Brain cancer (191, 192)	0/0.6	—	—	0/0.1	—	—
Hodgkin's disease (201)	0/0.2	—	—	0/0.02	—	—
Melanoma (172)	0/0.3	—	—	0/0.1	—	—
Ischemic heart disease (410–3)	4/7.1	0.6	0.2–1.4	1/2.1	0.5	0–2.6
Accidents (800–949)	8/7.2	1.1	0.5–2.2	0/0.6	—	—

[a]SIRs and 95% CIs were not calculated unless there were at least two observed or expected deaths.
[b]708 workers, 13,811 person years.
[c]160 workers, 1,652 person years.
[d]Observed number of cases/expected number of cases.

Table 4. Standardized incidence ratios (SIRs) for all cancer for employees with potential alachlor exposure (workplace and drinking water)

Duration of exposure/ time since first exposure	No. of workers[a]	Person-years[a]	O/E[b]	SIR	95% CI
All alachlor-exposed workers					
<5 years/<15 years	1,024[c]	6,585	10/5.2	1.9	0.9–3.6
<5 years/15+ years	193	871	1/1.9	0.5	0–3.0
5+ years/<15 years	481	4,122	5/4.6	1.1	0.3–2.5
5+ years/15+ years	383	2,076	8/5.5	1.5	0.6–2.9
Total	1,025	13,654	24/17.1	1.4	0.9–2.1
Workers with high alachlor exposure					
<5 years/<15 years	700[d]	6,787	6/5.8	1.0	0.4–2.3
<5 years/15+ years	387	1,676	5/3.3	1.5	0.5–3.5
5+ years/<15 years	159	1,455	3/1.7	1.8	0.4–5.3
5+ years/15+ years	138	1,179	4/3.8	1.0	0.3–2.7
Total	701	11,097	18/14.6	1.2	0.7–2.0

[a]No. of workers not mutually exclusive across groups, though person-years are.
[b]Observed number of cases/expected number of cases.
[c]One worker moved into the Iowa study area after achieving either 5 years of exposure or 15 years since first exposure.
[d]Two workers moved into the Iowa study area after achieving either 5 years of high exposure or 15 years since first high exposure.

presumed drinking water exposures during the 1968–1975 period. Cancer incidence was fairly similar for these workers and the Iowa population (18 observed, SIR = 1.2, 95% CI, 0.7–2.0) (Table 4). Analyses that considered only 1974–1975 as the period of drinking water exposure gave similar results (17 observed, SIR = 1.3, 95% CI, 0.8–2.1). Workers exposed 5 or more years with at least 15 years since first exposure had 4 observed and 3.8 expected cancers (SIR = 1.0, 95% CI, 0.3–2.7).

Results for specific cancers for workers with high exposure showed no observed cases or 1 case for most sites and elevated SIRs for colorectal cancer, chronic myeloid leukemia (CML), Hodgkin's disease, and melanoma based on 3, 2, 2, and 2 cases, respectively (Table 5). One of the CML cases was diagnosed soon after first employment at the plant, which, given the course of CML, indicates etiologic factors before employment at the plant. Among workers with 5 or more years of exposure, there were no cases of CML or Hodgkin's disease, 1 case of melanoma (0.2 expected), and 2 colorectal cancer cases (SIR 3.9, 95% CI, 0.5–14.2). The results were similar for all alachlor-exposed workers.

We did a further analysis of cancer incidence focusing on 429 alachlor production workers. Our definition of production workers allowed for a maximum of 90 days in maintenance jobs. Many of these workers were employed in formulation and packaging operations, where there was potential for high dermal exposure on a daily basis during the early years of production. Among workers with less than 5 years exposure, there were 7 observed versus 4.5 expected cancers (SIR = 1.6, 95% CI, 0.6–3.2), while for workers with 5 or more years exposure, there were 2 observed versus 2.2 expected cancers (SIR = 0.9, 95% CI, 0.1–3.3). Overall, there were no observed cases of colorectal cancer versus 0.6 expected, 1 case of CML versus 0.1 expected, and no cases of malignant melanoma versus 0.6 expected.

Table 5. Standardized incidence ratios (SIRs) for various cancers for employees with potential high alachlor exposure (work place and drinking water)[a]

	Total[b]			5+ years exposure; 15+ years since first exposure[c]		
Cancer site/type (ICD-0–2 codes)	O/E[d]	SIR	95% CI	O/E	SIR	95% CI
All cancers	18/14.6	1.2	0.7–1.9	4/3.8	1.0	0.3–2.7
Lung (C339–49)	1/1.9	—	—	1/0.8	—	—
Colorectal (C180–9,C260,C199,C209,C210–8)	3/1.6	1.9	0.4–5.6	2/0.6	3.9	0.5–14.2
Breast (C500–9)	1/1.2	—	—	0/0.2	—	—
Prostate (C619)	0/0.7	—	—	0/0.3	—	—
Kidney (C649)	0/0.5	—	—	0/0.2	—	—
Bladder (C670–679)	0/0.7	—	—	0/0.2	—	—
Hodgkin's disease (M9650–9667)	1/0.5	—	—	0/0.04	—	—
Non-Hodgkin's lymphoma (M9590–5,M9670–9714)	2/0.8	2.4	0.3–8.8	0/0.2	—	—
Chronic myeloid leukemia (M9863,M9868)	2/0.1	18.6	2.3–67.2	0/0.02	—	—
Other leukemias (M9800–9941, excluding M9863,9868)	0/0.4	—	—	0/0.1	—	—
Testes (C620–29)	1/0.8	—	—	0/0.1	—	—
Melanoma (C440–9, M8720–90 only)	2/1.1	1.9	0.2–6.7	1/0.2	—	—

[a]SIRs and 95% CIs were not calculated unless there were at least two observed or expected cases.
[b]701 workers, 11,097 person years.
[c]138 workers, 1,179 person years.
[d]Observed number of cases/expected number of cases.

Discussion

The purpose of this study was to monitor patterns of cancer mortality and incidence for alachlor workers, especially for cancer sites seen in chronic feeding studies of rats, and to follow-up on the slight colorectal cancer excess seen in the previous incidence study (*14*). We did not see a relationship between cancer incidence and years of alachlor exposure or time since first exposure, and there were no cancers of the thyroid, stomach, or nose and nasal sinuses among exposed workers. The numbers of observed and expected cases were small for most cancer sites, which makes the SMRs and SIRs imprecise and precludes informative exposure–response analyses for individual cancer sites.

There were no new colorectal cancer cases during the update period versus 0.6 expected, lessening the observed/expected ratio previously reported (*14*). This observation, in conjunction with the lack of any cases among workers in formulation and packaging and the minor involvement of the large bowel in alachlor metabolism and excretion, tends to support a noncausal interpretation of the colorectal cancer findings for this cohort. Further follow-up of these workers will be important to monitor incidence from colorectal and other cancers.

The major limitation of this study is the small numbers of incident cancers and cancer deaths. The cohort is still relatively young (74% of person years under observation were less than 40 years of age), and the follow-up period is relatively short. In terms of power, the study had more than 80% power to detect a relative risk of 2.0 for all cancers, but the power for major individual cancer sites would exceed 80% only for relative risks of 5 or higher (*20*).

A second limitation is the possibility of exposure misclassification due to the difficulty in estimating dermal occupational exposures, for which there is no accepted methodology even today, and exposures from plant drinking water. Exposure estimation, however, is more straightforward for these workers than for agricultural populations because the plant manufacturing history is well documented, there is a long standing industrial hygiene program, and work history records documenting departmental assignments and workers' jobs were fairly complete.

Despite the limitations of this study, the findings are useful for assessing potential alachlor-related health risks. The exposure circumstances for this manufacturing cohort are unique among alachlor-exposed workers, the vast majority of whom are involved in agricultural applications for a few days or weeks each year. It has been estimated that the relatively high daily exposures characteristic of early manufacturing operations exceed exposures in agriculture by several orders of magnitude (*8*). If this is true, then this study has exposure weighted years of observation equivalent to an extremely large study of agricultural workers. Periodic follow-ups of this cohort, in conjunction with an on-going, large, prospective study of farmers and applicators (*2*), should provide the most comprehensive assessment possible of potential health risks for workers with various levels of alachlor exposure. At present, however, the available data from manufacturing workers do not indicate an appreciable hazard during the study period related to alachlor exposure.

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Dioxinlike Properties of a Trichloroethylene Combustion-Generated Aerosol

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Conventional chemical analyses of incineration by-products identify compounds of known toxicity but often fail to indicate the presence of other chemicals that may pose health risks. In a previous report, extracts from soot aerosols formed during incomplete combustion of trichloroethylene (TCE) and pyrolysis of plastics exhibited a dioxinlike response when subjected to a keratinocyte assay. To verify this dioxinlike effect, the complete extract, its polar and nonpolar fractions, some containing primarily halogenated aromatic hydrocarbons, were evaluated for toxicity using an embryo assay, for antiestrogenicity using primary liver cell cultures, and for the ability to transform the aryl hydrocarbon receptor into its DNA binding form using liver cytosol in a gel retardation assay. Each of these assays detect dioxinlike effects. Medaka (*Oryzias latipes*) embryos and primary liver cell cultures of rainbow trout (*Oncorhynchus mykiss*) were exposed to concentrations of extract ranging from 0.05 to 45 µg/l. Cardiotoxicity with pericardial, yolk sac, and adjacent peritoneal edema occurred after exposure of embryos to concentrations of 7 µg/l or greater. These same exposure levels were associated with abnormal embryo development and, at the higher concentrations, death. Some of the fractions were toxic but none was as toxic as the whole extract. In liver cells, total cellular protein and cellular lactate dehydrogenase activity were not altered by *in vitro* exposure to whole extract (0.05–25 µg/l). However, induction of cytochrome P4501A1 protein and ethoxyresorufin *O*-deethylase activity occurred. In the presence of whole extract, estradiol-dependent vitellogenin synthesis was reduced. Of the fractions, only fraction 1 (nonpolar) showed a similar trend, although vitellogenin synthesis inhibition was not significant. The soot extract and fractions bound to the Ah receptor and showed a significantly positive result in the gel retardation/DNA binding test. Chemical analyses using GC-MS with detection limits for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and dibenzofuran in the picomole range did not show presence of these compounds. Our results indicate that other chemicals associated with TCE combustion and not originally targeted for analysis may also pose health risks through dioxinlike mechanisms. *Key words*: Ah receptor, antiestrogen, complex mixture, dioxinlike toxicity, dioxin-response element binding, embryo/cardiovascular toxicity, incomplete combustion by-products, liver, trichloroethylene, vitellogenin. *Environ Health Perspect* 104:734–743 (1996)

Incineration has been widely used as a means for disposal of municipal, hospital, and industrial hazardous wastes. Its use has been curtailed in recent years because of concern about the emission of toxic by-products associated with the soot particles, especially chlorinated phenols, aromatic hydrocarbons, polychlorinated dibenzodioxins, and dibenzofurans (*1–3*). These emissions arise from improper operation of incinerators or from transients (*4–6*) in operation during which inadequate temperature and mixing conditions in the combustion zone may lead to incomplete combustion. These transient discharges, also known as puffs, are characterized by large transient emissions of soot and toxic volatile organic hydrocarbons (*4–7*). Although they are relatively rare during incinerator operation, puffs contribute a major fraction of the toxic compounds in incinerator effluent. For example, Wendt (*3*) demonstrated in a toluene-fed kiln that puffs can emit approximately 10,000 ppm of hydrocarbons for a period of about 20 seconds. Depending on the precursor chemistry, additional reactions downstream of the high temperature regions may lead to the formation of dioxins (*8*). Atmospheric transport of incinerator emissions may result in wide-spread dispersal and subsequent deposition of these particles in various environmental matrices (*9*) including soil, water, and vegetation (*10*).

Dioxin and dioxinlike compounds constitute a diverse and important group of contaminants widely spread in the environment, where they persist as complex mixtures (*7,11,12*). One particular compound, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has been the subject of considerable concern with regard to incinerator emissions. TCDD and related halogenated aromatic hydrocarbons, including 2,3,7,8-tetrachlorodibenzofuran (TCDF), produce a wide variety of species- and tissue-specific toxic and biological effects, such as teratogenesis, immunotoxicity, hepatotoxicity, tumor promotion, and induction of numerous enzymes, including microsomal cytochrome P4501A1 (CYP1A1) (*7,13*).

Many hazardous waste sites contain chlorinated solvents, including trichloroethylene (TCE). For example, the McClellan Air Force Base in Sacramento (California) contains soil that is heavily contaminated by TCE; it was used as a cleaning agent on aircraft. Earlier experiments by Blankenship et al. (*14*) found that extracts from soot aerosols formed during the combustion of TCE exhibited a dioxinlike response when subjected to a keratinocyte bioassay. These experiments showed that all of the hazardous material was associated with the aerosol and that little was found in the gas phase of the flames. Chemical analyses of the soot extracts indicated that, at picomole levels, TCDD/TCDF were not detected, suggesting that chlorinated fulvalenes, among other chlorinated hydrocarbons, were major components of the mixture and that these may have been responsible for the toxic response. Because of its environmental importance and in view of the previous experience with toxic TCE aerosols, TCE was chosen as the model waste for this study.

Although conventional chemical analyses of incineration by-products identify compounds of known toxicity, they often fail to indicate the presence of other chemicals which may also pose health risks. The purpose of the present investigation was to verify whether materials with dioxinlike properties were present in the chemically

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Trout-specific antibodies and standards were gifts from Michael Miller, West Virginia University, and Ray Simon, formerly of the U.S. Fish Health Center, Kearneysville, West Virginia. Anti-scup CYP1A1 (Mab 1-12-3) was a gift of John Stegeman, Woods Hole Oceanographic Institution. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin was obtained from S. Safe, Texas A&M University. A.V. thanks Miguel González-Doncel and Swee Teh for their assistance in the histological preparations and evaluations. This research was supported by the NIEHS Superfund Basic Research Program (P42ESO4699), by the Ecotoxicology Program of the University of California Toxic Substances Research and Teaching Program, and by the US EPA-UC Davis Center for Ecological Health Research (R819658).
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complex TCE soot mixture and its fractions. Dioxinlike effects (e.g., cardiotoxicity and yolk sac edema) have been investigated in medaka (*Oryzias latipes*) embryos. Biological potency has been demonstrated *in vitro* both by measuring interference of compounds from the mixture with estrogen receptor using rainbow trout (*Oncorhynchus mykiss*) liver cells and by monitoring the mixture's binding affinity to the Ah receptor and further ability to convert it into its DNA binding form.

Methods

Flame Conditions and Chemical Analysis

Unmixed or poorly mixed combustion can be modeled in a well-defined laboratory experiment with a laminar diffusion flame. Poor mixing with relatively long residence times in an incinerator is then modeled by increasing the flame length beyond the point at which soot breaks through the flame tip. The nature of the compounds that are emitted from these flames is typical of the material that could be found in puffs from incinerators.

A mixture of TCE and methane (CH_4) was burned in a laminar diffusion flame. TCE vapor was generated by passing CH_4 through an impinger containing liquid TCE that was maintained at a constant temperature. The mole fraction of TCE in the methane was 0.51; the flow rates were 696 ml/min of CH_4 and 734 ml/min of TCE. This mixture was supplied to an axisymmetric laminar diffusion flame burner. The co-flow burner assembly consisted of a circular Plexiglas chamber with a 67-mm inside diameter. The round nozzle was made of thin-walled stainless steel tubing with a 6-mm outside diameter. Soot was collected from the post-flame gases with a 47-mm PTFE-coated glass fiber filter in line with a sorbent tube.

The sorbent tube was prepared by packing 100 mm lengths of Pyrex glass tubing (12 mm O.D.) with 3.5 g of Carbotrap C. Glass wool plugs were inserted into both ends. The filters were Soxhlet extracted for 16 hr with 250 ml of dichloromethane (CH_2Cl_2) using anhydrous sodium carbonate (Na_2CO_3) to neutralize adsorbed acids. CH_2Cl_2 extracts were roto-evaporated to a volume of 10 ml, divided into 10 aliquots, and stored at - 20°C. Each aliquot was dry-evaporated under a stream of nitrogen at 25°C and reconstituted in 1 ml of analytical grade dimethylsulfoxide (DMSO) for bioassays.

An individual aliquot was applied to a silica gel column and four fractions were eluted with different solvents including fraction 1 (nonpolar compounds) with *n*-hexane, fraction 2 (primarily PAHs and chlorinated PAHs) with *n*-hexane/CH_2Cl_2 (3:2 v:v), fraction 3 (intermediate polarity) with CH_2Cl_2, and fraction 4 (polar compounds) with methanol. Control fractions, prepared by Soxhlet extractions of blank cellulose extraction thimbles, were obtained using identical laboratory procedures.

Analyses were performed on extracts and fractions using a VG Trio-2 mass spectrometer coupled to a Hewlett Packard 5890 gas chromatograph. Separations were performed using a 30-m DB-17 capillary column with helium as carrier gas. Electron ionization (70 eV) mass spectra were obtained; compounds were quantified based upon average molar response factors obtained for a series of PAHs and chlorinated aromatic standards.

Embryo Toxicity Assay

Egg collection and broodstock maintenance followed the procedure described by Marty et al. (*15*). Medaka female broodstock, maintained at 25°C under a 16 hr light:8 hr dark photoperiod stimulating continuous egg production, were individually netted and eggs <5 hr old were carefully removed from extruded clusters. Filaments that attached adjacent eggs were broken by gently rolling clusters between moistened finger tips. Individual (blastula stage) eggs were kept in continuously aerated embryo rearing medium (ERM) (*16*).

Embryo exposures were repeated until the whole TCE soot extract and individual fractions were tested. Each exposure was conducted as a completely randomized design (*17*) which consisted in pooling eggs and distributing them (n = 8) by stratified random assortment to individual 20 ml borosilicate vials (Fisher Scientific, Pittsburgh, Pennsylvania) in each of four replicates. Each vial contained 2 ml of solution and 18 ml of air space. A double layer of teflon tape (Scientific Instruments, Randallstown, Maryland) and screw-type lid were used to hermetically seal each vial. For each experiment, vials were coded for blind study except for one additional ERM replicate (known control, not included in statistical analysis), which served as a reference for time of normal development. Due to the hazardous nature of this complex mixture and to the blind randomized experimental design, embryos were maintained in vials under static (non-renewal) conditions for duration of embryonic development (8 days). After exposure and rinsing in clean ERM, embryos were transferred to clean vials and allowed to complete their development. Static non-renewal conditions have been used when testing dioxin, dioxin-like compounds, and other complex mixtures (*17–20*). Oxygen requirements during medaka development in a closed system (no access to free air), are approximately 23 ml of ERM/egg (*21*). Since dissolved oxygen in air is 25–30 times greater than in ERM, sufficient aeration was provided given the eggs:ERM/eggs:air ratio.

For exposure, soot whole extract (WE) and fraction stock solutions were dissolved in ERM (pH 7 ± 0.2) using DMSO (WE) or DMSO/fraction as vehicle solvents. All vehicle concentrations were restricted to 500 µl/l (0.05% v/v). This concentration has shown in pilot tests to produce no embryonic toxicity. Estimated maximum concentration of incomplete combustion by-products was 0.09 µg/µl of vehicle (i.e, 500 µl/l × 0.09 µg/µl = 45 µg/l). The range of interest in these pilot studies was determined between a stock solution of 500 µl carrier (containing WE soot) in 1 liter ERM, and a respective dilution of 1:100 (1 ml of stock in 100 ml ERM). The intermediate concentrations were chosen so that there could be 5 equidistant intervals in a log scale, based on the absolute difference (2.0) between log 45 µg/l, and log 0.45 µg/l. This conversion resulted in intervals of 0.4 log units, which when reconverted (antilog) to a linear scale gave concentrations of 45, 18, 7.2, 2.7, 0.9, and 0.45 µg/l. Controls consisted of embryos exposed to vehicle or ERM alone.

Embryos were observed daily under a dissecting microscope for normal and abnormal development. Mortality and sublethal endpoints including pericardial and peritoneal edema, eye and/or subdermal edema, hemostasis, yolk resorption, cephalic and spinal deformities, and hatching success were observed. The transparent chorion of medaka embryonated eggs permits direct visualization of heart beat. Cardiac activity was monitored by averaging heart rate (in beats per minute ± SD) of at least three embryos per vial. This monitoring was done daily until hatching. Evaluation was continued through the first 4–5 days after hatching. Development, including swim (or air) bladder inflation and swimming activity, was monitored. A hatchling was considered normal if it swam vigorously, and had normal gross morphology and an inflated swim bladder. Medaka hatchlings inflate swim bladders within 24 hr (*15*). To confirm and extend observations with the dissecting microscope, a limited number of normal and abnormal embryos/larvae were fixed in 10% buffered formalin, dehydrated in a graded ethanol series and embedded in complete glycolmethacrylate monomer (*22*). Sections (4 µm thickness) were cut on an LKB Historange microtome, mounted to glass slides, and stained with hematoxylin and eosin (H&E) or toluidine blue.

Serial sectioning was performed to validate locations within a given embryo/larva.

For statistical purposes, all embryos that failed to hatch were considered abnormal. Differences from the controls were identified with Wilcoxon's sign-rank test ($p<0.05$), using the JMP statistical software package (SAS Institute, Cary, North Carolina). The additional ERM replicate was excluded from statistical calculations.

Liver Cell Assays

Sexually immature male and female rainbow trout (400–600 g mean weight) from Mt. Lassen trout farm (Red Bluff, California) were housed in a large (4 × 1.7 × 1 m) concrete tank at the Institute of Ecology aquaculture facility at UC-Davis. Gonadosomatic indices (gonad weight/body weight × 100) ranged between 0.25 and 0.75%. Fish were held under natural photoperiod in constant flow (Lake Berryessa, California) water at temperatures between 14 and 15°C and fed Silver Cup trout pellets at approximately 1% body weight/day. Fish were acclimated to the above holding conditions at least 2 weeks before experimentation.

Medium 199, L-glutamine, antibiotic–antimycotic solution, buffer salts, anti-rabbit IgG alkaline phosphatase conjugated antibodies, *p*-nitrophenyl phosphate (PNPP), pyruvate, NADH, and NADPH were purchased from Sigma (St. Louis, Missouri). 17β-Estradiol was purchased from Steraloids (Wilton, New Hampshire). Anti-mouse IgG horseradish peroxidase-conjugated antibody was purchased from Amersham (Arlington Heights, Illinois). Tween 20, enzyme immunoassay grade nonfat dry milk, and 3,3′,5,5′-tetramethylbenzidine (TMB) solution were purchased from Bio-Rad (Burlingame, California). Diethanolamine was purchased from Aldrich (Milwaukee, Wisconsin), collagenase (269 U/mg) from Worthington Biochemicals (Newark, New Jersey), and 7-ethoxyresorufin and resorufin from Molecular Probes (Eugene, Oregon). All other chemicals were of analytical grade.

Cells were isolated following a two-step perfusion technique (*23*) with the following modifications: no heparin was injected into the animals and the perfusion medium was a calcium-free HEPES buffered Hank's salt solution, pH 7.6 (*24*). Following liver digestion and tissue disassociation, cells were washed two times and resuspended in medium 199 (see below). Viability was assessed by phase microscopy and trypan blue dye exclusion. Typically 90% or more of the cells were viable.

Cell cultures followed procedures of Pesonen and Andersson (*25*) with one exception: HEPES buffered medium 199 at pH 7.6 contained no additional Na_2HPO_4 because high concentrations caused precipitation and interfered with the ELISA assays. Cells were plated on 60- or 100-mm diameter Falcon polystyrene tissue culture dishes (Beckton Dickinson, Oxnard, California) at a concentration of approximately 1.65×10^5 cells/cm^2 and placed in a humidified Ambi-Hi-Low incubator (Baxter, McGaw Park, Illinois) at 15°C in air atmosphere.

Cells were allowed to attach to tissue culture dishes and acclimate to culture conditions for 24 hr before the first media change and dosing. Cells were then treated with fresh medium 199 containing either DMSO alone (control), WE (0.6–25 µg/l), or each of the fractions (in DMSO). Due to the use of 4 fractions and testing of each with cells from a single trout, a single concentration (11.25 µg/l) was used. The total concentration of DMSO in the media was maintained at 0.05% (v/v) as described above. Simultaneously, 1 µM 17β-estradiol or an equivalent volume of ethanol (carrier control) was added to the medium. Cells from control and treatment groups were always obtained from the same fish.

After 48 hr of exposure, cells were gently scraped off the dishes with a teflon rod and placed in individual centrifuge tubes. Tubes were centrifuged at 150*g* for 2 min at 4°C to separate media from cells. Resultant cell pellet was resuspended in 1 ml of 0.1 M phosphate buffer, pH 7.5 (80mM Na_2HPO_4, 20 mM NaH_2PO_4) with 20% glycerol and sonicated for 5 sec on ice. Cell homogenates and media were immediately frozen on dry ice and stored at -80°C until assays were performed.

Determinations of vitellogenin (Vg) and albumin (Alb) released into the cell culture media and cellular CYP1A1 content were estimated by indirect ELISA as described (*26,27*) using monoclonal (MAb) anti-trout Vg (MAb SD6C) (*28*), polyclonal rabbit anti-trout Alb, and anti-scup CYP1A1 (MAb

Table 1. List of the major incomplete combustion by-products present in trichloroethylene whole extract

Retention time (min)	Monoisotopic m/z	Tentative identification	% of TIC area[a]
17.17	248	C_6HCl_5, pentachlorobenzene	5.1
20.37	282	C_6Cl_6, hexachlorofulvene	1.0
20.75	282	C_6Cl_6, hexachlorobenzene	12.3
21.32	272	C_8HCl_5	0.5
21.62	296	$C_7H_2Cl_6$, heptachlorobicyclo-[2.2.1]hepta-2,5-diene	0.5
21.85	310	$C_8H_4Cl_6$	1.6
22.15	296	$C_7H_2Cl_6$, heptachlorobicyclo-[2.2.1]hepta-2,5-diene	0.7
23.08	342	C_8HCl_7	0.9
24.13	306	C_8Cl_6	1.0
24.23	342	C_8HCl_7	1.0
24.55	330/264	$C_7HCl_7/C_{10}H_4Cl_4$	0.9
25.62	330	C_7HCl_7	1.4
25.85	322	$C_9H_4Cl_6$	0.6
26.20	376	C_8Cl_8	3.6
27.08	300/376	$C_{10}H_5Cl_5/C_8Cl_8$	1.2
27.23	298	$C_{10}H_3Cl_5$	2.1
27.55	300	$C_{10}H_5Cl_5$	0.8
27.67	300	$C_{10}H_5Cl_5$	1.0
27.90	298	$C_{10}H_3Cl_5$	0.9
28.17	298	$C_{10}H_3Cl_5$	0.6
29.58	298	$C_{10}H_3Cl_5$	0.9
30.92	334	$C_{10}H_4Cl_6$	3.2
31.05	334	$C_{10}H_4Cl_6$	2.3
32.07	332	$C_{10}H_2Cl_6$	1.4
33.02	332	$C_{10}H_2Cl_6$	1.0
33.40	332	$C_{10}H_2Cl_6$	1.6
36.23	366	$C_{10}HCl_7$	5.5
37.02	366	$C_{10}HCl_7$	3.7
43.30	400	$C_{10}Cl_8$	5.2
45.15	390	$C_{12}HCl_7$	0.8

[a]Listed peaks account for 63% of area of total ion chromatogram (TIC), with $C_{10}H_xCl_{8-x}$ compounds representing ~30%. The remaining area is distributed among at least 200 smaller peaks.

1-12-3). Dilutions of media or cell extracts (10–100-fold) in phosphate buffered saline, pH 7.5 (PBS: 80mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl) were used.

Ethoxyresorufin *O*-deethylase (EROD) activity of whole cell homogenates followed method of Burke et al. (*29*) adapted for microplate format (Cambridge microtiter plate fluorometer, model 7620). Briefly, fluorescence (excitation 530 nm and emission 585 nm) in 80–100 µg of whole cell homogenates, incubated in 100 mM potassium phosphate buffer, pH 8.0 (90 mM K_2HPO_4, 10 mM KH_2PO_4, 0.25 µM ethoxyresorufin, and 0.5 mM NADPH) to a final reaction volume of 0.2 ml, were recorded at 30–40 sec intervals over 5 min at 24°C. Determinations of cellular lactic dehydrogenase (LDH) activity were made following the method of Bergmeyer and Berndt (*30*). Protein concentrations of cell homogenates were determined using the Bio-Rad DC protein assay kit, with bovine serum albumin (BSA) as the standard.

Each exposure group of liver cells consisted of three to four dishes per treatment, with duplicate determinations per dish. Significant differences between means of various treatment groups were determined by ANOVA ($p<0.05$) and means were contrasted using Dunnett with control group and Tukey-Kramer methods. All statistical analyses were performed using the JMP procedure of SAS software (SAS Institute).

Gel Retardation/DNA Binding Assay

Based on the ability of Ah receptor (AhR) ligands to convert this receptor to its DNA binding form, a gel retardation assay was used to measure the amount of inducible protein [^{32}P]DNA-complex. This provided an indirect way to detect dioxinlike chemical(s). Guinea pig hepatic cytosol was used as the source for the receptor, based on previous determinations which indicated that this species is the most optimal for the transformation and DNA binding analyses of ligand:AhR complexes (*31*).

In the assay, hepatic cytosol prepared from male Hartley guinea pigs (250–300 g; Michigan Department of Public Health, Lansing, MI), was suspended in ice-cold HEDG buffer (25 mM HEPES, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 10% (v/v) glycerol) and aliquots were stored at -80°C as previously described (*32,33*). Protein concentrations were measured by the method of Bradford (*34*) using BSA as the standard. For gel retardation analysis, 125 µl cytosol (16 mg of protein/ml) was incubated with DMSO (20 µl/ml), 15 nM TCDD in DMSO or an aliquot (2.5 µl) of the soot WE or fractions (in DMSO) for 2 hr at 20°C. Gel retardation analysis of the samples was carried out using [^{32}P]-labeled dioxin-responsive element (DRE)-containing DNA oligonucleotide as described by Helferich and Denison (*33*) and the resulting protein–DNA complexes were detected following autoradiography of dried gels. Quantitation of the inducible protein–DNA complex was carried out as described by Denison and Yao (*32*). The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was obtained from S. Safe (Texas A&M University) and [γ-^{32}P] ATP (6,000 Ci/mmol) from New England Nuclear. Molecular biological reagents were obtained from New England Biolabs.

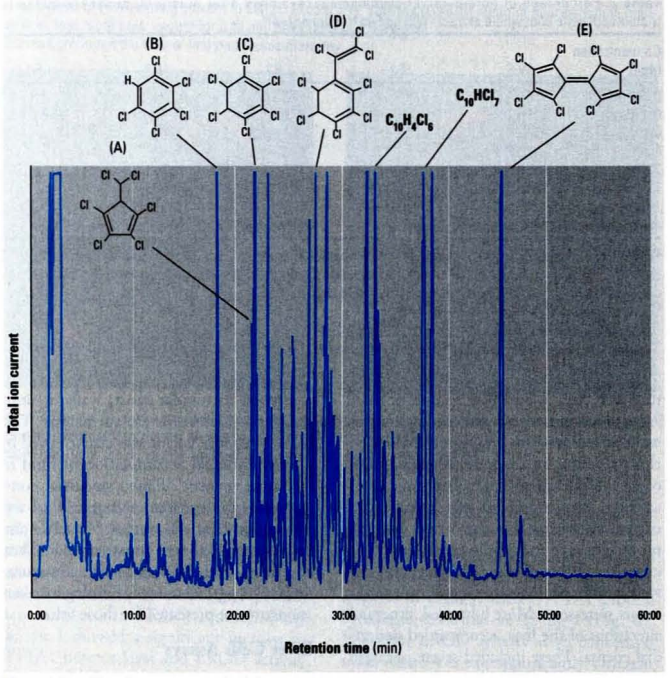

Figure 1. Total ion chromatogram of trichloroethylene soot whole extract. More than 250 incomplete combustion by-products were formed during pyrolysis. (A) Hexachlorofulvene, (B) pentachlorobenzene, (C) hexachlorobenzene, (D) octachlorostyrene, (E) octachlorofulvalene.

Results

Analytical Chemistry

Combustion of the TCE/CH_4 mixture produced a flame characterized by heavy soot production, approximately 100 mg/g of fuel burned. CH_2Cl_2 extracts of the soot had a dark blue color, which may be attributed to the presence of significant amounts of chlorinated fulvalenes ($C_{10}H_xCl_{8-x}$, depending on number of H and Cl substitutions, or $C_{10}Cl_8$ = octachlorofulvalene), structural isomers of naphthalenes (*14*). $C_{10}H_xCl_{8-x}$ compounds represented ~30% of the total ion chromatogram (Table 1). GC/MS analysis of the WE indicated that over 250 organics (Fig. 1) were formed during TCE pyrolysis. Nearly all were chlorinated mono- and polyunsaturated aliphatics, cyclic polyenes 1-, 2-, and 3-ring aromatics, phenols, fulvenes (structural isomers of benzene), and the above mentioned fulvalenes. With a limit of detection of about 1 pM in the extract, no polychlorinated biphenyls (2,3,7,8-TCDD or TCDF) were detected.

Embryo Toxicity Assay

The combined percentage of normal development for all controls was above 90 (Table 2). Greatest toxicity was seen after exposure to WE (Table 2 and Fig. 2). Based on the nominal concentrations previously estimated, the observed WE concentration in which 50% of larvae (EC_{50}) showed signs of abnormality was 7.2 µg/l, while at 2.7 µg/l, no effect was observed. The calculated EC_{50} was ~4.3 µg/l (y = -51.64 log x + 136.75, r^2 = 0.92). The two higher WE concentrations proved lethal to most embryos. The few embryos that hatched were extremely weak and did not inflate swim bladders. Statistical analyses (Wilcoxon's sign-rank test) showed

significant differences at and above 7.2 µg/l. The toxicity trend observed after exposure to the individual fractions indicated that fractions 3 and 1 were the most toxic, while fractions 2 and 4 had no significant effects. However, none of the toxic fractions were as toxic as WE. For fraction 1, approximately 40% of embryos exposed to 45 µg/l developed abnormally, while lower concentrations showed variable effects (Tables 2 and 3; Fig. 2). Results with fractions 2 and 4 were similar: no more than 29% of the exposed embryos developed abnormally regardless of concentration (Table 2). Fraction 3 was slightly more toxic than WE over the range of 0.45–2.7 µg/l, and became less toxic at higher concentrations (approximately 65% and 50% of embryos exposed to 18 and 45 µg/l were abnormal) (Tables 2 and 3; Fig. 2).

The predominant embryonic defect was edema, pronounced in pericardial cavity but also present in the peritoneal cavity and yolk sac (Table 3 and Fig. 3). Embryonic mortality was rarely seen. Within the first 6 days of exposure, 13 (1%) out of a combined total of 1280 embryos died. Of these, only 6 (4 deaths in 48 hr or less and 2 delayed hatchings) were observed in controls. During these first 6 days, no symptoms of cardiovascular toxicity (i.e., bradycardia or tachycardia) that would indicate formation of edema were apparent (data not shown). Two to four days later, depending on concentration, mild pericardial edema appeared and progressed rapidly, often leading to death before hatching (Fig. 3). In these severely affected embryos, the process of heart chamber formation observed as a shunt of blood from left to right was apparently terminated, and a pulsatile single tube had appeared in individual fish who had earlier shown evidence of more developed heart formation. Other lesions included hemostasis, a severe darkening over brain, and larger than normal yolk sac. Cephalic/spinal abnormalities were rare (<0.5%). The highest concentration of WE compatible with control hatch frequency was 7.2 µg/l. Fifty percent of hatchlings exposed to this concentration showed normal structure and were able to inflate swim bladders and move about. The remainder could not inflate swim bladders; edemas became more severe, often extending from pericardial and peritoneal/yolk sac areas to the eyes (Fig. 4). Finally, these hatchlings could not swim or maintain equilibrium.

In embryos showing no evidence of gross alterations, light microscopy revealed additional lesions. The lower concentrations of WE (0.45 and 0.90 µg/l) caused no apparent lesions, but 2.7 µg/l was associated with mild hepatocyte glycogen depletion in liver hepatocytes. At concentrations of 7.2 µg/l and above, changes of greater magnitude were seen in both liver and heart. Since 7.2 µg/l was the experimental EC_{50}, analysis was divided into two groups, depending on the presence or absence of pericardial edema. Moderate glycogen depletion characterized livers of embryos which showed no edema, suggesting that the former was the more sensitive morphologic indicator of exposure. Although heart, kidney, and gut were examined, no other significant alterations were seen. More advanced structural alterations of the liver accompanied pericardial edema. These included severe glycogen depletion, mild lipidosis, and occasional enlarged hepatocytes. In embryos which developed pericardial edema but showed no regression to tubular heart, walls of sinus venosus and atrium were edematous. This localized cardiac edema was characterized by a subendothelial accumulation of fluid in the sinus venosus, dilated sinoatrial compartment, and apparent enlargement of several endothelial cell nuclei. Ventricle and bulbus arteriosus were apparently not affected. Since death followed when concentrations >7.2 µg/l were used, histological alterations are not presented for those fish.

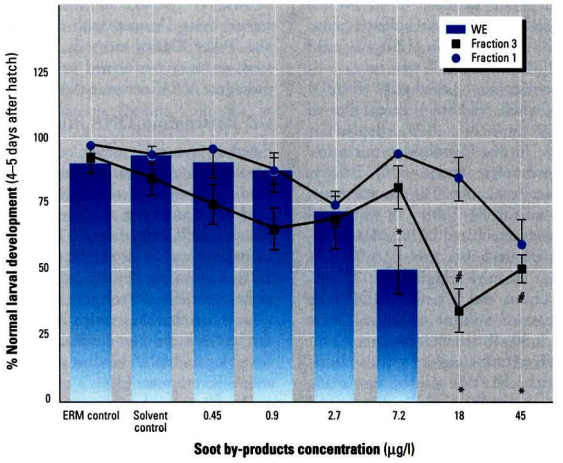

Figure 2. Effect of incomplete combustion by-products from trichloroethylene soot whole extract (bars) and fractions 1 and 3 on the development of medaka after static non-renewal exposures at embryonic stages. Each point represents the mean of four replicates ± SE, eight embryos per replica. In fraction 1 (0.45 µg/l), one replicate was lost due to bacterial infection. Significant ($p<0.05$, Wilcoxon's sign-rank test) abnormalities compared to controls were seen at concentrations ≥7.2 µg/l for whole extract (*), 45 µg/l for F1 (+), and >18 µg/l for F3 (#).

Table 2. Percentage of normal larval development (4–5 days after hatch) of medaka exposed to trichloroethylene soot whole extract (WE) and its fractions (1–4)[a]

Concentration (µg/l)	WE	F1	F2	F3	F4
0 Control[b]	90.6 ± 3.1	96.9 ± 3.1	87.5 ± 5.1	92.5 ± 5.6	87.5 ± 7.2
0 Solvent[c]	93.8 ± 3.5	93.8 ± 3.6	87.5 ± 5.1	84.4 ± 6.0	93.8 ± 6.3
0.45	90.6 ± 6.0	95.8 ± 3.6	78.1 ± 6.0	75.0 ± 7.2	71.9 ± 11.8
0.90	87.5 ± 5.1	87.5 ± 7.2	81.3 ± 8.1	65.6 ± 7.9	87.5 ± 7.2
2.7	71.9 ± 6.0	75.0 ± 1.0	71.9 ± 6.0	68.8 ± 10.8	90.6 ± 6.0
7.2	50.0 ± 9.0*	93.8 ± 3.6	78.1 ± 6.0	81.3 ± 8.1	87.5 ± 5.1
18.0	0*	84.4 ± 7.9	84.4 ± 7.9	34.4 ± 7.9*	84.4 ± 7.9
45.0	0*	59.4 ± 9.4*	71.9 ± 6.0	50.0 ± 5.1*	71.9 ± 12.9

[a]Values represent the mean of 4 replicates ± SE; number of embryos per replica = 8. One replicate from F1 (0.45 µg/l) was lost due to possible bacterial infection. Static non-renewal exposures on embryos ~10 hr old (blastula stage), until 8 days (after completion of organogenesis).
[b]Control was embryo rearing medium (ERM).
[c]ERM-DMSO or ERM-eluting solvent in DMSO (0.05% v/v).
*Statistically significant ($p<0.05$), Wilcoxon's sign-rank, compared to respective controls.

Liver Cells Assays

At concentrations between 0.05 and 1.2 µg/l and in the absence of 17β-estradiol in the culture media, WE induced EROD

Table 3. Percent (%) of medaka embryos/larvae with selected abnormalities after continuous exposure to trichloroethylene soot whole extract (WE) and fractions 1 and 3 (F1, F3)

Concentration (µg/l)	Pericardial/other edema	Abnormal larval activity	Death resulting from edema	Delayed/ incomplete hatch
WE				
Control[a]	0	0	0	3
Solvent[b]	3	3	3	3
0.45	6	0	6	3
0.90	0	9	0	3
2.70	0	18	0	0
7.20	50	44	50	0
18.0	100	6[c]	88	6
45.0	100	3[c]	88	9
F1				
Control[a]	0	3	0	0
Solvent[b]	0	3	0	0
0.45	3	0	3	0
0.90	0	9	0	3
2.70	0	12	0	0
7.20	0	3	0	0
18.0	3	9	0	3
45.0	12	34	9	0
F3				
Control[a]	0	6	0	0
Solvent[b]	0	9	0	3
0.45	0	25	0	0
0.90	3	28	3	0
2.70	9	22	0	0
7.20	0	19	0	0
18.0	9	38	6	0
45.0	6	22	6	3

[a]Control was embryo rearing medium (ERM).
[b]ERM-DMSO or ERM-eluting solvent in DMSO (0.05% v/v). Values represent mean of nearest whole number from four replicates, except for 0.45 µg/l of F1 (loss of 1 replicate).
[c]Edemas produced the bulk of late embryonic mortality.

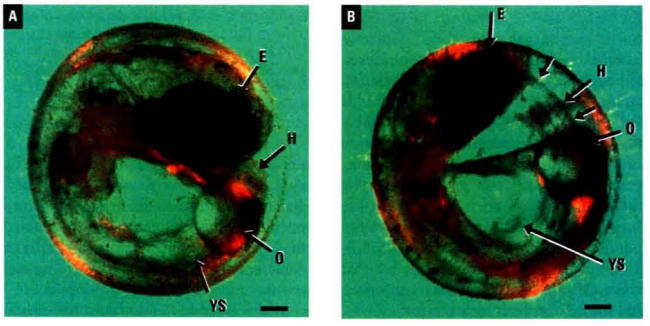

Figure 3. Normal (A) and abnormal (B) late-stage (216 hr) medaka embryos after control and trichloroethylene whole extract treatments. Note how pericardial edema (small arrows) results in separation of embryo proper from yolk sac. E, Eye; H, heart; O, oil droplet; YS, yolk sac. Bar = 100 µm.

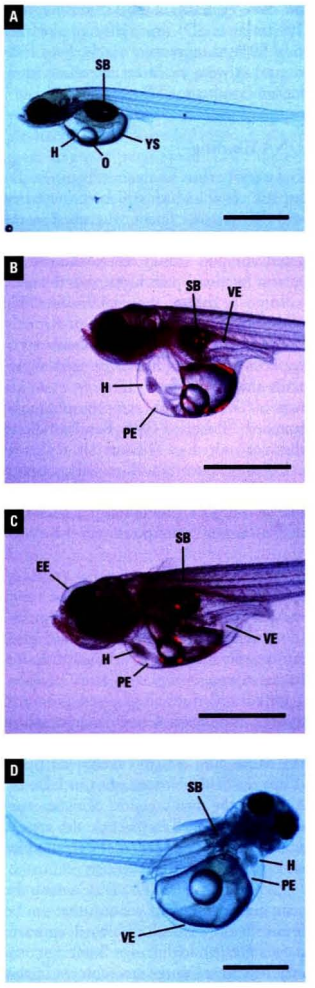

Figure 4. Normal (A), and abnormal (B–D) medaka larvae after control and trichloroethylene whole extract treatments. Note in B and C pericardial (PE), peritoneal/visceral (VE), and eye (EE) edema in larvae that managed to inflate swim bladder (SB) and all of the above plus no swim bladder inflation in D. H, Heart; O, oil droplet; YS, yolk sac. Severe edema preceded death. Bar = 1 mm.

activity. This induction was maximal at 0.6 µg/l. CYP1A1 protein synthesis was significantly increased at the three higher concentrations (Fig. 5). More WE was required to cause a detectable rise in CYP1A1 protein than for a rise in EROD activity. Only fraction 1 showed a significant increase in CYP1A1 protein level and EROD activity (29 and 45%, respectively).

All concentrations from 0.6 to 25 µg/l of WE depressed trout liver cell response to 17β-estradiol relative to the 17β-estradiol-only positive control. Inversely, CYP1A1 protein was induced in a concentration dependent manner with increasing concentrations of extract, with CYP1A1 protein synthesis maximal at 25 µg/l (Fig. 6). However, EROD activity at all concentrations tested was not significantly different from carrier or positive (17β-estradiol-only) controls (data not shown). Mean CYP1A1 protein level was higher, but not significantly, at the 3.95 µg/l WE in the absence of 17β-estradiol (Fig. 6). Significant depression of albumin synthesis (20–30%) was seen only at the higher concentrations (3.95–25 µg/l) of WE. However, the viability of cells exposed to all concentrations of WE was confirmed by phase contrast microscopy, cellular protein, and cellular LDH activity

per dish. Typically, of the $322 \times 10^6 \pm 73 \times 10^6$ (mean ± SD) liver cells harvested per fish, 90% or more were viable. Soot fractions 1–4 were tested for effects on vitellogenin synthesis as above; only fraction 1 depressed mean vitellogenesis (30%).

DNA Binding

Gel retardation analysis of guinea pig hepatic cytosol which had been incubated with WE or soot fractions resulted in the formation of a soot-inducible protein-^{32}P-DNA complex (compared to the control solvent fractions) that migrated to the same position as that of the TCDD-inducible complex (Fig. 7). We have previously shown (*32*) that the TCDD-inducible protein-DNA complex in this position represents the high affinity binding of transformed TCDD-(AhR) complex to double-stranded ^{32}P-labeled DRE. Results indicate that not only does WE contain a chemical(s) which exhibits dioxinlike activity (i.e., it binds to AhR activating its transformation and DNA binding), but that each of the fractions tested positive in this assay.

Discussion

Transient emissions of soot and toxic volatile organic hydrocarbons or "puffs" (*4–6*) were modeled in a well-defined laboratory experiment with a laminar diffusion flame. A very complex mixture of halogenated and nonhalogenated aromatic hydrocarbons was found in association with the aerosol that escaped the flame in the same manner that transient puffs escape the oxidation zone of an incinerator. Although the total amounts of these emissions may be small in practice, the present analysis has revealed that their potential toxicity may be significant.

While dioxins and furans are among the compounds of greatest concern that can be found in the effluent of hazardous waste incinerators, and while significant amounts may be released to the environment in this way (*35*), attention should not be exclusively directed toward these compounds. Harris et al. (*19,20*) found that certain PCB congeners and dioxin, extracted from Lake Ontario rainbow trout skeletal muscle, were toxic to medaka embryos. These compounds are present in Great Lakes biota at concentrations ranging from parts per trillion to parts per billion. It has been proposed that these non-*ortho*-substituted PCBs may contribute more to the overall toxicity than dioxins, which are present at lower orders of magnitude.

Nearly all chemical species in the mixture studied herein were heavily chlorinated (4-, 5-, 6-Cl) and sometimes perchlorinated. They included benzenes, styrenes, fulvenes, butadienes, fulvalenes, cyclopentadienes, naphthalenes, acenaphthylenes, and phenols. Although many compounds still remain unidentified, it is very likely that these as yet unidentified organics were configurational isomers of the main compounds just mentioned, given the possible mathematical combinations of chlorine substitutions across the many double bonds. Despite the absence of 2,3,7,8-TCDD and -TCDF, it is conceivable that other chlorinated dioxins, dibenzofurans, and related chemicals were present.

Results of this study confirm and extend previous work showing the presence of dioxinlike compounds (*14*) in this complex soot mixture and demonstrate that the WE and fractions 3 and 1 (products of mixed polarity and no polarity, respectively) caused toxicity and exhibited biological activity. The major developmental toxicity endpoint of this study was edema of pericardial cavity with extension to peritoneal cavity and yolk sac. Severe pericardial edema was accompanied by an uncoiling of the fused endocardial tube. This defect resulted in a reversal of initial chamber formation to that of a single, pulsatile tube. The latter, normally seen at an earlier stage of development, was also accompanied by apparent rupture of the posterior pericardial membrane with release of fluid into peritoneal cavity. These changes resembled those reported after exposure to dioxin or dioxinlike compounds (*12,38–41*) by late embryo and larval stages of rainbow (*36*) and lake trout (*37*), medaka (*18*), chick, fish-eating birds (terns, herons, double crested cormorants, and herring gulls), and rodents. Furthermore, the generation of toxicity in medaka embryos exposed to TCE soot resembled that of TCDD, where early development proceeded normally and was followed by a gradual progression of cardiotoxicity.

Histopathological studies have suggested that edema of endothelial cells and myocardial interstitium was an important early stage in cardiotoxicity (*37*). Interestingly, juvenile yellow perch (*Perca flavescens*), respond more aggressively with myocyte necrosis, hypertrophy, and hyperplasia of pericardial mesothelium as well as fibrinous

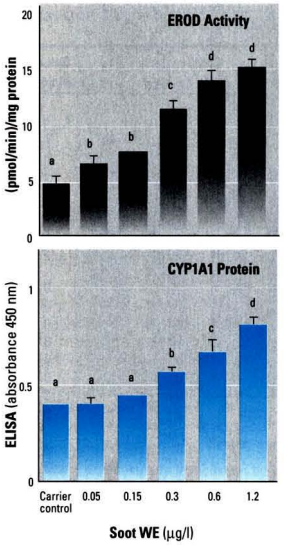

Figure 5. Soot whole extract (WE) induces ethoxyresorufin *O*-deethylase (EROD) activity and CYP1A1 protein in rainbow trout liver cells. Error bars = standard deviation. Means with the same letter are not significantly different ($p<0.05$, ANOVA). Number of dishes per treatment = 3–4, with duplicate determinations per dish.

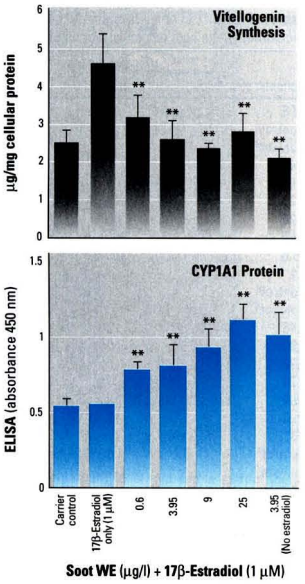

Figure 6. Effect of various concentrations of whole extract (WE) on vitellogenin and CYP1A1 protein levels in rainbow trout liver cells simultaneously exposed to 1 µM 17β-estradiol or carrier control. Error bars = standard deviation. Significant ($p<0.05$, ANOVA) depression of vitellogenin (all concentrations) and increase in CYP1A1 protein (all concentrations), indicated by asterisks, is relative to 17β-estradiol-only control. Number of dishes per treatment = 3–4, with duplicate determinations per dish.

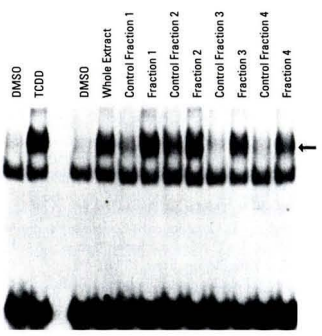

Figure 7. Soot and soot fractions stimulate arylhydrocarbon receptor (AhR) transformation and DNA binding. Guinea pig cytosol, incubated in the presence of 15 nM TCDD, whole extract, various control solvents or soot solvent fractions, was mixed with ^{32}P-labeled DRE oligonucleotide and specific protein–DNA complexes resolved by gel retardation as described in Methods. The arrow indicates the position of the inducible AhR:DNA complex. The following are densitometry readings (%) of the chemically induced bound complexes relative to that obtained with TCDD. Control readings were subtracted as background. TCDD (100%); whole extract (55%), F1 (69%), F2 (52%), F3 (62%), F4 (34%).

pericarditis (*42*). Although our initial histologic analyses have not revealed altered endothelial morphology, it is possible that fluid loss occurred through this tissue into pericardial and peritoneal cavities. The occurrence of edema in mammals, birds, and fish by TCDD and related compounds (*37*) suggests a common mechanism related to endothelial dysfunction (*43*). Immunohistochemical studies have localized CYP1A1 to endothelium of heart in scup (*Stenotomus chrysops*) (*44*) and salmonids, and embryonic induction occurs commonly in endothelial cells (*45*). It is possible that CYP1A induction (mediated through AhR activation) in our study could have led to oxidative injury and loss of endothelial integrity. Octachlorofulvalene appears to be a potent inhibitor and substrate of certain glutathione *S*-transferases (GSTs) (*46*), as are many extensively chlorinated compounds. Perhaps some embryo toxicity may be related to changes in cellular redox status resulting from depletion of reduced glutathione or GST inactivation.

Embryonic chick edema after TCDD or toxic PCB congener exposure suggested that increased prostaglandin synthesis, as a sequel to AhR activation, could mediate CYP1A induction and cardiotoxicity. Such a relationship was suggested by the ability of benoxaprofen, an anti-inflammatory drug, to reduce toxicity in 3,4,3′,4′-tetrachlorobyphenyl-treated embryos without affecting CYP1A induction, supporting a role for arachidonic acid metabolites (prostaglandins, leukotrienes, etc.) as mediators in toxicity, rather than induction itself (*38*). Wisk and Cooper (*47*) exposed medaka embryos to dioxin (≥10 ng/l) or beta-naphthoflavone (BNF; 50 µg/l) and found increased activity of benzo(*a*)pyrene hydroxylase. Induction of these CYP1A-associated enzymes over a period of days suggests that embryos have an intact AhR-mediated activation pathway. However, while benzo(*a*)pyrene hydroxylase induction, hemorrhage, and edema were seen in some medaka after dioxin treatment, others showed similar induction but no vascular changes at nontoxic levels of BNF. This suggests that CYP1A induction is not a prerequisite of cardiotoxicity. Nevertheless, the importance of AhR mediated events in embryonic cardiovascular toxicity needs further study.

While we are not aware of these types of studies in fish, investigations in other animal models have shown interaction between the CYP1A-AhR system and other CYP isoforms. These linkages involve metabolic alterations of endogenous substrates through biochemical pathways, which include antioxidant enzymes, metallothioneins, heat shock proteins, steroid receptors, oncogenes, tumor suppresor genes, glutathione, and GSTs (*45*). Possible involvement of rodent CYP1B1 in edematous lesions and overall dioxinlike toxicity, depending on tissue-specificities, is being investigated. Although highly inducible by TCDD/PAHs (via AhR) and involved in PAH metabolism (*48*), the presence of CYP1B1 in fish remains to be demonstrated.

Edematous spaces, devoid of cells, were observed in heart, peritoneum, and skin of medaka embryos, a condition similar to that of chickens exposed to TCDD and toxic PCBs (*38,39*). We cannot state whether the developing medaka has white blood cells capable of emigration into extravascular spaces, and we cannot rule out a compound-induced cytopenia. Other possible mechanisms underlying edema continue to be investigated. *In situ* nuclear magnetic resonance analyses from our laboratory suggest that transient depression of certain energy phosphate metabolite levels (mainly ATP) may lead to deficient ion translocation and consequent edema (Villalobos, in preparation). We are also focusing attention on the relative abundance of basement membrane components in control versus treated medaka embryos.

While WE adversely affected normal development in a concentration dependent manner, various concentrations of fractions 3 or 1 did not exhibit such a relationship. This finding may be related to solubility but has persisted over repeated assays. Perhaps combustion by-products of nonpolar and/or intermediate polarity act synergistically in the WE to produce effects whose impact was not apparent when a single fraction was assayed. However, synergism has not been specifically tested. Moreover, direct comparisons of the toxicity of combined fractions with WE are complicated by losses of volatile compounds or the reactivity of constituents like the chlorinated fulvenes and fulvalenes. Thus, evaluation of the toxicity of individual fractions should be viewed as a qualitative guide indicative of the polarity of the most toxic components of WE.

In vitro observations revealed no direct cellular toxicity but vitellogenin in medium was reduced. Fish liver cells are sensitive indicators of exposure to aquatic pollutants that have dioxinlike activity (*49–52*). Hepatocytes and biliary epithelial, and endothelial cells contain the readily inducible enzyme CYP1A1 (*45*). The liver plays a key role in reproduction in fish, being a component of the hypothalamic, pituitary, gonadal, and liver reproductive axis (*53*). In these oviparous vertebrates, the egg yolk precursor protein vitellogenin is synthesized in the liver and transported by the circulatory system to the developing oocytes. Vitellogenesis is under direct control of estrogens (*54*), and since CYP1A1-inducing compounds such as dioxin are known antiestrogens in mammals (*55*), the possibility exists that vitellogenesis and gonadal maturation could be disrupted in exposed fish.

At the concentrations tested, WE was not overtly toxic to liver cells but induced dioxinlike effects. EROD activity and the amount of CYP1A1 protein increased in a concentration-dependent manner, confirming the dioxinlike activity of component(s) of the extract. The EROD activity assay proved more sensitive in detecting significant changes in CYP1A1 expression at low WE concentrations than the CYP1A1 ELISA assay. 17β-Estradiol may have had an inhibitory or antagonistic effect upon EROD and CYP1A1 protein induction in cultured liver cells, as has been previously demonstrated *in vivo* with feminized brook trout (*56*) and in mouse fetal cell cultures (*57*). Higher concentrations of WE significantly increased CYP1A1 protein, but EROD activity remained unchanged. At concentrations above 0.6 µg/l, components of the WE may have competitively inhibited binding of ethoxyresorufin to CYP1A1. Substrate inhibition by PCBs in fish liver cell EROD assays has been demonstrated *in vivo* and *in*

vitro (*58,59*). These effects underscore the importance of conducting direct measurements of enzyme concentration in addition to enzyme activity.

Trout liver cells exposed simultaneously to noncytotoxic concentrations of 17β-estradiol and WE showed much less vitellogenin in medium than did similar cells exposed to 17β-estradiol alone. Vitellogenin levels and CYP1A1 protein appeared to be negatively correlated. Higher concentrations (3.95–25 μg/l) of the extract may affect the secretory capacity of liver cells; however, even at the 0.6 μg/l concentration (where albumin synthesis was not depressed) vitellogenin production was still compromised. From the fractions, only fraction 1 showed an effect on CYP1A1 protein or EROD activity (both increased), or vitellogenin (reduced). CYP1A1 inducing compounds may suppress vitellogenin production in fish liver cells by an antiestrogenic mechanism mediated through the AhR, similar to that described in mammals (*60*). We investigated whether this mechanism might apply to teleost liver, since AhR has been identified in this organ (*45*).

Numerous studies have revealed that most of the critical and sensitive toxic and biological responses to TCDD and related compounds are mediated by its soluble AhR, to which these chemicals bind with high affinity (*7,13,61*). After ligand binding, the halogenated aromatic hydrocarbon:AhR complex undergoes transformation into its DNA binding form and translocates into the nucleus (*62,63*). The transformed complex associates with a specific DNA sequence, the dioxin responsive element (DRE), resulting in transcriptional activation of adjacent responsive genes (*63–66*). Since previous studies have demonstrated a high correlation between binding of a chemical to the AhR and its degree of toxicity, the relative biological/toxicological potency of complex mixtures of chemicals can be estimated by measuring the ability of an unknown chemical/mixture to activate the AhR or an AhR-dependent response (*61,67*). Previously, we have utilized a gel retardation DNA binding assay to demonstrate that transformed TCDD:AhR complexes, formed *in vitro*, can bind to a DRE oligonucleotide specifically and with high affinity, mimicking that which occurs *in vivo* (*32,65,66*). Since there appears to be an excellent correlation between the ability of a given chemical to stimulate AhR transformation/DNA binding and its ability to activate gene expression, this technique has been utilized as a sensitive bioassay for the detection of dioxinlike chemicals (*33*).

The gel retardation assay results indicated that WE contains dioxinlike chemicals which not only bind to the AhR but also induce its transformation and DNA binding. The formation of inducible protein–DNA complexes by each soot fraction implies that the soot must contain numerous AhR ligands. Given the correlation between the ability of a given chemical to stimulate AhR transformation/DNA binding and its ability to activate gene expression, our results suggest that WE and fractions might also alter gene expression in mammals. In addition, given the role of the AhR in mediating toxicity of these chemicals (*7,13,61*), it is very likely that some of the toxicity produced by these compounds was AhR-mediated. Fractions 2 and 4 were not associated with developmental cardiotoxicity but did bind to the AhR inducing its transformation and DNA binding. While these processes may lead to cardiotoxicity, mediating factors are not known and need investigation.

In summary, CH_2Cl_2 extracts of TCE combustion aerosol proved toxic/bioactive using a battery of bioassays. The pattern of toxicity was identical to that previously reported for dioxin. Chemical analyses performed herein documented the presence of at least 250 chlorinated incomplete combustion by-products in the whole soot extract, but the obvious target compounds, TCDD and TCDF, were not present at detectable (picomole) levels. These results indicate that an array of toxic effects may arise from substances other than those targeted by conventional chemical analyses. They also suggest a need for bioassay-directed assessments of toxicity/biological potency in complex mixtures.

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Effect of an Antimitotic Agent Colchicine on Thioacetamide Hepatotoxicity

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In an earlier study we established that timely and adequate tissue repair response following the administration of a six-fold dose-range of thioacetamide (TA; 50, 150, and 300 mg/kg) prevented progression of injury and led to recovery and animal survival. Delayed and attenuated repair response after the 600 mg/kg TA dose resulted in a marked progression of injury and 100% lethality. The objective of the present study was to further scrutinize this concept in an experimental protocol in which we hypothesized that a selective ablation of the tissue repair response should lead to lethality from the nonlethal, moderately toxic doses of 150 and 300 mg/kg TA. In this study we investigated the effect of the antimitotic agent colchicine (CLC, 1 mg/kg) on the outcome of TA hepatotoxicity. Male Sprague-Dawley rats (175–225 g) were injected intraperitoneally (ip) with 150 and 300 mg/kg TA. We assessed liver injury by serum enzyme elevations and histopathology. Tissue regeneration response was measured by ^{3}H-thymidine incorporation into hepatonuclear DNA and by proliferating cell nuclear antigen (PCNA) assay. S-Phase stimulation, as indicated by ^{3}H-thymidine incorporation, was noted at 36 and 48 hr following the administration of 150 mg/kg TA, whereas with the 300 mg/kg TA S-phase stimulation was elicited at 48 hr following treatment. Therefore, two doses of CLC (30 hr and 42 hr, 1 mg/kg, ip) were administered to the 150 mg/kg treated group while a single dose of CLC (42 hr, 1 mg/kg, ip) was administered to the 300 mg/kg group. CLC treatment resulted in 100% lethality in both groups. Thus, CLC administration converted nonlethal doses into lethal doses. The 150 mg/kg TA dose was then chosen to further investigate the underlying mechanism. Rats treated with TA alone recovered from injury by 36–48 hr while CLC treatment resulted in a progression of injury as indicated by serum enzyme elevation and histopathology. Tissue repair, as evidenced by ^{3}H-thymidine incorporation and PCNA studies explained this dichotomy. Antimitotic intervention with CLC resulted in a significantly diminished repair response leading to unrestrained progression of injury and lethality even from nonlethal doses. This model demonstrates the critical role of tissue repair response in determining the final outcome of toxicity. *Key words:* colchicine, mitosis, necrosis, thioacetamide, tissue repair. *Environ Health Perspect* 104:744–749(1996).

Thioacetamide (TA), originally used as a fungicide, is a potent hepatotoxicant that has been much studied since the first report of its toxic properties (*1–4*). Earlier literature (*5–8*) suggests that an obligate intermediate metabolite of TA binds to proteins with the formation of acetylimidolysine derivatives (*7*) responsible for TA-induced hepatotoxic effects. Accordingly, it was reported that the hepatotoxic effects of TA are manifested only after metabolic conversion to thioacetamide-*S*-oxide, which undergoes further oxidative metabolism to an as yet unidentified toxic metabolite (*7*). TA has been reported to stimulate DNA synthesis and mitosis in the liver of rats at doses that produce limited necrosis (*9–11*). The rise in DNA synthesis induced by TA has been shown to follow a time course that is similar to that seen following partial hepatectomy (*12*). Rats treated with a single dose of 50 mg/kg TA show non-neoplastic hepatocellular proliferation indicated by ^{3}H-thymidine (^{3}H-T) incorporation and cell cycle progression to mitosis after administration (*9,12–14*).

Hepatocellular regeneration and tissue repair mechanisms have been implicated in the ultimate outcome of toxicity, after injury from a variety of structurally and mechanistically dissimilar chemicals including carbon tetrachloride (*15–18*), acetaminophen (*14*), and thioacetamide (*19*).

These studies have thus far illustrated two important biological events. They are the role of tissue repair in the ultimate outcome of toxicity and a dynamic coexistence of the processes of tissue repair and tissue injury as parallel but opposite biological responses and the collective influence of their interaction on the ultimate outcome of toxicity.

Intervention with the repair processes has been reported to result in unrestrained progression of hepatic injury leading to hepatic failure and animal death (*20,21*). Colchicine (CLC) is a widely used antimitotic agent (*20–23*). A single intraperitoneal (ip) administration of CLC (1 mg/kg) results in antimitosis against TA (*11,13*) with minimal side effects because of very rapid elimination of the compound (*23–25*). The objective of this study was to investigate the effect of colchicine antimitosis in a dose–response paradigm. We hypothesized that if timely and adequate tissue repair was indeed the critical underlying mechanism for the nonlethality observed with the lower six-fold dose-range of TA (*19*), then intervention with the repair response should convert these nonlethal doses to lethal doses. For this study we chose two doses of TA (150 and 300 mg/kg) from the 12-fold dose-range reported earlier (*19*). These two doses were chosen because of the accompanying high rates of tissue repair (*19*). After conducting lethality studies in the presence and absence of colchicine, we carried out further time course (0–96 hr) studies with the 150 mg/kg TA to quantitate tissue repair and injury. Colchicine antimitotic intervention resulted in the abolishment of the tissue repair response that led to 100% lethality from an ordinarily nonlethal dose of 150 mg/kg TA. These findings establish that the key difference between moderately toxic nonlethal doses and a lethal dose of TA is the presence or absence, respectively, of adequate and timely tissue repair response.

Materials and Methods

Male Sprague-Dawley rats (175–225 g, 7–8 weeks of age) were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) and were maintained over woodchip bedding free of any chemical contaminants for 10 days on a 12-hr photoperiod in our central animal facility. A 21 ± 1°C temperature and 50% relative humidity were maintained at all times. The animals had free access to water and normal commercial rat chow (Teklad, Madison, WI, diet #7001) *ad libitum*.

Thioacetamide (TA) and [^{3}H-methyl] thymidine (^{3}H-T, specific activity 1.7 Ci/mmol) were obtained from Sigma Chemical Co. (St. Louis, MO). The scintillation fluid (Scintiverse SX 16-4) was purchased from Fisher Scientific (Baton Rouge, LA). All other biochemicals and chemicals were obtained from Sigma Chemical Co.

After acclimation, rats were divided into four groups (Table 1). Groups I and II

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Table 1. Protocol to study the effect of antimitosis in the dose–response paradigm*

Groups	TA (mg/kg)	Saline (1 ml/kg)	Colchicine (1 mg/kg)	No. of Rats	% Survival
I	150[a]	+	–	5	100
II	150[a]	–	+	5	0
III	300[b]	+	–	5	100
IV	300[b]	–	+	5	0

[a]Colchicine was administered 30 and 42 hr after TA (150 mg/kg).
[b]Colchicine was administered 42 hr after TA (300 mg/kg).
*Route of administration for both the doses and vehicle-treated groups was ip. Thioacetamide was dissolved in vehicle (normal saline, 1 ml/kg). Rats were observed for 14 days daily. All deaths occured between 4–6 days after treatment. + and – indicate the presence and the absence of the stated treatments respectively.

received 150 mg/kg TA ip at 0 hr. After 30 and 42 hr, Group I received control vehicle (1 ml/kg) while group II received CLC (1 mg/kg). Groups III and IV received 300 mg/kg TA at 0 hr. After 42 hr, Group III received control vehicle (1 ml/kg) while group IV received CLC (1 mg/kg). Rats of all groups were given normal diet and water *ad libitum* during and after treatment until termination.

One experiment was designed to determine the lethality of TA both in the presence and absence of CLC treatment in rats following the administration of each dose of TA (150, 300 mg/kg). CLC (1 mg/kg in normal saline) was administered at 30 and 42 hr (6 hr prior to peak S-phase activities at 36 and 48 hr) following the administration of 150 mg/kg TA, while CLC (1 mg/kg in normal saline) was administered 42 hr (6 hr prior to peak stimulation of cell division) following treatment with 300 mg/kg TA. The respective controls received normal saline (1 ml/kg) at the respective times. The rats were observed twice daily for 14 days and survival/lethality was recorded in each group.

Blood was collected from the dorsal aorta of rats under diethyl ether anesthesia at 0, 12, 24, 36, 48, 72, and 96 hr after TA or vehicle administration; the serum was separated for the estimation of serum enzymes alanine aminotransferase (ALT, EC 2.6. 1.2.) and sorbitol dehydrogenase (SDH, EC 1.1.1.14.) as markers of liver injury using kit # 59 UV (ALT) and kit # 50 UV (SDH), respectively, from Sigma Chemical Co.

Portions of liver from each group collected at various periods after TA treatment were washed with normal saline (0.9% NaCl), cut into small slices, and fixed in phosphate buffered 10% formaldehyde solution for 48 hr. The tissues were then transferred to 70% ethyl alcohol until they were processed. These slices were then embedded in paraffin after processing. The liver sections (5 µm thick) were stained with hematoxylin-eosin (H&E) for histological examination under the light microscope.

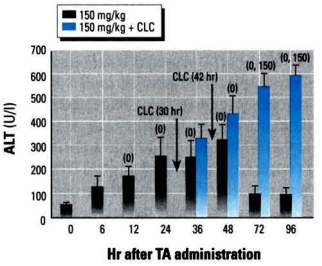

Figure 1. Serum alanine aminotransferase (ALT) was measured as a marker of liver injury over a time course (0–96 hr) following each treatment. Male Sprague-Dawley rats (175–225 g) were divided into two groups. At time zero both groups received 150/kg TA in normal saline (1 ml/kg, ip). Controls received normal saline only (1 ml/kg, ip). One group received CLC (1 mg/kg, ip) at 30 hr and 42 hr following administration of 150 mg/kg TA. The other group received normal saline (1 ml/kg) at the same time points. Results are expressed as mean ± SE for four rats in each group at each time point. Numbers above the error bars indicate significant differences from control (0 hr) and 150 mg/kg TA treated groups, respectively ($p \leq 0.05$). Control ALT value:54 U/l.

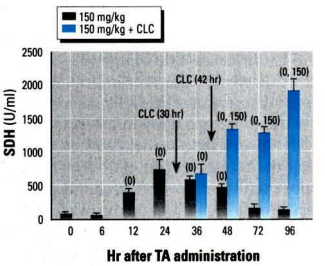

Figure 2. Serum sorbitol dehydrogenase (SDH) was measured as a marker of liver injury over a time course (0–96 hr) following each treatment (treatment protocol as in Fig. 1). Results are expressed as mean ± SE for four rats in each group at each time point. Numbers above the error bars indicate significant differences from control (0 hr) and 150 mg/kg TA treated groups, respectively ($p \leq 0.05$). Control SDH value:78 U/ml.

^{3}H-T incorporation into hepatonuclear DNA was measured using the procedure of Chang and Looney (*26*) as modified by Chauveau et al. (*27*). The DNA content of the supernatant fraction was estimated by the diphenylamine reaction (*28*).

The proliferating cell nuclear antigen (PCNA) study was done as described by Greenwell et al. (*29*) and as reported earlier (*14,19*).

Mean ± SEM were calculated for all values. Statistical differences were determined by one-way analysis of variance followed by Duncan's multiple range test to determine which means were significantly different from each other or from controls. In all cases, a $p \leq 0.05$ was used as the statistical criterion to determine significant differences.

Results

All groups were observed twice a day for survival/lethality for a period of 14 days. A 100% survival was observed in the groups that did not receive CLC (groups I and III) while a 100% mortality was noted in the groups that received TA followed by CLC (groups II and IV). All the deaths occurred between 4 and 6 days.

Serum ALT and SDH were estimated as markers of liver injury over a time course of 0–96 hr after treatment with 150 mg TA/kg. Figures 1 and 2 show the ALT and SDH activities at various times after the administration of TA in the presence and the absence of CLC treatment. In the group receiving 150 mg/kg TA alone, ALT activity increased up to 48 hr and then declined to normal, indicating an initial infliction of injury followed by recovery; this is consistent with 100% animal survival. Colchicine intervention significantly altered the course of these biochemical events. Marked progression of injury, as indicated by significantly higher elevation of serum ALT, was evident from 48 hr onward. This progression of injury was associated with 100% animal mortality in this group. Sorbitol dehydrogenase elevation measured in a similar fashion exhibited the same trend (Fig. 2).

The liver sections stained by H&E were examined for necrotic cells, extent of inflammation, and neutrophil infiltration. Swollen cells and a few pyknotic nuclei started appearing around the centrilobular region between 6 and 12 hr in the rats treated with 150 mg/kg TA. Pericentral hepatocellular necrosis was clearly evident at 12 hr and was progressive between 12 and 24 hr. Periportal cells remained unaffected. Moderate centrilobular necrosis observed in the 150 mg/kg TA treated group progressively declined between 24 and 48 hr. Centrilobular damage accompanied by extensive neutrophil infiltration was evident between 36 and 48 hr. In sharp contrast, CLC treatment resulted in marked centrilobular necrosis at 36 hr. This was associated with extensive neutrophil infiltration and vacuolation. Between 48 and 72 hr, progression of injury was accelerated as evidenced by

widespread necrosis, crumbling centrilobular columns, and damaged portal tracts. By 96 hr, fulminant liver injury with no zonal specificity was observed, indicating complete destruction of the liver architecture.

^{3}H-T incorporation into hepatonuclear DNA over a time course (0–96 hr) following the administration of 150 mg/kg TA alone and over 36–96 hr following treatment with TA + CLC, measured as a marker of S-phase stimulation, is represented in Figure 3. Peak ^{3}H-T incorporation after the administration of 150 mg/kg TA occurred between 36 and 48 hr. Administration of CLC (1 mg/kg, ip) resulted in a significant reduction of S-phase stimulation (Fig. 4). This suppression sustained until 96 hr and ultimately resulted in 100% animal death. Examination of the H&E-stained sections revealed a significant mitotic activity in the rats treated with 150 mg/kg TA alone between 36 and 72 hr in sharp contrast to no mitotic activity in the rats receiving TA and CLC. Thus, timely stimulation of tissue repair was consistent with animal survival while suppression of repair activity led to a dramatic reversal of events leading to 100% animal lethality.

The results of thymidine incorporation were further corroborated by the PCNA immunohistochemical procedure (Figs. 5, 6). Normally, most cells in the liver are in the resting phase (G_0; Fig. 5A), and a small proportion of cells are in other phases of the cell cycle. After administration of TA (150 mg/kg), cell cycle progression is stimulated; this results in a large number of cells in the G_1 phase by 24 hr and S-phase between 36 and 48 hr. The maxi-

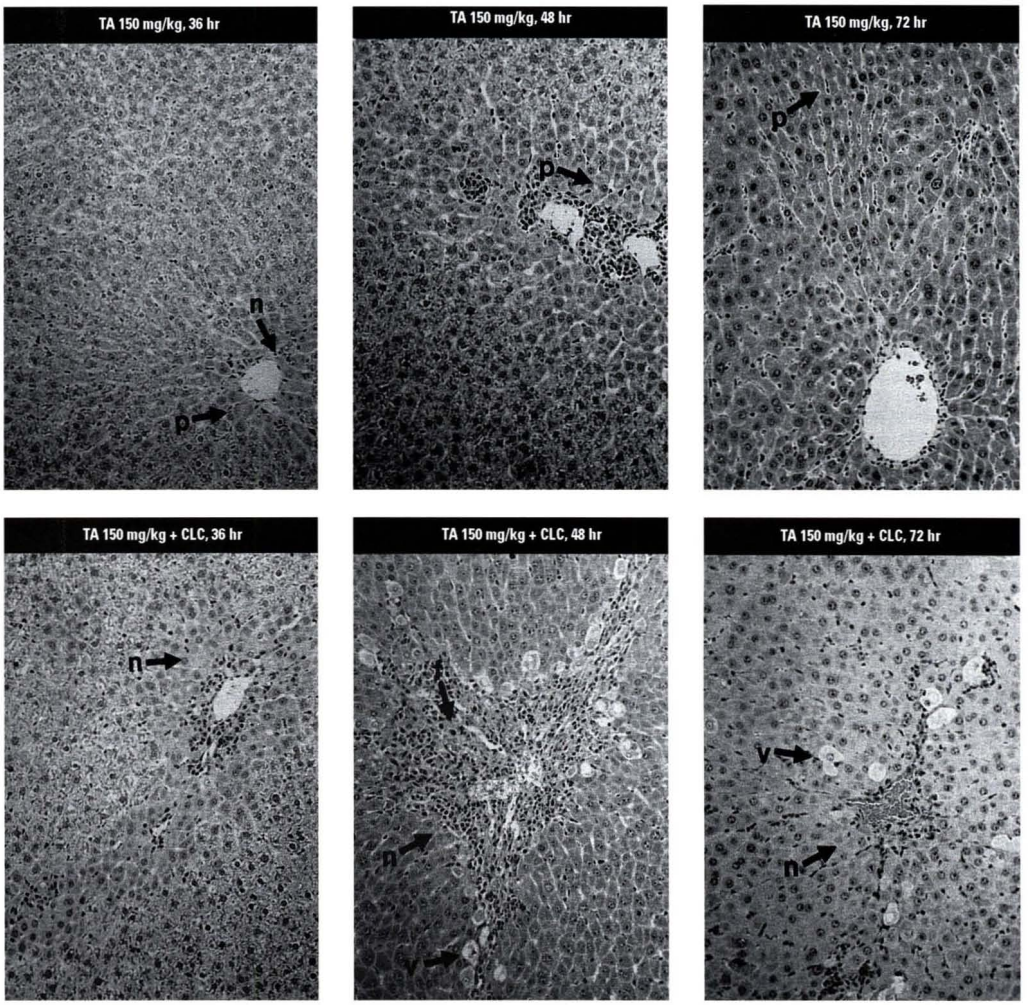

Figure 3. Representative liver histopathology during a time course after thioacetamide treatment. Top panel represents photomicrographs of liver sections from rats receiving 150 mg/kg TA taken at 36 hr, 48 hr, and 72 hr after treatment. Bottom panel represents photomicrographs of liver sections from rats receiving 150 mg/kg TA+CLC at 36 hr, 48 hr, and 72 hr. c, central vein; p, pyknotic nuclei; v, vacuolization; n, areas of necrosis; f, fibrosis; m, mitosis.

mum number of S-phase cells seen by this method is in agreement with S-phase stimulation observed by ^{3}H-T incorporation. Most of the cells progress to the G_2 phase between 36 and 48 hr. The maximum number of cells are seen in the M-phase at the 48 hr. By 96 hr the liver returned to normal quiescence as evidenced by the predominant number of G_0 cells. In sharp contrast, CLC intervention blocked the G_0–G_1 transition and thereafter arrested the progression of the cell cycle. S-phase synthesis and mitotic activity were ablated almost completely. Progression of the cell cycle and timely stimulation of tissue repair were consistent with animal survival, while ablation of the repair process by colchicine intervention led to unrestrained progression of injury and culminated in 100% animal mortality from an otherwise nonlethal and moderately toxic dose; this indicates that stimulated cell division and tissue repair are key events in the outcome of toxicity.

Discussion

Earlier studies have amply demonstrated that tissue repair is a biological response that accompanies chemical-induced injury (*13–19,30*). Other studies (*11,13,14,22, 31–36*) indicate that the G_2-subpopulation of cells in the liver undergo mitosis after treatment with low doses of toxicants such as thioacetamide, galactosamine, dichlorobenzene, $CHCl_3$ or CCl_4. Additionally, several investigators (*37–41*) have observed that newly divided cells are significantly more

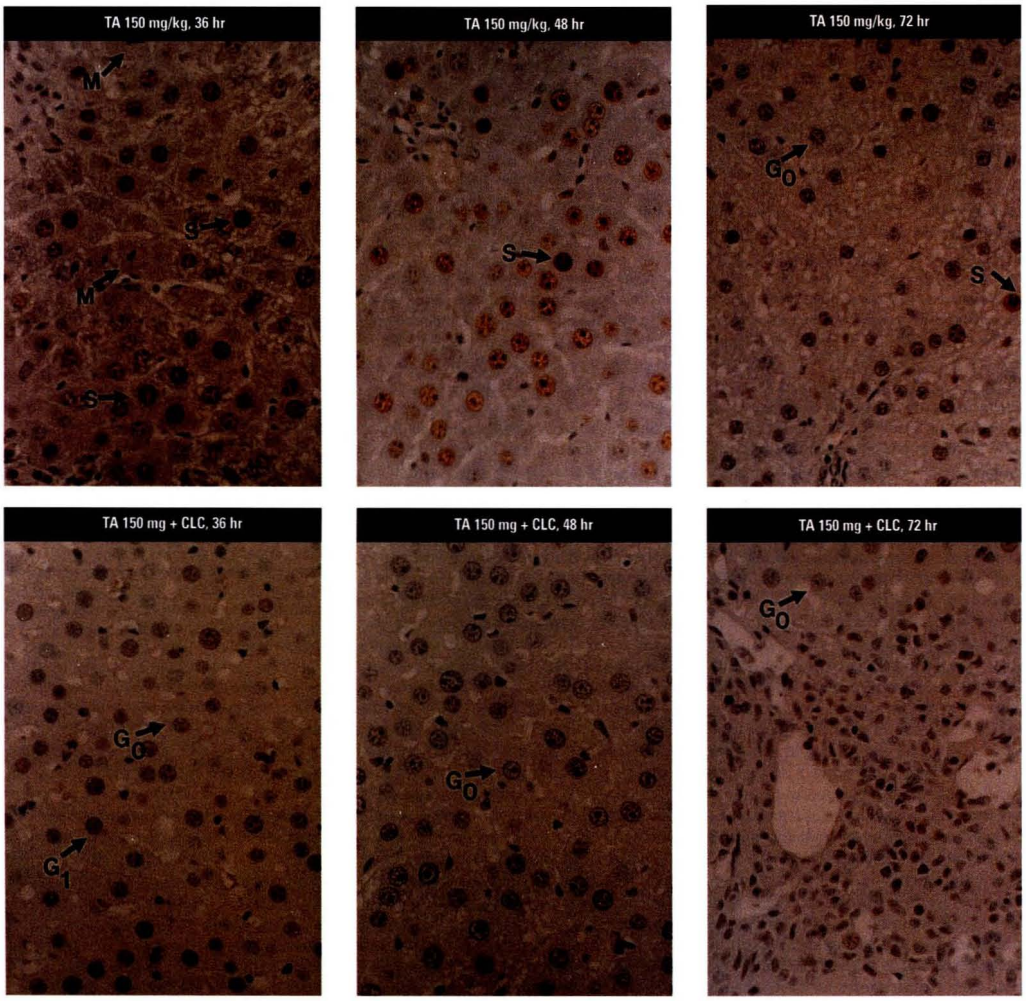

Figure 5. Results of the proliferating cell nuclear antigen study after intraperitoneal treatment with 150 mg/kg TA+vehicle and 150 mg/kg TA+CLC (1 mg/kg). Representative photomicrographs of liver sections from rats at 36 hr, 48 hr, and 72 hr after 150 mg/kg TA+vehicle treatment and 36 hr, 48 hr and 72 hr after 150 mg TA/kg+CLC treatment. G_0, cells with blue nuclear staining; G_1, cells with light-brown nuclear staining; S, cells with deep brown nuclear staining; G_2, cells with or without speckled nuclear staining and with diffused cytoplasmic staining; M, cells with diffuse cytoplasmic and deep blue chromosomal staining. Significant S-phase stimulation at 36 and 48 hr following 150 mg/kg TA is contrasted with inhibited S-phase stimulation with 150 mg/kg TA+CLC.

resistant to cytotoxic effects of a variety of toxic chemicals. Using TA as a model compound, we have also reported that the repair response can be measured in a dose-dependent and temporal manner in parallel with injury (*19*). The toxicodynamic interaction between tissue repair and injury in a dose–response paradigm and its effect on the ultimate outcome of toxicity has major implications. Measuring the parallel but opposing biological responses of repair and injury may provide new insights on the use of dose–response relationships in predictive toxicology and risk assessment.

The objective of this study was to subject the concept that stimulated tissue repair plays a critical role in the ultimate outcome of TA toxicity to further experimental scrutiny in a dose–response paradigm. We hypothesized that if stimulated cell division and tissue repair were indeed the mechanism of animal survival, then intervention with the repair mechanism should convert nonlethal doses into lethal doses. To test this hypothesis, we chose two doses (150 and 300 mg/kg) of TA, a model hepatotoxicant. These two doses cause moderate injury but are nonlethal by virtue of their ability to maximally stimulate hepatocellular division and tissue repair (*19*). CLC (1 mg/kg) was used as an antimitotic tool to block stimulated cell division and tissue repair. Earlier studies have demonstrated that CLC used in this dose does not disrupt hepatobiliary function (*20,21*).

A lethality study was designed (Table 1) to investigate the effect of colchicine treatment. It should be noted that 150 and 300 mg/kg TA are otherwise nonlethal in the absence of CLC treatment. While liver injury occurs, simultaneous dose-related stimulation of cell division and tissue repair allows the animals to overcome this injury and survive. However, a 100% lethality was observed in the presence of CLC treatment in both the groups. For further mechanistic inquiry, we chose the 150 mg/kg TA dose. Liver injury and tissue repair were the two parallel but opposing responses measured and were used as predictors of regression or progression of liver injury leading to animal recovery or death, respectively.

Serum enzyme (ALT, SDH) elevation and liver histopathology (Figs. 1, 2, 3) were used as indices of liver injury following the administration of 150 mg/kg TA both in the presence and absence of CLC intervention over a time course of 0–96 hr and 36–96 hr, respectively. In the absence of CLC treatment, a moderate elevation of serum enzyme was observed between 24 and 48 hr. Thereafter, the animals recovered from liver injury, as evidenced by the

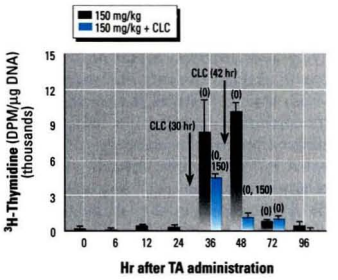

Figure 4. ^{3}H-T into hepatonuclear DNA after TA treatment. ^{3}H-T (35 µCi) was administered 2 hr prior to sacrifice at each time point. One group received CLC (1 mg/kg, ip) at 30 hr and 42 hr following administration of 150 mg/kg TA. The other group received normal saline (1 ml/kg) at the same time points. Results are expressed as mean ± SE for four rats at each time point. Number above error bars represents significant differences from control (0 hr) and 150 mg/kg TA treated groups, respectively ($p \leq 0.05$). Control ^{3}H-T value = 150 dpm/µg DNA.

prompt decline of enzyme activity between 72 and 96 hr to control level (Figs. 1, 2). In contrast, CLC treatment resulted in a dramatic progression of injury as indicated by progressive elevation of serum enzyme activity between 48 and 96 hr. Under a light microscope, liver sections revealed identical injury in both groups at 36 hr (Fig. 3). Thereafter, injury progressed remarkably between 36 and 96 hr in the rats receiving CLC after 150 mg/kg TA, as evidenced by severe centrilobular necrosis, vacuolation, and congestion; injury regressed in the rats receiving 150 mg/kg TA alone. An interesting observation here is that the 36 hr injury was not significantly different between the CLC-treated and CLC-nontreated groups. This indicates that the inflictive phase of liver injury from TA was not affected by CLC treatment.

Tissue repair indicated by the S-phase DNA synthesis and PCNA assay (Figs. 4, 5, 6) explains the dichotomy between the outcomes of TA treatment with or without CLC. Following the administration of the 150 mg/kg TA alone, there is a peak and sustained ^{3}H-T incorporation into hepatonuclear DNA between 36 and 48 hr (Fig. 4). These rapidly dividing cells form new resilient hepatic parenchymal cells that continuously replace the dead cells, thereby restoring hepatolobular architecture and function. Consequently, injury is not only restrained from progression, liver structure and function are restored which eliminates liver injury altogether between 48 and 72 hr. In contrast, CLC treatment results in an ablation of this compensatory mechanism as shown by blocked S-phase stimulation, as

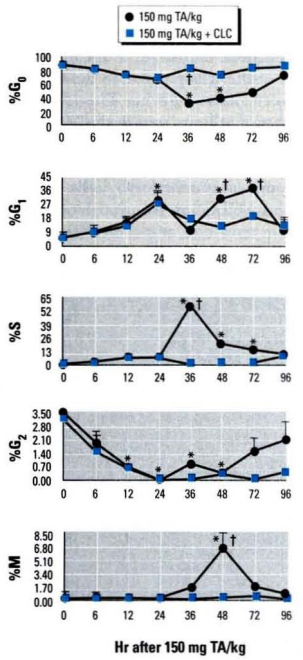

Figure 6. Graphical representation of cell cycle progression as measured by proliferating cell nuclear antigen immunohistochemical procedure. Percentage was calculated from a total of 1000 viewed cells in the centrilobular region of the liver for each animal. Each time point had four rats per group. Rats received a single dose of 150 mg/kg TA or 150 mg/kg TA+CLC, intraperitoneally. Percent cells in different phases of cell cycle were then counted during a time course of 0–96 hr. *, significant difference from control; †, significant difference between groups ($p \leq 0.05$). Control rats received vehicle only (normal saline, 1 ml/kg, intraperitoneally).

well as cell cycle progression. Failure in the timely appearance of an adequate number of new cells allows unrestrained progression of injury on the one hand and deteriorating hepatic structure and function on the other, leading to lethality from an otherwise nonlethal dose. It should be noted that, only after failure of cell division to occur, the progressive phase of injury flares in the group that receives CLC.

These findings have the potential to affect current risk assessment and predictive toxicology methods. A knowledge of the mechanisms of infliction of injury by toxic chemicals has given us the ability to predict with a degree of confidence that a particular chemical or physical agent will or will not inflict tissue injury under a given set of exposure circumstances. However, that the

ultimate outcome of the injury is determined by the initiation of timely and adequate tissue repair calls for a clearer understanding of the underlying biology to attain greater precision in predictive toxicology.

These findings have two broad implications. First, an increased understanding of the biological events that follow infliction of injury (toxicodynamic phase) helps to bridge the link between mechanisms responsible for the injury (Stage I) and the ultimate outcome of that injury (Stage II) (*42*). Secondly, the finding that the repair response can be manipulated and that such manipulation leads to a complete reversal in the outcome of toxic injury opens up avenues for therapeutic applications in the realm of biomedicine.

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Air Pollution and Lung Cancer in Trieste, Italy: Spatial Analysis of Risk as a Function of Distance from Sources

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To investigate the relationship between four sources of environmental pollution (shipyard, iron foundry, incinerator, and city center) and lung cancer risk, we conducted a case–control study of deceased men in Trieste, Italy. We identified 755 cases of lung cancer and 755 controls through the local autopsy registry. Information on smoking habits, occupational history, and place of residence were obtained from the subject's next of kin. The case–control design was used to properly account for subject-specific confounders, which represent a major problem in geographical analysis. Spatial models were used to evaluate the effect of sources of pollution on lung cancer after adjustment for age, smoking habits, likelihood of exposure to occupational carcinogens, and levels of air particulate. The models are based on distance from the sources and enable estimation of the risk gradient and directional effects separately for each source. The risk of lung cancer was highly related to the city center ($p = 0.0243$), with an excess relative risk at zero distance of 2.2 and a smooth decrease moving away from the source (-0.015), and related to the incinerator ($p = 0.0098$), with an excess relative risk of 6.7 in the source and a very steep decrease (-0.176). These results are consistent with findings of previous analyses and provide further evidence that air pollution is a moderate risk factor of lung cancer. *Key words:* air pollution, epidemiology, geographical analysis, lung cancer. *Environ Health Perspect* 104:750–754 (1996)

Results of a case–control study on air pollution and lung cancer in Trieste, Italy, were reported by Barbone et al. (*1*). That study confirmed a moderate elevation in risk of lung cancer in polluted areas and showed a variation by histologic type and category of air pollution. Trieste, which had approximately 250,000 inhabitants in the mid-1980s, is a border city located in the northeast of Italy and is characterized by a major port and a high concentration of industries. Air pollution has been monitored since the early 1970s. Higher total particulate deposition levels (i.e., >0.3 g/m^2/day) were documented in the center of the city and in the industrial area in the 1970s. Currently, higher levels of carbon monoxide (monthly average 3.6 mg/m^3) and nitrogen oxides (218 µg/m^3) are found in the center of the city, and higher levels of ozone (32–39 µg/m^3) and sulfur dioxide (50–59 µg/m^3) are present near an incinerator and an iron foundry. The presence of suspended asbestos fibers was documented near a shipyard. Here we present analyses of the spatial pattern of risk of lung cancer with regard to four sources, shipyard, iron foundry, incinerator, and the city center, while adjusting for known risk factors.

Geographical investigations are hampered by the difficulties in properly accounting for confounders (*2*). However, methods based on the case–control design have been proposed in the statistical literature that allow the collection of data at individual level, avoiding the ecologic bias (*3*). The merit of the analysis presented here is in relaxing the *a priori* categorization of the subject residence in given areas and in using the distance from a source as a proxy for exposure. Second, the method we used allows for directional effects and estimates the risk gradient in order to properly describe the specific pattern of risk for each source.

Materials and Methods

The Cancer Registry and the Department of Pathology of the Province of Trieste identify 99% of cancer cases and conduct autopsies on approximately 73% of all the deaths of the region. From these institutions, 938 histologically confirmed cases of lung cancer were identified among males resident in the province of Trieste, who died from 1979 to 1981 or from 1985 to 1986. The two enrollment periods were chosen to cover an extended time span at a reasonable cost. The study had been originally designed to investigate environmental and occupational risk factors for lung cancer. This, together with statistical power considerations, was the reason we restricted the study to male cases only. We excluded 182 cases because we failed to trace the next of kin and 1 case because his residence was outside the Province of Trieste.

For each case, one male control resident in the Province of Trieste, who died within the same 6-month period, at the same age (± 2 years), was randomly selected from the same archive at the Department of Pathology. The causes of death of the controls were not chronic lung diseases or cancer of the upper aerodigestive tract, urinary tract, pancreas, liver, or gastrointestinal system. The sampling probabilities for the control series are usually varied according to the proportion of cases by some relevant variable such as age or sex (*4,5*). The baseline spatial intensity would be therefore distorted, compared to a random sample of death controls. Use of death controls instead of living ones is widely discussed in the epidemiological literature (*6*). Our choice is justified by minimizing selection biases with special reference to residential history.

The present study was based on 755 case–control pairs, determined by age. Each subject's next of kin was interviewed within 1–3 years of the subject's death by means of a structured questionnaire to obtain information on demographic characteristics, smoking habits, occupational history, and last place of residence. Likelihood of exposure to occupational carcinogens was obtained from expert evaluation based on the type of job and also for people working in the iron foundry, shipyard, and incinerator. This summary variable was chosen to increase statistical power, since to include several variables for each job would have led to sparse data and results would have been affected by excess random variation.

Length of residence was not individually assessed; we only assessed if any subject moved from his place of residence in the last 10 years. A detailed description of data collection procedures and exposure coding has been published elsewhere (*1*).

Geographical. The boundaries of the Province of Trieste were coded using the geographical coordinates (Mercatore projection) as provided by the Italian Army Geographical Institute (Florence, Italy; map

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1:10,000). The subject's last residence was identified in the same map, and the geographical coordinates were read directly. The location of the incinerator, the iron foundry, and the shipyard was identified similarly. The city center corresponded to the location of the central square of the town.

For the analysis, we calculated the distance and the angle from each subject location to each pollution source (north orientation). Maps with point locations were produced using ARC/Info 6.1 (*7*); contour plots of relative risk gradient were constructed using Gauss 2.2 (*8*).

Point-source analysis. The present analysis focuses on the spatial intensity $\lambda(x)$ i.e., the frequency of events by unit area at location x. This is the spatial counterpart of the usual concept of rate, having substituted unit time with unit area. When we deal with heterogeneous population denominators, the spatial intensity is expressed in terms of intensity of the population (density of inhabitants) instead of person-years. The spatial intensity as function of the distance from a source is expressed as:

$$\lambda(x) = \lambda_p(x)\rho(x-x_0;\theta)$$

where $\lambda_p(x)$ indicates the population intensity at the location x and $\rho(x-x_0;\theta)$ is the risk as a function of the distance $x-x_0$ from the location of the source (x_0), modeled by the parameters θ.

The case–control design is used to bypass the task of obtaining valid estimates of the population density at each location x. The spatial intensity for the control series (i.e., non-cases) is:

$$\lambda_{CN}(x) = k\lambda_p(x)[1-\rho(x-x_0;\theta)]$$

and for the case series:

$$\lambda_{CS}(x) = c\lambda_{CN}(x)\frac{\rho(x-x_0;\theta)}{1-\rho(x-x_0;\theta)}$$

where k and c are constants determined by study design (sampling fraction and case–control ratio, respectively). The spatial intensity of disease is therefore a function of the odds of disease (the odds being the probability of being ill over the probability of not being ill). To overcome the difficulty in estimating $\lambda_{CN}(x)$, Diggle and Rowlingson (*3*) proposed conditioning the analysis on the observed case and control locations [further details are in Lagazio (*9*)]. We define a logistic regression model in which the odds of disease is:

$$\text{odds}[\rho(x-x_0;\theta)] = w[1 + f(x-x_0;\theta)]$$

assuming an additive scale for the relative risk [where w is a proportionality factor and $f(\cdot)$ is a function to be defined later]. This is plausible because, with a suitable choice of $f(\cdot)$, the risk is unchanged at infinite distance from the source. In the case of multiple sources the model becomes:

$$\text{odds}[\rho(x-x_0;\theta)] = w[1 + \Sigma_s f(x-x_{0s};\theta)]$$

and individual risk factors can be modeled in the following way:

$$\text{odds}[\rho(x-x_0, z;\theta,\gamma)] = w\Pi_j \exp(z_j\gamma_j)[1 + \Sigma_s f(x-x_{0s};\theta)]$$

where s denotes the sth source and γ_j is the log odds ratio for the jth risk factor, z_j. The adjusted excess risk gradient for each source has been modeled as follows:

$$f(x-x_{0s};\theta) = \alpha_s \exp(\beta_s d_s)$$

where the parameter α_s models the excess relative risk at the source location, d_s is the distance (in meters) from the sth source, and the parameter β_s models the exponential decrease of the excess relative risk for longer distances. To allow for directional effects, we define the following model for a given source:

$$f(x-x_0;\theta) = \alpha \exp[\beta_1 d + \beta_2 \sin(\vartheta) + \beta_3 \cos(\vartheta)]$$

where d is the distance and ϑ is the angle between the case or control location and the source location. This is of particular importance when considering a situation like that in Trieste, where the city is located between the coast (southwest) and hills (northeast). Although Trieste is famous for a strong northeast to southwest wind (*bora*) the moderate winds from the sea toward the hills are more relevant for the spread of air pollution.

The model-based spatial analysis was conducted to allow for the contribution of relevant risk factors. These terms were considered in the multiplicative scale in the model: age, smoking habit (nonsmoker, 1–19, 20–39, and >40 cigarettes/day), and exposure to occupational carcinogens (none, possible, likely). Moreover, we included the levels of air particulates as defined in a previous paper (*1*) (tertiles of distribution, 1972–1977: <0.175; 0.175–0.298; >0.298 g/m^2/day). Each subject was assigned the average value measured by the nearest among the 28 stations that covered the city.

In the appendix, we report point estimates and likelihood ratio tests for the significance of the spatial terms in the model. The likelihood surface for those parameter estimates has an odd shape, and therefore their relative standard errors are poorly estimated. In this situation it is preferable to rely on likelihood ratios (*10*). These models are known as mixed additive–multiplicative models for excess relative risks and can be fitted using Epicure software (*11*).

Crude analysis. To describe the observed pattern of relative risk within the study area, we estimated the spatial intensity, $\lambda(x)$ nonparametrically, following the suggestions of Bithell (*12*) and Lawson and Williams (*13*). The spatial intensities for the case and control series are estimated separately as follows:

$$\hat{\lambda}(x_i) = \sum_{j=1}^{n} h_i^{-2} G\left(\frac{x_i - x_j}{h_i}\right)$$

where the kernel function, $G(\cdot)$, has the Epanechnikov functional form (*14*). The terms h_i are smoothing parameters that allow for local variation of the degree of smoothing. They are obtained as $h_i = \eta_i h$, where h is fixed in advance (500 m for our application), and η_i is a previous estimate obtained using the simple nearest-neighbor technique (*14*).

The ratio of the kernel estimates for cases and non-cases is the odds of being a case, given the observed sample (this quantity differs from the odds of being ill because it also depends on the case–control ratio). To obtain easily interpretable contour plots, we back-transformed it to probability; i.e.,

$$\hat{p}(x) = \hat{g}(x)/[1+\hat{g}(x)]$$

where $\hat{g}(x)$ represents the odds of being a case. Because in our study the case–control

Table 1. Relative risks for lung cancer in Trieste: smoking habits, occupational exposures, and levels of air particulates

Variable	Cases	Controls	Odds ratio[a]	95% CL
Smoking (cigarettes/day)	0	22	199	1.0
1–19	225	272	6.7	4.2–11
20–39	302	198	12.8	7.9–21
≥40	206	86	21.3	13–36
Occupational exposure to carcinogens				
No	255	351	1.0	
Possible	282	279	1.4	1.1–1.9
Probable	218	125	2.5	1.8–3.4
Air particulates (g/m^2/day)				
<0.175	188	219	1.0	
0.175–0.298	256	274	1.1	0.8–1.5
>0.298	311	262	1.4	1.1–1.8

[a]Adjusted for smoking, likelihood of occupational exposure to carcinogens, and air pollution.

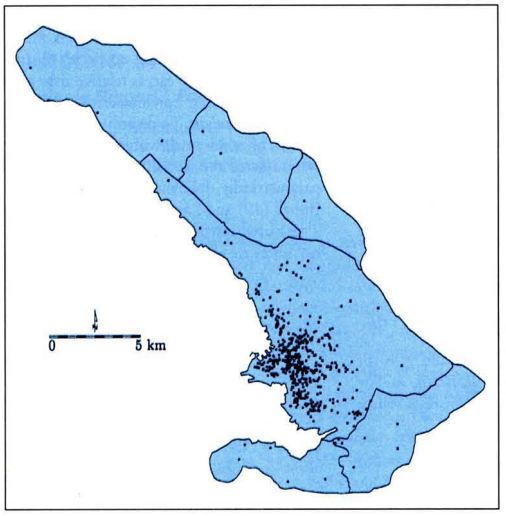

Figure 1. Locations of cases.

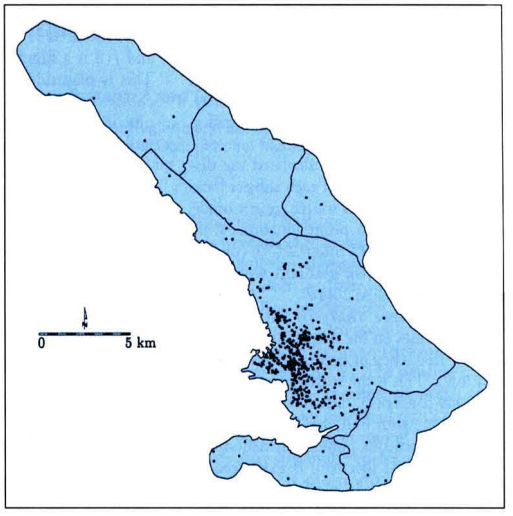

Figure 2. Locations of controls.

ratio is 1, the areas with a probability >0.5 of being a case are characterized by higher risk of disease.

Results

Descriptive statistics and odds ratios for the relevant variables are shown in Table 1. Figures 1 and 2 show the locations of the case and control series. Figure 3 reports the location of the pollution sources and the contour plot of the probability of being a case obtained using adaptive kernel estimators with a 500-m bandwidth. There appears to be a wide risk area in the eastern part of the city with a spot near the city center and two peaks northeast and southeast from the incinerator.

The appendix reports the estimates of the spatial parameters α and βs for each source. The highest excess relative risks is shown by the city center along with the most slowly declining gradient. All these sources appeared to be highly statistically significant.

The distances from the four sources are highly correlated. We chose to consider the city center, the most important source from a statistical point of view, as part of the model and assess the significance of the inclusion of each other source in turn.

The appendix reports the estimates of the spatial parameters of the other sources, adjusting for the effect of individual risk factors and for the effect of city center. The effect of shipyard is no longer statistically significant after adjustment. The iron foundry was of borderline significance (p = 0.09), with an excess relative risk of 5.9 at the source location. The incinerator was highly significant (p = 0.0098), with an excess relative risk of 6.7 and a very rapid decay moving away from the source. No other sources reached statistical significance when city center and incinerator had been included in the model.

Finally, we investigated if there were directional effects with regard to the effect of the incinerator. The appendix shows the results of fitting that model. Although not statistically significant, the point estimates for the directional effects suggested a wind effect from southwest to northeast.

Incidentally, we note the estimates for the levels of particulate: the odds ratios were 1.1 (95% CL, 0.8–1.5) for the second tertile and 1.4 (1.1–1.8) for the highest tertile. When we took into account the distance from the city center and the incinerator, the effect of particulate vanished: second tertile, OR = 1.2 (0.9–1.4); highest tertile, OR = 1.0 (0.7–1.4).

Discussion

The present analysis supports and validates the geographical areas defined in a previous study (*1*). Indeed, the use of the distance between residential location and sources of pollution as a continuous variable provided a more sensitive approach to spatial modeling of risk than the classification of the residences into four areas on the basis of their proximity to each source. Furthermore, the evidence of higher risk in the neighborhood of the incinerator has been confirmed. The excess relative risk estimated at the city center and at the location of the incinerator appears to be consistent as well as the shallow and steep descent, respectively.

The model adopted is simple, allowing an exponential decrease by distance from the source. Although several alternatives could be specified (*15*), we chose the model described here because it could be extended to include more than one source. The peculiar spatial location of the four sources complicate the analysis. The sources appear to be highly correlated, and the geography of the city is heavily affected by its proximity to the coast.

For these reasons we adopted a forward strategy to select the best-fitting model. The final model contains terms for spatial effects of the city center and of the incinerators. This could be due to the indistinguishable effects of the shipyard, the city center, and, to a lesser degree, the iron foundry, which lie on the same line along a north–south direction. The incinerator effects retained statistical significance even when adjusting for individual risk factors and spatial effects of the city center.

The previous analysis based on histological subtypes of lung cancer showed higher relative risks for small cell and large cell carcinoma among residents close to the city center, whereas the relative risk for squamous cell carcinoma and adenocarcinoma was elevated among those residents who lived close to the incinerator (*1*). The presence of a linear trend by level of particulate deposition was significant for small and large cell cancers. In the present study, for all lung cancers there was a significant increase in risk for those resi-

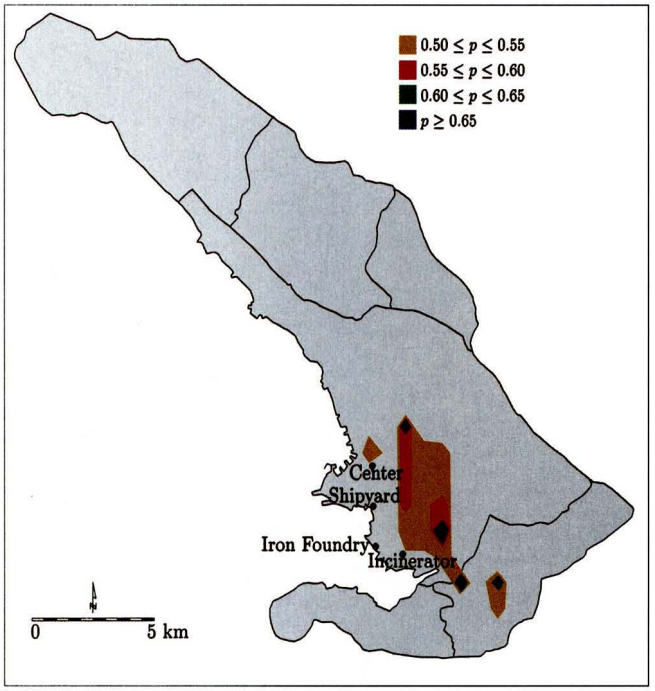

Figure 3: Locations of pollution sources and contour plot of the probability of being a case.

dent in areas in the highest tertile of particulate (>0.298 g/m^2/day, OR = 1.4; 95% CL, 1.1–1.8). This effect appeared to be fully explained once distance from city center and incinerator had been included in the model.

This study was mainly a geographical investigation with characterization of environmental exposure by adjustment for total particulate deposition and residence location. Although the spatial pattern of the risk was adjusted for relevant confounders, residual confounding due to other unmeasured exposure cannot be excluded. Background radiation should not be a problem in this area because it is known that radiation follows a gradient, with a minimum at the city center and a maximum in the rural area at the boundary of the province. A selection bias due to the chosen frame of cases and controls cannot be excluded in principle; however, it should be noted that the subject list is derived from the Cancer Registry, which guarantees the coverage of the resident population and provides high-quality data, including 73% of all deaths autopsied. It was impossible to obtain a complete residential history for each subject enrolled. Therefore, misclassification bias due to change in residence cannot be excluded (we note that eventually this error would push the risk estimates toward the null value; nondifferential misclassification or selective migration of cases, e.g., of terminally ill people, outside the risk areas). The results shown here are coherent with the hypothesis of an independent effect of residing close to the incinerator and the city center. Further investigations should be undertaken to characterize the types and levels of pollutants from the incinerator and the center of the city.

Appendix

Excess risk of lung cancer as a function of distance from city center, shipyard, foundry, and incinerator considered separately

Null model: $\text{odds}[\rho(z;\gamma)] = w\Pi_j \exp(z_j\gamma_j)$

Includes terms for age, smoking habits, occupational exposure and levels of air particulate.

Model 1: $\text{odds}[\rho(x - x_0, z;\theta,\gamma)] = w\Pi_j \exp(z_j\gamma_j)[1 + \alpha_{ce}\exp(\beta_{ce}d_{ce})]$

α_{ce} = risk excess in the source (city center) = 2.209
β_{ce} = risk decay moving away from city center = -0.0151
Likelihood ratio statistic model 1 vs. null model = 7.435, $df = 2$
$p = 0.0243$

Model 2: $\text{odds}[\rho(x - x_0, z;\theta,\gamma)] = w\Pi_j \exp(z_j\gamma_j)[1 + \alpha_{sh}\exp(\beta_{sh}d_{sh})]$

α_{sh} = risk excess in the source (shipyard) = 2.033
β_{sh} = risk decay moving away from the shipyard = -0.01922
Likelihood ratio statistic model 2 vs. null model = 7.868, $df = 2$
$p = 0.0196$

Model 3: $\text{odds}[\rho(x - x_0, z;\theta,\gamma)] = w\Pi_j \exp(z_j\gamma_j)[1 + \alpha_{if}\exp(\beta_{if}d_{if})]$

α_{if} = risk excess in the source (iron foundry) = 1.702
β_{if} = risk decay moving away from the iron foundry = -0.01692
Likelihood ratio statistic model 3 vs. null model = 5.273, $df = 2$
$p = 0.0716$

Model 4: $\text{odds}[\rho(x - x_0, z;\theta,\gamma)] = w\Pi_j \exp(z_j\gamma_j)[1 + \alpha_{in}\exp(\beta_{in}d_{in})]$
α_{in} = risk excess in the source (incinerator) = 1.484
β_{in} = risk decay moving away from the incinerator = - 0.01505
Likelihood ratio statistic model 4 vs. null model = 4.736, $df = 2$
$p = 0.0937$

Excess risk of lung cancer as a function of distance from city center and from either the shipyard, foundry, or incinerator

Null model: $\text{odds}[\rho(x - x_0, z;\theta,\gamma)] = w\Pi_j \exp(z_j\gamma_j)[1 + \alpha_{ce}\exp(\beta_{ce}d_{ce})]$

Includes terms for age, smoking habits, occupational exposure, levels of air particulate and excess risk as function of distance from the city center.

Model 1: $\text{odds}[\rho(x - x_0, z;\theta,\gamma)] = w\Pi_j \exp(z_j\gamma_j)[1 + \alpha_{ce}\exp(\beta_{ce}d_{ce}) + \alpha_{sh}\exp(\beta_{sh}d_{sh})]$

α_{ce} = risk excess in the source (city center) = 0.9091
β_{ce} = risk decay moving away from city center = -0.01855
α_{sh} = risk excess in the source (shipyard) = 1.242

β_{sh} = risk decay moving away from the shipyard = -0.02208
Likelihood ratio statistic model 1 vs. null model = 1.089, $df = 2$
$p = 0.5803$

Model 2: $\text{odds}[\rho(x - x_0, z; \theta, \gamma)] = w\Pi_j \exp(z_j\gamma_j)[1 + \alpha_{ce}\exp(\beta_{ce}d_{ce}) + \alpha_{if}\exp(\beta_{if}d_{if})]$

α_{ce} = risk excess in the source (city center) = 1.857
β_{ce} = risk decay moving away from city center = -0.02439
α_{if} = risk excess in the source (iron foundry) = 5.858
β_{if} = risk decay moving away from the iron foundry = -0.1615
Likelihood ratio statistic model 2 vs. null model = 4.889, $df = 2$
$p = 0.0868$

Model 3: $\text{odds}[\rho(x - x_0, z; \theta, \gamma)] = w\Pi_j \exp(z_j\gamma_j)[1 + \alpha_{ce}\exp(\beta_{ce}d_{ce}) + \alpha_{in}\exp(\beta_{in}d_{in})]$

α_{ce} = risk excess in the source (city center) = 1.959
β_{ce} = risk decay moving away from city center = -0.03523
α_{in} = risk excess in the source (incinerator) = 6.740
β_{in} = risk decay moving away from the incinerator = -0.1762
Likelihood ratio statistic model 3 vs. null model = 9.241, $df = 2$
$p = 0.0098$

Excess risk of lung cancer as a function of distance from city center and incinerator, including an angular component associated with the incinerator

Null model: $\text{odds}[\rho(x - x_0, z; \theta, \gamma)] = w\Pi_j \exp(z_j\gamma_j)[1 + \alpha_{ce}\exp(\beta_{ce}d_{ce}) + \alpha_{in}\exp(\beta_{in}d_{in})]$

Includes terms for age, smoking habits, occupational exposure, levels of air particulate and excess risk as function of distance from the city center and incinerator.

Model 1: $\text{odds}[\rho(x - x_0, z; \theta, \gamma)] = w\Pi_j \exp(z_j\gamma_j)\{1 + \alpha_{ce}\exp(\beta_{ce}d_{ce}) + \alpha_{in}\exp[\beta_{in}d_{in} + \beta_2\sin(\vartheta) + \beta_3\cos(\vartheta)]\}$

α_{ce} = risk excess in the source (city center) = 1.873
β_{ce} = risk decay moving away from city center = -0.03885
α_{in} = risk excess in the source (incinerator) = 4.045
β_{in} = risk decay moving away from the incinerator = -0.1661
β_2 = - 0.6621
β_3 = - 0.1669
Likelihood ratio statistic model 1 vs. null model = 0.5005, $df = 2$
$p = 0.7786$

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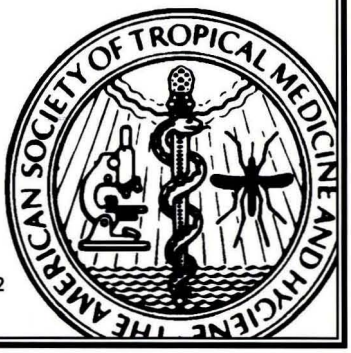

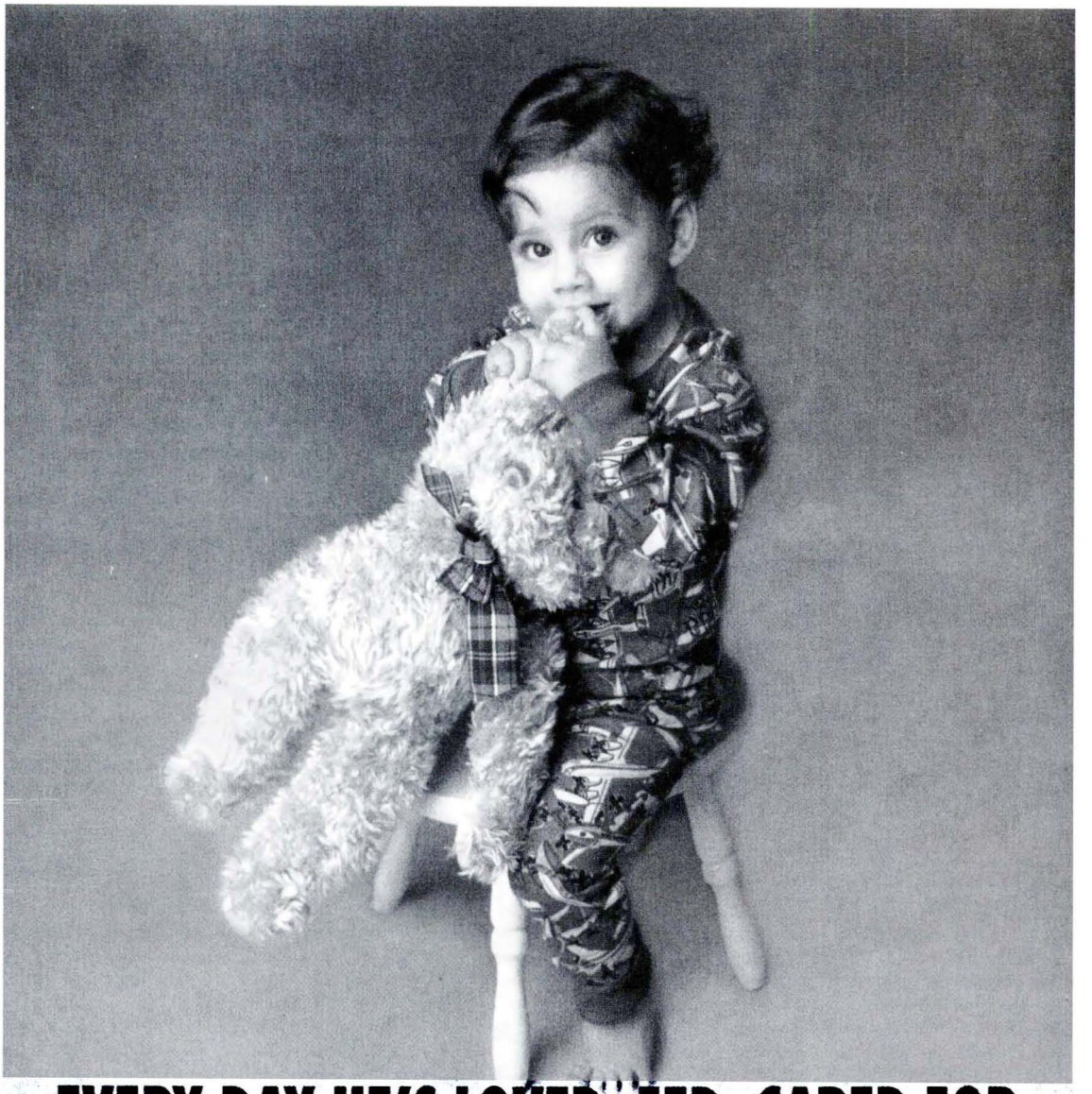

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PCDDs, PCDFs, and PCBs in Human Blood in Relation to Consumption of Crabs from a Contaminated Fjord Area in Norway

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Consumption of fish and shellfish from contaminated areas may be an important source of human exposure to persistent organohalogen compounds such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). We determined concentrations of 2,3,7,8-substituted PCDDs and PCDFs and 19 PCB congeners in whole blood samples from three groups of men, 40–54 years of age, with different consumption levels of crabs from a fjord area in southern Norway polluted with organochlorine compounds from a magnesium production plant. A significant increase of many PCDD/PCDF congeners was found in the blood when comparing the referents, moderate-, and high-intake groups. The greatest difference was observed for several of the PCDFs that are characteristic for the contamination of the marine biota of the fjord. PCBs, in general, play a minor role in the contamination of the fjord by the magnesium production process, except for the highly chlorinated congeners such as PCB-209. Nevertheless, almost all PCBs increased from the referents to the high-intake group. However, the relative concentrations of several highly chlorinated PCBs (particularly PCB-209) in blood are unexpectedly low compared to their abundance in crabs, indicating low uptake of these congeners. The exposure to PCDDs/PCDFs from crab consumption calculated from individual body burdens of these compounds were in good agreement with the intake estimated from previously measured concentrations in crabs, reported fishing sites, and consumption. Almost all subjects in the high-intake group exceeded the tolerable weekly intake of 35 pg TEQ/kg body weight/week proposed by a Nordic Expert Group. *Key words:* blood, crab consumption, polychlorinated biphenyls, polychlorinated dibenzofurans, polychlorinated dibenzo-*p*-dioxins, . *Environ Health Perspect* 104:756–764 (1996)

A magnesium factory, situated in the inner part of the Frierfjord in southern Norway, produces considerable amounts of organochlorine compounds such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) during the production of metallic magnesium (Fig. 1). As much as 50–100 kg TCDD toxic equivalents (TEQs) may have been discharged to the Frierfjord during the past 35 years (*1*). As part of the National Program on Pollution Monitoring, PCDDs/PCDFs, PCBs, and non-*ortho*-PCBs have been monitored in sediments and marine organisms at various distances from the source since 1986, 1989–1990, and 1992, respectively (*2–4*). In spite of a reduction in the emissions of organochlorine compounds by more than 98% from 1990 to 1992 (*1*), considerable amounts of PCDDs and PCDFs are still observed in most marine organisms of the area. Restrictions on commercial fishing and recommendations to the general public to reduce consumption of fish and shellfish have been established for the contaminated fjord area. However, the area is popular for recreational purposes, and some residents still catch and consume considerable amounts of local fish and shellfish. Particularly during the summer and autumn, the crab species *Cancer pagurus* is a popular food item in this area. These crabs contain high concentrations of organochlorine compounds, revealing the characteristic isomer pattern of the magnesium process (*5*) with tetra- to hexa-CDFs being the predominant PCDD/PCDF congeners and decachlorobiphenyl (PCB-209) the most abundant PCB congener.

PCDDs, PCDFs, and PCBs are complex mixtures of persistent lipophilic substances and tend to accumulate in marine and terrestrial food chains. The general population is mainly exposed to these substances through fatty food, leading to a background body burden of these substances (*6–8*). People consuming large amounts of contaminated seafood may have elevated concentrations of organochlorine compounds in their tissues compared to the general population (*9–14*). Due to low biodegradation and excretion in humans, these substances accumulate in the body fat, and their concentrations reflect external exposure (*6–8,15,16*). Cumulative exposure has been assessed by analyzing adipose tissue, human milk, and/or blood (*17–20*).

The patterns of PCDDs, PCDFs, and PCBs in humans do not directly reflect the patterns of discharge to the environment owing to differences in physical properties, e.g., lipophilicity and volatility, and biodegradability of the individual compounds in the food chain. Of the PCDD/PCDF congeners, the 2,3,7,8-substituted PCDD and PCDF congeners are the most resistant to metabolism and are generally the only congeners found in human tissue (*7,8*). The hepta- and octa-CDD congeners are by far the most abundant in samples from the general population (*8*). In contrast, fish and other organisms from the aquatic environment usually contain quite low concentrations of these congeners (*21,22*).

For risk assessment purposes and to assist in risk management, the concept of toxic equivalency factors (TEFs) for the 2,3,7,8-substituted congeners has been developed to express the toxic potency of complex mixtures of PCDDs/PCDFs in biological samples by a single value, the 2,3,7,8-TCDD toxic equivalent (TEQ) (*23,24*). In addition, several of the non-, mono-, and di-*ortho*-substituted PCBs, which induce effects similar to those caused by PCDDs and PCDFs, have been given provisional TEF values (*25*). Even though the application of the proposed TEFs to health risk assessment has been criticized, particularly for the PCBs (*26*), they are widely used for risk management.

The objective of the present study was to assess the role of consumption of crabs from the contaminated fjord area for the exposure to PCBs, PCDDs, and PCDFs. We therefore determined blood concentrations of these compounds in 24 male crab consumers and 10 referents and recorded information on crab consumption and fishing site as well as consumption of fish and other food items to answer the following questions: 1) Does the consumption of crabs from the Frierfjord area lead to increased body burdens of PCDD/PCDF

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and PCB compounds? 2) Are the patterns for PCDDs/PCDFs and PCBs found in human blood changed by the exposure through crab consumption? 3) Do exposure estimates based on measured blood concentrations agree with those based on reported crab intake? 4) What is the individual exposure level compared to present recommended tolerable intake of toxic equivalents?

Materials and Methods

Study group and sample collection. It is well documented that the body burden of PCDD/PCDF and PCB compounds increases with age and for women decreases with the number of breastfed children (*7,18,27*). To avoid variation of body burden with age and sex, we restricted the study to male subjects in a relatively close age range. The study was approved by the Regional Committee of Medical Research Ethics, and informed consent was obtained from all participants. A total of 34 male volunteers, age 40–54 years, participated in the study. All subjects were living in the Frierfjord area of Norway. Male crab consumers (high and moderate intake) were recruited nonrandomly through announcements in the newspapers and other media. Of the 24 crab consumers, 20 had eaten crabs for more than 10 years, 2 for 5–10 years, and 2 did not report the duration of crab consumption. The male referents were drawn randomly from the Register of Population. Blood was sampled after overnight fasting. About 250 ml venous blood was drawn from each subject into thoroughly cleaned glass bottles (Duran, Schott, Germany) and kept frozen at -20°C until analyzed. A questionnaire including information on crab and fish consumption, intake of other fatty food items, and other relevant factors was completed by each donor. All subjects reported being healthy. Information concerning the subjects of the three groups is summarized in Table 1.

Standards and chemicals. Acetone was glass-distilled grade and all the other organic solvents were pestiscan grade from Labscan (Dublin, Ireland) or Merck (Darmstadt, Germany). Sulfuric acid (Scanpure, 98.3%) and nitric acid (Scanpure, 65%) were purchased from Chem Scan A/S (Elverum, Norway). The $^{13}C_{12}$-labeled PCB-77, PCB-126, PCB-169, and 2,3,7,8-substituted PCDDs and PCDFs as well as the native PCDDs and PCDFs and ^{37}Cl-2,3,7,8-TCDD were from Cambridge Isotope Laboratories (Woburn, Massachusetts). The normal PCB standards were purchased from Cambridge Isotope Laboratories or from Restek (Sulzbach, Germany). Silica gel, aluminum oxide, sodium sulfate, and potassium hydroxide were from Merck and the activated carbon AX-21 was from Anderson Development Company (Adrian, Michigan). All adsorbents were prepared as previously described by Smith et al. (*28*) and Oehme et al. (*21*). All glassware for organochlorine analysis was washed in 2.5% RBS detergent and distilled water, then heated in an oven at 500°C overnight.

Determination of PCDDs, PCDFs, and non-ortho PCBs. The extraction was performed after a method described by Päpke et al. (*29*). We packed 100 g of Hydromatrix (plankton marine diatomite; Varian Sample Preparation Products, Harbor City, California) and 50 g of sodium chloride in alternating layers into glass columns (30 cm × 5 cm i.d.). Thereafter, the adsorbents were washed with 375 ml of *n*-heptane/isopropanol (3:2, v/v) and 375 ml of methylene chloride and dried at 50°C overnight. We transferred 45–50 g of whole blood, spiked with internal standards and diluted with water and ethanol (1:0.66:0.13, v/v/v) to the column. The extraction was performed by eluting with 650 ml of *n*-heptane/isopropanol (3:2, v/v). The eluate was concentrated under a gentle stream of purified nitrogen and dissolved in about 2 ml of cyclohexane. The extract was transferred to glass columns (10 cm × 2 cm i.d.) filled with sodium sulfate for removal of any precipitated salt and evaporated to dryness with a gentle stream of nitrogen before gravimetric determination of the lipids.

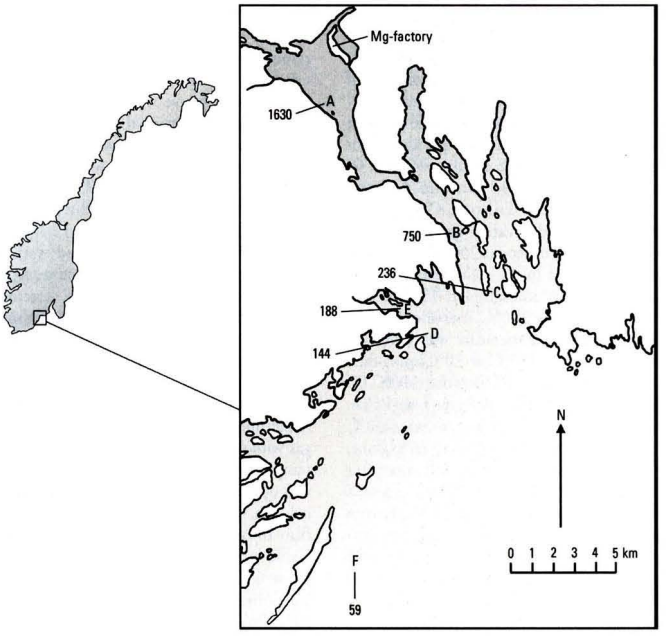

Figure 1. Map of Norway and the Frierfjord fjord area, with concentrations of PCDDs/PCDFs (pg TEQ/g wet weight) in crab hepatopancreas obtained during the National Program on Pollution Monitoring 1992 (*3*) at the regular sampling sites A to F.

Table 1. Mean and range of age, body mass index (BMI), crab and fish consumption, % fat in the blood samples, and polyunsaturated fatty acids (PUFA) in serum phosphatidylcholine for the three groups

	Referents (n = 10)	Moderate intake (n = 15)	High intake (n = 9)
Age (years)	46.7 (41–50)	45.33 (40–54)	46.22 (41–52)
BMI (kg/m^2)	25.4 (22.2–30.7)	26.2 (21.7–32.7)	27.3 (21.5–30.9)
Crab intake (no./year)	0	11.9 (10–38)	77.2 (40–150)
Crab equivalents (no./year)	0	19.6(10–115)	93.2 (24.8–156)
Fish intake (meals per week)	1.1 (0.5–2.5)	1.4 (0.25–2.5)	1.8 (0.5–3.5)
Extracted fat from whole blood (weight %)	0.39 (0.29–0.48)	0.37 (0.28–0.55)	0.47 (0.33–0.69)
Σn-3 PUFA (weight %)	11.72 (8.95–16. 01)	12.98 (8.3–20.46)	11.64 (8.66–15.5)
Σn-6 PUFA (weight %)	35.25 (30.43–38.89)	34.31 (24.55–38.8)	35.88 (31.32–38.88)

The clean-up was carried out in a multicolumn system according to a slight modification of the method described by Smith et al. (*28*). In brief, the fat extract was passed through two columns in series using a total of 825 ml of dichloromethane/cyclohexane (1:1, v/v) followed by 50 ml of dichloromethane/methanol/benzene (15:4:1, v/v/v). The first column contained sodium sulfate, potassium silicate, and silica gel, and the second contained activated carbon (AX-21) dispersed on glass fibers. PCDDs and PCDFs were removed from the carbon column by reversed elution with 100 ml of toluene. The crude dioxin fraction was further purified by chromatography using two Pasteur pipettes in series filled with acidic silica and basic alumina, respectively. ^{37}Cl-2,3,7,8-TCDD was added to the final extract as a recovery control standard and 10 µl of *n*-nonane as a keeper before concentrating to about 15 µl.

The GC-HRMS instrument consisted of a VG AutoSpec high-resolution mass spectrometer with Opus Quan software program and a Hewlett Packard 5890 gas chromatograph with a DB-5 MS capillary column, 60 m × 0.25 mm i.d., 0.1-µm film thickness (J&W Scientific, Folsom, California). Helium was used as carrier gas with a linear velocity of 28 cm/sec (at 200°C). Injections were performed in the splitless mode using an HP 7673 A autosampler. The injector temperature was 280°C. For dioxins, the initial column temperature was 90°C (1-min hold), then 197°C at 20°C/min, then 250°C at 2°C/min, and thereafter 300°C (5-min hold) at 5°C/min. For non-*ortho*-PCBs, the initial column temperature was 130°C (1-min hold), then 200°C at 20°C/min, then 212°C at 0.6°C/min, and thereafter 300°C (10-min hold) at 5°C/min. Selected ion detection was carried out in the electron impact mode with an MS ion source temperature of 250°C, an electron energy of 35 eV, and a resolution of 10,000 and 9,000 for the dioxins and the non-*ortho*-PCBs, respectively. Dwell time for each ion was 80 msec. Perfluorokerosene was used to provide suitable lock masses. The transfer line was held at 280°C.

Quantitation. ^{13}C-labeled analogues of the 2,3,7,8-substituted PCDDs and PCDFs, except OCDF, were added as internal standards before extraction of samples. ^{37}Cl-2,3,7,8-TCDD was added to the final extract as a recovery control standard. Multilevel calibration was performed. Detection limits were 0.5–8 pg/g fat, depending on the congener. Recoveries of the internal standards were between 70 and 100%.

For non-*ortho*-PCBs, ^{13}C-labeled analogues were used as internal standards. Multilevel calibration was performed before or directly after each analysis series. PCB-189 was added to the final extract to measure the recovery of the ^{13}C-labeled standards. Detection limits were 1–7 pg/g fat for different congeners and sampling conditions. Recoveries were between 50 and 80%. PCB-77 could not be quantitated due to coelution with contaminants. However, this non-*ortho*-substituted PCB does not contribute significantly to the dioxin-related toxicity as both the concentration in human samples is generally low (*16,19*), and its WHO-TEF value is very small.

Determination of PCBs. Mono- and multi-*ortho*-PCBs were extracted from 15 ml of whole blood by the same extraction procedures as the PCDDs and PCDFs and the non-*ortho*-PCBs. The lipid extracts were dissolved in cyclohexane (about 0.1 g lipid/ml) and treated twice with an equal volume of concentrated sulfuric acid to remove the major part of the lipids and other interfering organic compounds. The sulfuric acid phase was reextracted with cyclohexane. Additional purification was performed by chromatography on basic alumina using hexane/dichloromethane (80/20, v/v) as an eluent. The organic phase was reduced to 1 ml by a gentle stream of purified nitrogen.

GC analysis was performed with a Perkin Elmer 8700 gas chromatograph equipped with an electron capture detector, an AS-8300 autosampler and a PE Nelson Model 1020 personal integrator (Perkin-Elmer Corp., Beaconsfield, UK). Hydrogen was used as carrier gas with a linear velocity of 28 cm/sec (at 100°C). Argon/methane (5%) was used as make-up gas with a flow rate of 50 ml/min. Injector temperature was 270°C and the detector was operated at 330°C. A DB-5 capillary column, 50 m × 0.25 mm i.d., 0.25 µm film thickness (J&W Scientific, Folsom, California) was temperature-programmed from 60°C (1-min hold) to 200°C at 20°C/min, then to 280°C (10-min hold) at 2.0°C/min.

PCB-116 was used as an internal standard for the mono-*ortho* and multi-*ortho* PCB congeners (IUPAC nos. 28, 52, 74, 99, 105, 118, 128, 138, 153, 156, 157, 170, 180, 187, 194, 206, 209). Detection limits for the multi-*ortho*- and mono-*ortho*-substituted PCBs were 0.01 to 0.05 ng/g fat, depending on the congener.

Toxic equivalency factors. Concentrations expressed in 2,3,7,8-TCDD toxic equivalents (TEQs) were calculated according to a model proposed by a Nordic expert group (*30*) for PCDDs/PCDFs and by an expert group convened by the World Health Organization (WHO) (*25*) for the PCBs. The Nordic model differs from the international TEFs (*31*) in that the TEF for 1,2,3,7,8-penta-CDF is 0.01 (i.e., a factor of 5 lower than in the international model).

Quality assurance of organochlorine compound analysis. All samples were analyzed coded. For each set of five samples, a method blank was prepared using the same extraction and preparation procedures. New glass columns and adsorbents were used for each sample to avoid cross-contamination. For PCDDs/PCDFs, most blank samples contained less than 1 pg of all analytes, except for the ubiquitous octachlorodibenzo-*p*-dioxin (OCDD). The repeatibility of the entire method, the recovery rates of isotopically labeled internal standards, and the detection limit for the method were in good agreement with those values reported by Päpke et al. (*29*), who used the method successfully in WHO interlaboratory control studies.

For PCBs, recovery rates for all quantitated congeners throughout the procedure were in the range of 45–80%. Good analytical quality for determination of PCBs in fish oil and of PCDDs/PCDFs in human milk was confirmed by successful participation in interlaboratory quality control studies organized by the Swedish Environmental Protection Agency in 1991 and by WHO/EURO in 1991–1992, respectively.

Determination of fatty acids. We allowed 10 ml of venous blood to clot and immediately separated serum by centrifugation, cooled it, and froze it within 6 hours at -70°C until analysis. The concentration of fatty acids in plasma phospholipids was measured essentially as described elsewhere (*32*). Briefly, plasma lipids were extracted with *n*-butanol (*33*) and phosholipids were isolated from the lipid extracts by column chromatography on Sep-Pak C_{18} cartridges (Waters Corp., Milford, Massachusetts). Diheptadecanoylglycerophosphocholine and butylated hydroxytoluene were added as internal standard and antioxidant, respectively. Phospholipids were transmethylated and quantitated by gas chromatography (*34*). A reference human serum sample was included as a control to monitor analytical performance. The day-to-day coefficient of variation for 20:4 (*n*-6), 20:5 (*n*-3), and 22:6 (*n*-3) fatty acids were 3.8, 3.7, and 4.7%, respectively. The results were quantitated as milligrams of phospholipid fatty acid per liter of serum.

Statistical analysis. Nonparametric tests were chosen for statistical analysis because lack of normality was found in several distributions of PCB, PCDD, and PCDF concentrations. The Mann-Whitney *U*-test in the statistical program Statview SE (Abacus Concepts, Inc.

Berkeley, California) was used to compare groups. Significant difference was set at $p < 0.05$. Correlations were calculated using the Pearson correlation coefficients.

Results

The crab consumers were divided into two groups, a moderate-intake group (10–38 crabs per year) and a high-intake group (>40 crabs per year). These two groups and referents were compared with respect to age, body mass index, fish intake, fat in whole blood, and relative levels of 22:5 (*n*-6) polyunsaturated fatty acids (PUFA), total *n*-3 PUFA, and total *n*-6 PUFA in blood (Table 1). In addition to differences in crab intake, there was a slight nonsignificant increase in the intake of fish, particularly for the high consumers of crabs. There were no differences with respect to consumption of milk and dairy products or other sources of animal fat. There was no other significant difference in age of the subjects, ranging from 40 to 54 years, and no correlation between the age and the level of PCDDs and PCDFs in blood was observed.

In addition to the the number of crabs eaten, exposure to organochlorine compounds (OCs) is highly dependent on the location of the fishing sites and which parts of the crabs are consumed. The hepatopancreas of the crab has a high fat content (about 15–20%) compared to the rest of the crab meat and accordingly contains most of the OCs. All but two of the subjects in our study group reported eating whole crabs, including the hepatopancreas.

There was a considerable decrease (about 25 times) of PCDD/PCDF in crab hepatopancreas from the inner part of the fjord (site A), close to the magnesium factory, to sampling site F, about 35 km from the source (Fig. 1). To account for this gradient in organochlorine content of crabs as a function of distance from the source, we introduced equivalency factors according to the relative PCDD/PCDF content of the crab hepatopancreas. These factors ranged from 10 at site A (corresponding to 1630 pg TEQ/g wet weight) to 0.36 at site F (corresponding to 59 pg TEQ/g wet weight). The reported number of crabs consumed was then multiplied with the factors closest to the reported fishing sites. In cases where the fishing sites were at approximately equal distances from two monitoring sites, interpolated factors were used. Crab equivalents consumed per year in the different groups are given in Table 1.

Concentrations of 17 2,3,7,8-substituted PCDDs and PCDFs, given as mean, median, and ranges, divided into three groups according to the reported crab intake, are listed in Table 2. Blood concentrations for many PCDD and PCDF congeners in crab consumers are significantly raised compared to the referents, particularly for the penta- and hexa-CDFs. The most pronounced difference between the control and high-intake groups (more than 14 times) was observed for 1,2,3,4,7,8-hexa-CDF. There was also a significant increase in the level of several PCDDs, mostly the lower-chlorinated ones. In contrast, the concentrations of hepta- and octa-CDDs tended to decrease from the control to the high-intake group, but not significantly.

In Figure 2, the profile of 2,3,7,8-substituted congeners in crab hepatopancreas is compared with concentrations found in the blood from persons with no and high crab consumption. The PCDD/PCDF profile in the high-intake group is clearly influenced by the profile found in crab hepatopancreas. The PCDD profiles of the blood samples from both the high-intake group and the referents are dominated by octa-CDD which contributes little to the sum of PCDDs/PCDFs in crab hepatopancreas; however, this congener is clearly less dominant in the crab eaters.

When plotting blood concentrations of individual congeners against the intake of crab equivalents, good linear correlations (*r*

Table 2. Mean, (median), and range of PCDDs and PCDFs congeners in blood samples for the three groups of men with different crab intake[a]

Congener	Referents (*n* = 10)	Moderate intake (*n* = 15)	High intake (*n* = 9)
2,3,7,8-TCDD	3.6 (3.1)* 0.2–7.0	7.7 (6.8) 3–13.6	11.0 (9.2) 6.3–22.4
1,2,3,7,8-PeCDD	5.9 (5.6)* 0.5–10.6	17.3 (15.0)** 6.9–34.8	28.3 (24.8) 15.4–45.13
1,2,3,4,7,8-HxCDD	2.4 (2.2)* 0.9–3.4	8.0 (6.3) ND–30.1	10.8 (11.4) 4.0–17.4
1,2,3,6,7,8-HxCDD	14.7 (14.2)* 2.7–24.5	27.6 (24.8) 13.1–48.2	39.1 (34.4) 16.9–63.7
1,2,3,7,8,9-HxCDD	4.3 (3.8) 0.6–7.3	8.6 (6.7) ND–43.5	9.9 (8.8) 5.9–20.9
1,2,3,4,6,7,8-HpCDD	54.1 (34.7) 10.0–179.1	45.5 (39.8) 20.8–77.4	33.3 (31.1) 16.9–63.7
1,2,3,4,6,7,8,9-OCDD	477.9 (470.3) 51.7–951.1	335.6 (350.9) 157.1–440.4	266.8 (284.9) 104.2–362.9
ΣPCDDs	562.8 (532.5)	450.3 (450.3)	399.3 (404.6)
PCDDs, Nordic-TEQs	9.7 (8.7)	21.5 (18.8)	31.8 (27.7)
2,3,7,8-TCDF	2.8 (2.9) 0.6–5.0	5.1 (4.1) ND–12.7	7.2 (6.4) 1.7–16.5
1,2,3,7,8,-PeCDF	1.8 (1.6)* 0–10.9	7.2 (6.3) 1.5–19.5	13.4 (13.3) 1.29–34.59
2,3,4,7,8-PeCDF	17.1 (15.5)* 4.9–33.5	54.0 (52.8)** 17.6–111.9	102.2 (103.6) 51.5–147.8
1,2,3,4,7,8-HxCDF	8.7 (7.4)* 1.9–21.4	54.9 (55.2)** 10.8–107.1	130.1 (130.2) 34.3–232.6
1,2,3,6,7,8-HxCDF	9.7 (7.7)* 2.3–21.9	44.8 (46.9)** 8.8–90.3	102.7 (77.8) 26.6–217.1
2,3,4,6,7,8-HxCDF	4.3 (3.8)* 1.6–6.7	8.9 (7.9) ND–33.0	14.3 (10.9) 3.2–29.4
1,2,3,7,8,9-HxCDF	0.9 (0.9)* 0–1.0	3.6 (1.1) ND–34.3	2.3 (2.0) 0–5.4
1,2,3,4,6,7,8-HpCDF	18.0 (13.3) 2.5–53.3	44.3 (46.8)** 0.1–117.5	93.0 (92.6) 26.8–201.2
1,2,3,4,7,8,9-HpCDF	1.0 (1.1) ND–0.9	4.8 (1.5) ND–46.2	2.6 (2.4) ND–5.3
1,2,3,4,6,7,8,9-OCDF	8.6 (6.1) 1.3–30.0	13.4 (7.4) ND–93.9	5.4 (6.1) 2.1–8.8
ΣPCDFs	72.8 (60.2)	241.0 (230.1)	473.3 (446.3)
PCDFs, Nordic-TEQs	11.4 (10.2)	39.3 (38.5)	77.9 (75.7)
ΣPCDDs/PCDFs	631.1 (589.4)	691.3 (680.4)	872.5 (850.9)
PCDDs/PCDFs, Nordic-TEQs	21.1 (18.9)	60.8 (57.3)	109.6 (103.4)

ND, not dectected.
[a]Concentrations are given in pg/g fat.
*Significantly different as compared with the moderate-intake group ($p<0.05$ by Mann-Whitney U-test).
**Significantly different as compared with the high-intake group ($p<0.05$ by Mann-Whitney U-test).

>0.6) were obtained for 2,3,4,7,8-penta-CDF, 1,2,3,4,7,8-hexa-CDF, and 1,2,3,6,7,8-hexa-CDF, as well as TCDD and penta-CDD. The correlations were lower (r = 0.4–0.6) for 2,3,7,8-tetra-CDF, 1,2,3,7,8-penta-CDF, 1,2,3,4,7,8-hexa-CDF and 2,3,4,6,7,8-hexa-CDF (not shown).

Mean, median, and ranges of blood concentrations of 2 non-*ortho*-PCBs, 4 mono-*ortho*-PCBs, and 13 other PCBs for the three study groups are given in Table 3. Almost all PCB congeners increased slightly from the referents to the high-intake group with significant increases for PCB-99, 128, 156, 157, 170, 180, 194, 206, and 209. However, no significant correlation was observed between the concentrations of sum PCBs (dominated by PCB-138, 153, 170, and 180) and crab intake (r = 0.19).

A comparison of the PCB profiles in crab hepatopancreas (*3*) from the Frierfjord area and in blood samples of the referents and the high-intake group is shown in Figure 3. There are only minor differences in the profiles for the referents and the high-intake group, and there is little agreement between the blood profiles and the profile found in crabs. The high concentration of PCB-209 found in crabs from the Frierfjord area is not reflected in the blood of the crab consumers.

The contribution of different dioxinlike compounds to the total TEQs for the three study groups is presented in Figure 4. While there is only a slight increase in TEQs for the PCBs with increasing crab consumption, the PCDD/PCDF-related TEQs are about five times higher in the high-intake group compared to the referents. Thus, the relative contribution of PCBs to the total TEQs drops from 65% in the referents to about 35% in the high-intake group.

When plotting the PCDD/PCDF-related TEQs in blood against the intake of crab equivalents, a good correlation was observed (Fig. 5, r = 0.75). When only numbers of crabs were used, the correlation factor was 0.68, and several of the data points for subjects who caught crabs close to extreme sites B and F (see Fig. 1) were remote from the regression line. This emphasizes the usefulness of crab equivalents, which accounts for the gradient in PCDD/PCDF concentrations of crabs as a function of the distance from the source.

Based on the measured blood concentrations, we wanted to estimate the total body burden of PCDDs /PCDFs, expressed as TEQs. We assumed that the whole dose is evenly distributed in the body fat compartment (*35–37*). The percentage of body fat was calculated from the body mass index

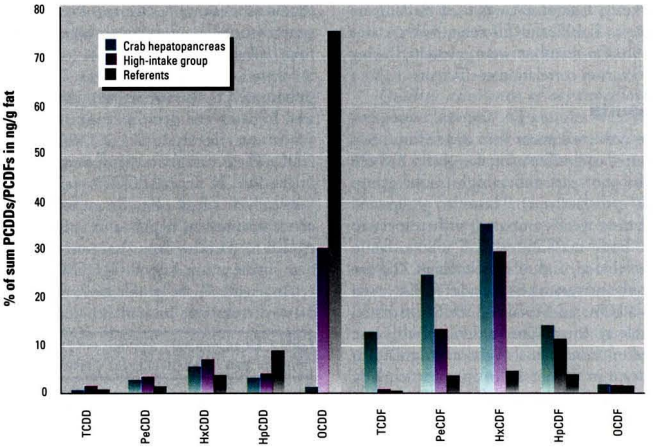

Figure 2. Profile of PCDDs/PCDFs in blood samples from referents and high-intake group compared to the profile in crab hepatopancreas from sampling site D.

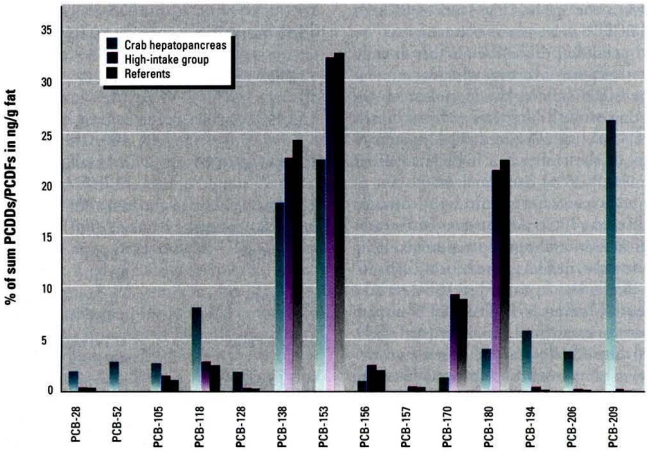

Figure 3. Profile of PCBs in blood samples from referents and high-intake group compared to the profile in crab hepatopancreas from sampling site D.

and age, according to an empirical equation developed by Deurenberg et al. (*38*). The mean body burdens and ranges for the referents, the moderate-intake group and the high-intake group were 5.2 (1.1–11.5), 15.5 (5.5–30.4), and 28.7 (12.7–45.9) ng/kg body weight, respectively. Based on these calculated body burdens, we wanted to estimate the average weekly intake. Several assumptions were made. Although the kinetics differ between the individual compounds (*39*), PCDD/PCDF concentrations were expressed as a single number, the TEQ. We use a single-compartment model with first-order kinetics. Several half-life values for 2,3,7,8-TCDD elimination have been reported varying from 3 to 11 years (*40,41*). We assumed an average half-life of 7 years and assumed that PCDD/PCDF concentrations in the subjects had reached a steady state. Assuming complete absorption, we used the following equation to calculate the mean weekly intake:

$$WI = C \times BF \times \left[\frac{\ln 2}{7(52\ \text{weeks})}\right]$$

where WI = weekly intake (pg/kg body weight/week), C = concentration of

Table 3. Mean, (median) and range of PCB congeners in blood samples for the three groups of men with different crab intake[a]

Congener	IUPAC no.	Referents (n = 10)	Moderate intake (n = 15)	High intake (n = 9)
2,4,4′	28	2.9 (3.3) 1–4.3	4.1 (4.8) 1.3–9.4	4.2 (3.2) 1.8–10.4
2,2′,5,5′	52	0.4 (0.5) ND–0.6	0.7 (0.6) ND–1.9	0.6 (0.6) ND–0.9
2,4,4′,5	74	14.1(15.5) 5.2–20.5	11.6 (12.3) 0.7–24.6	17.5 (18.3) 4.2–21.8
2,2′,4,4′,5	99	12.1 (11.1)* 4.5–18.2	12.1 (10.1) 3.8–21.8	16.1 (14.7) 5.1–23.9
2,2′,3,3′,4,4′	128	1.5 (1.4)* 1.1–2.8	1.5 (1.4) 0.3–2.8	3.7 (3.3) 1.9–6.8
2,2′,3,4,4′,5′	138	287.4 (223.9) 145.9–359.5	329.8 (289.2) 220.1–478.1	334.2 (299.1) 180.7–516.4
2,2′4,4′,5,5′	153	385.3 (361.9) 188.6–553.6	438.8 (390.3) 236.1–699.6	479.2 (417.3) 289.2–751.5
2,2′,3,3′,4,4′,5	170	105.5 (100.4)* 63.9–141.9	124.7 (116.7) 34.9–229.0	140.5 (133.4) 86.6–314.8
2,2′,3,4,4′,5,5′	180	264.3 (237.9)* 135.3–346.0	283.5 (256.5) 239.9–610.9	317.4 (315.9) 239.0–650.9
2,2′,3,4′,5,5′,6	187	34.1 (36.6) 5.6–25.4	39.8 (25.1) 1.4–38.9	46.8 (44.2) 8.0–29.2
2,2′,3,3′,4,4′,5,5′	194	1.6 (0.53)* ND–4.8	4.2 (4.3) ND–9.0	6.2 (5.6) 3.1–11.4
2,2′,3,3′,4,4′,5,5′,6	206	1.5 (1.4)* 0.3–0.9	3.6 (3.5) ND–11.9	3.2 (4.2) ND– 5.61
2,2′,3,3′,4,4′,5,5′,6,6′	209	0.9 (1.2)* 0–2.3	2.1(1.8) 1.4–4.7	3.4 (4.7) 1.2–9.9
2,3,3′,4,4′	105	11.7 (9.2) 1.9–19.9	17.5 (18.3) 4.3–49.8	21.9 (19.9) 11.7–41.9
2, 3′,4,4′,5	118	29.3 (32.2) 11.1–42.6	35.6 (34.2) 2.3–72.1	41.7 (39.2) 38.9–74.8
2,3,3′,4,4′,5	156	23.7 (26.2)* 8.7–34.6	31.5 (29.7) 6.7–46.3	37.4 (35.6) 22.3–53.7
2,3,3′,4,4′,5′	157	4.2 (4.4) 1.9–6.0	3.6 (3.5) 0.4–9.6	7.2 (6.5) 3.7–13.3
3,3′4,4′	77	NA	NA	NA
3,3′4,4′,5	126[b]	93.4 (100.7) 8.02–173.2	93.03 (92.6) 14.8–222	94.5 (98.1) 20.2–286.8
3,3′4,4′,5,5′	169[b]	70.1 (72.7) 4.9–108.9	94.7 (92.8) 18.3–199.1	119.6(89.7) 23.4–254.1
ΣPCBs	1180.6 (1067.8)	1344.2 (1202.5)	1481.4 (1365.9)	

ND, not detected.
[a]Concentrations are given in ng/g fat, if not stated otherwise.
[b]Value in pg/g fat.
*Significantly different as compared with the high-intake group (p<0.05 by Mann-Whitney U-test).
NA, not analyzed due to interfering compounds.

PCDD/PCDF in blood (pg/g fat), and BF = body fat fraction (g/kg body weight). It is obvious that this approach can only give a rough estimate of the weekly intake, particularly due to the assumption of a single half-life for all PCDD/PCDF congeners. Calculation of intake of PCDD/PCDF from measured blood levels gives for the referents a weekly intake of 9.7 (2–22) pg/kg body weight (mean and ranges), for the moderate-intake group 31 (10–61) pg TEQ/kg body weight, and for the high-intake group 62 (24–114) pg TEQ/kg body weight/week.

We further calculated the yearly intake of PCDDs/PCDFs from crab consumption using the differences between the blood concentrations of the individual crab consumers and mean blood concentrations of the referents. The values were 63–317 ng TEQ/year for the high-intake group and 2–244 ng TEQ/year for the moderate-intake group. These values were compared to estimates of yearly intakes based on previously measured concentrations in crabs, reported fishing site, and consumption. The linear correlation coefficient was r = 0.61 (Fig. 6). Given the uncertainty of crab consumption over the years and reported fishing sites, there is, except for a few subjects, remarkably good agreement between the two methods of estimating the intake.

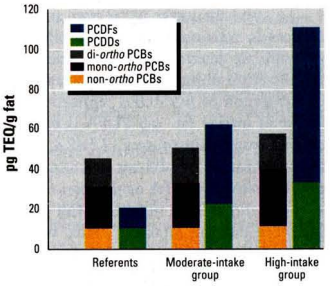

Figure 4. Mean blood concentrations (pg TEQs/g fat) of non-*ortho*-, mono-*ortho*- and di-*ortho*-PCBs, PCDDs, and PCDFs for the referents and the two crab-consuming groups.

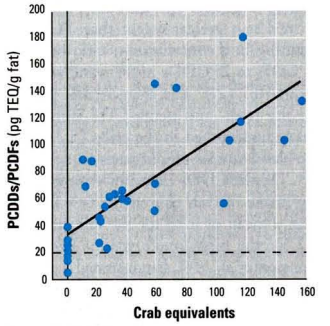

Figure 5. Relationship between intake of crab equivalents and blood concentrations of PCDDs and PCDFs (pg TEQ/g fat) in 34 subjects (r = 0.75).

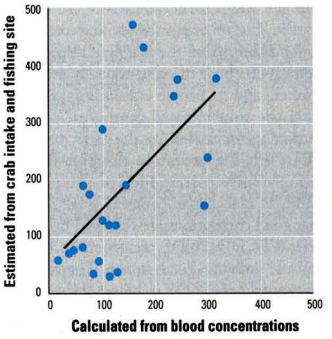

Figure 6. Relationship between estimates of PCDD/PCDF intakes (in ng TEQ/year) from crab consumption using either PCDD/PCDF blood concentrations or reported crab consumption and fishing site.

To assess the intake of fat from marine sources in relation to the PCDD/PCDF exposure, we determined the fatty acid profile in serum phospholipids. Whereas the concentration of *n*-3 PUFA represents the intake of marine fat, *n*-6 PUFA is mainly derived from plant sources (*12,42,43*). In the present study, we did not find a correlation between *n*-3 PUFA or the ratio of *n*-3 to *n*-6 PUFA and the number of fish meals per week nor the intake of crabs, which were mostly consumed more than 6 months before blood sampling. Consequently, we have not been able to find any correlation between the levels of *n*-3 PUFA and concentrations of PCDDs/PCDFs.

Discussion

During magnesium production, PCDFs are formed to a greater extent than PCDDS, resulting in a concentration ratio between ΣPCDFs to ΣPCDDs in the waste water of about 10:1 (*21*). The characteristic pattern for the tetra- and penta-CDF isomers is nearly undisturbed in the crabs (*5,21*). For the blood samples in our study, the ratio of ΣPCDFs:ΣPCDDs increases with increasing intake of crab equivalents (r = 0.699), with a mean of 0.13 for the referents and 1.2 for the high-intake group. Recently, Hansson et al. (*44*) demonstrated a similar increase of the ΣPCDFs:ΣPCDDs ratio in blood of workers from the same magnesium production plant with the number of years of employment in the plant giving a mean of 0.21 for the control group and 1.1 for the workers. This strongly indicates that changes occur in the PCDD/PCDF blood profile due to exposure to characteristic contaminants from the magnesium production process both in occupationally exposed workers and in people consuming seafood from the contaminated fjord area. This is further substantiated by the facts that the PCDD/PCDF profile in the high-intake group clearly corresponds to the profile found in crab hepatopancreas (Fig. 2) and that good correlations are observed between several PCDD/PCDF congeners in blood and the individual crab intake. The relative low content of TCDF in blood of the high-intake group could indicate that this congener has a shorter half-life.

In general, a high abundance of octa-CDD is observed in the fat fraction of human tissues, but its source has not yet been identified. Schecter et al. (*8*) reported octa-CDD ranging from 50% to 80% of the ΣPCDDs/PCDFs in blood samples from the general population living in different parts of the world. This corresponds well with the results of this study, where octa-CDD accounted for 74% of all PCDDs/PCDFs in the referents. In contrast, the relative contribution from octa-CDD was reduced to 32% for the high-consumer group due to the considerably higher concentrations of PCDFs in blood (Fig. 2), demonstrating the change in PCDD/PCDF profiles that follows crab consumption.

Levels of total PCDDs and PCDFs in our referents (563 and 73 pg/g fat) are comparable with mean concentrations of 461 and 85 pg/g fat for the control group of workplace exposure study of Hansson et al. (*44*) and with values of 489 and 53 pg/g fat reported in whole blood samples from Germany by Päpke et al. (*45*). Thus, the mean blood concentrations of PCDF found for our crab consumers (473 pg/g fat in the high-intake group) were clearly different from both Norwegian and German referents, but similar to those found in the magnesium plant workers (491 pg/g fat).

Previous studies from areas near the Baltic Sea with fish as a main source of dioxin exposure have reported strong correlations between blood levels of *n*-3 PUFA and dioxins as well as fish intake (*12,42,43*). In contrast, we did not find any association between *n*-3 PUFA and fish intake. This might be explained by the predominant consumption of lean fish in our study and the narrow range of fish intake (Table 1) in a relatively small population. Furthermore, the lack of a correlation between reported crab intake and *n*-3 PUFA is probably due to the limited season for crab consumption. Crabs are usually caught and consumed about 4 months a year, starting in August. The blood samples were collected in June, just before the crab season started. Because the half-lives of *n*-3 PUFA are quite short, an influence by former crab consumption on the *n*-3 PUFA concentrations is not expected. As a consequence, presuming that crab consumption is the main source of PCDD/PCDF exposure, no correlation was found between *n*-3 PUFA and PCDDs/PCDFs in blood.

Recently we reported on the congener-specific determination of PCBs in crabs from the Frierfjord area (*4*). Sum PCBs in the crabs, excluding PCB-209, was not much above background levels found in crabs from diffuse polluted areas along the coast [0.5–1 μg/g fat (*46*)]. However, the perchlorinated biphenyl PCB-209 showed a high abundance in the crabs, ranging from 2.9 to 0.05 μg fat in a gradient from fishing site A to F (*27,28*) (see Fig. 1). This congener has been identified as one of the major chlorinated components in the wastewater from the magnesium factory (*46*).

Surprisingly, the high concentrations of PCB-209 found in crabs from the Frierfjord area are not reflected in the blood of the crab consumers (Fig. 3; Table 3). This could be due to limited bioavailibility (*47*). Studies on mammals have shown that organochlorine compounds with a log *n*-octanol/water partition coefficient (log K_{ow}) >6.5 are poorly absorbed in the gastrointestinal tract (*47–50*). PCB-209, with a log K_{ow} of 9.6, is thus expected to have a low degree of absorption. Furthermore, the linear correlation coefficient between ΣPCDDs/PCDFs and ΣPCBs in the blood samples was low (r = 0.39). This fact and the similarity of PCB profiles for the referents and the high-intake group indicates that PCB exposure in our study group is only increased somewhat by crab consumption, but predominantly arises from other sources.

Blood concentrations of the major PCB congener, PCB–153 (189–554 ng/g fat in referents), as well as some other congeners (PCB-105, 118, 156 and 157), found in our study are in good agreement with the results obtained from control persons in a Swedish study [PCB-153, 220–760 ng/g fat for referents (*11*)]. On the other hand, our results for non-*ortho*-PCBs (PCB-126: 8–173 ng/g fat and PCB-169: 5–109 ng/g fat) seem to be considerably lower than for the Swedish nonconsumers of fish (PCB-126: 100-450 pg/g fat and PCB-169: 100-340 pg/g fat).

The calculated mean PCDD/PCDF exposure of the referents of 9.7 pg/kg body weight is in good agreement with an estimated intake of about 8–10 pg TEQ/kg body weight/week based on measurements of PCDDs/PCDFs in different food items in Norway (*51*). Consumption of contaminated crabs increases the total exposure to PCDDs/PCDFs considerably. The average total exposure calculated for the group with moderate crab consumption is with 31 pg/kg body weight, close to the tolerable weekly intake (TWI) (35 pg TEQ/kg body weight) proposed by a Nordic Expert Group (*30,52*), and only few subjects in this group exceeded this value. In contrast, nearly all subjects in the high-intake group exceeded the Nordic TWI. However, only two persons slightly exceeded the TWI of 70 pg TEQ/kg body weight recommended by a WHO expert group (*53*).

The use of the TEF concept for PCBs is controversial (*26*). However, based on measured concentrations in blood (see Fig. 4), PCBs in the referents would contribute twice as much as PCDDs/PCDFs to the total TEQs. In the moderate-intake group, PCBs and PCDDs/PCDFs would contribute equally to the total TEQs, whereas in the high-intake group PCDDs/PCDFs would contribute twice as much as the PCBs. With the inclusion of PCBs in the calculation of TEQs, the mean for the ref-

erents would be close to the Nordic TWI, whereas both groups of crab consumers would exceed the TWI.

Conclusion

The present study shows that among male recreational fishermen, consumption of crabs from the contaminated Frierfjord area is an important source of exposure to PCDDs/PCDFs. This is based on two findings: the PCDD/PCDF profile in blood of the high-intake group is clearly changed in the direction of that found in crabs from this area, and the measured blood concentrations of most PCDDs/PCDFs correlate strongly with the reported crab consumption and fishing site. No other major source of exposure to PCDDs/PCDFs could be identified through the questionnaire. It appears that the external exposure can be estimated both from reported consumption and fishing sites and from the PCDD/PCDF blood concentrations with fairly good agreement. Calculations of average weekly exposures from body burdens show that about half of the crab consumers exceeded the Nordic TWI of 35 pg TEQ/kg body weight/week, but only a few slightly exceeded the WHO TWI of 70 pg TEQ/kg body weight/week.

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Monograph based on conference to be published in EHP

Sixth International Meeting on the

Toxicology of Natural and Man-Made Fibrous and Non-Fibrous Particles

September 15–18, 1996
Lake Placid, New York, USA

Organized by:
Department of Environmental Medicine
University of Rochester
School of Medicine and Dentistry
Rochester, New York

Co-Chairs:
Kevin E. Driscoll, Ph.D.
Günter Oberdörster, D.V.M., Ph.D.

Objectives

The purpose of the Sixth International Meeting is to provide a forum to rigorously discuss and debate the most recent scientific findings on the toxicology of fibrous and non-fibrous particles. Rapid advances have been made in our understanding of the processes underlying the pathogenesis of neoplastic and non-neoplastic lung disease resulting from particle exposure. In addition, significant progress is being made regarding the molecular mechanisms by which particles interact with different populations of lung cells to elicit responses. These insights into basic mechanisms have the potential to impact on regulatory decision-making. The meeting will include state-of-the-art lectures on these and related areas of critical importance to particle toxicology. The lectures will be accompanied by thematic platform and poster/discussion sessions presenting the most recent findings from investigators in the field.

Topics to be covered include:

- The role of growth regulatory factors and cytokines in the pathogenesis of particle-induced lung disease.
- Direct and indirect mechanisms of particle carcinogenesis.
- The contribution of reactive oxygen and nitrogen species to particle lung toxicity.
- Mechanisms of particle-induced cell activation.
- The physical and chemical properties of fibrous and non-fibrous particles and their correlation with biological activity.
- *In vitro* and *in vivo* approaches for assessing toxicity and performing interspecies extrapolation.
- Toxicology of mineral, man-made vitreous and organic fibers.
- Regulatory issues in classifying and assessing health risks for inhaled mineral and man-made particles.
- Occupational and environmental exposures, epidemiological studies.
- Dosimetry-deposition, retention biopersistence and clearance of inhaled particles.

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Methylsulfonyl Metabolites of PCBs and DDE in Human Milk in Sweden, 1972–1992

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A multicomponent method used for analysis of organochlorine pesticides, polychlorinated biphenyls (PCBs), naphthalenes, dibenzo-*p*-dioxins, and dibenzofurans was adapted for the analysis of methylsulfonyl metabolites of chlorinated biphenyls ($MeSO_2$-CBs) and of *p,p′*-DDE ($MeSO_2$-DDE) in human milk. The extraction and initial purification was made by liquid-gel partitioning. Additional purification and separation steps were achieved by adsorption and gel permeation chromatography. The mean recoveries of 23 $MeSO_2$-CBs and $MeSO_2$-DDE standards, added to the milk before extraction, were 80–97%. Human milk sampled in Stockholm during 1972, 1976, 1980, 1984/85, 1990, 1991, and 1992 was analyzed by GC–MS. During the time course studied, the concentrations of $MeSO_2$-CBs decreased from approximately 9 to 2 ng/g lipids and of $MeSO_2$-DDE from 5 to 0.4 ng/g lipids. The concentrations of $MeSO_2$-CBs and $MeSO_2$-DDE correlated to the levels of total PCB and *p,p′*-DDE, respectively. 3-$MeSO_2$-DDE was the major isomer of the aryl methyl sulfones studied in the milk. PCB methyl sulfones with five and six chlorine atoms in the molecule were predominant among the PCB methyl sulfones. Generally, the concentrations of 4-$MeSO_2$-CBs were higher than the corresponding 3-$MeSO_2$-CB compound. The major $MeSO_2$-CBs in the milk were 4-$MeSO_2$-2,5,2′,3′,4′-pentaCB (4-87) and 4-$MeSO_2$-2,3,6,2′,4′,5′-hexaCB (4-149). *Key words:* DDE, environmental pollutants, human milk, methyl sulfone, polychlorinated biphenyls. *Environ Health Perspect* 104:766–773 (1996)

Polychlorinated biphenyls (PCBs) and 1,1-bis(4-chlorophenyl)-2,2-dichloroethene (*p,p′*-DDE) are among the most widely spread environmental contaminants known. PCBs have mainly been used as plasticizers, dielectric fluids, and hydraulic oils. They were first detected in a white-tailed sea eagle in 1966 (*1*) and soon after were detected in human milk (*2*). In Sweden the use of PCBs was restricted in 1972 and was fully phased-out in 1995. Despite worldwide restrictions of these compounds, PCBs are still distributed to the environment and circulate between different compartments of the system. The persistence and lipophilic character of PCBs lead to their ubiquitous distribution as environmental contaminants [e.g., found in blood (*3*) and mother's milk (*4*)].

DDE is a metabolite of 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT), which was a commonly used insecticide from the 1940s to the 1960s. In Sweden the use of DDT as an insecticide in homes, gardens, and agriculture was prohibited in 1970, with an exception made for use on conifer plants until 1974. However, this pesticide is still used in certain countries, particularly where malaria is a problem of great concern (*5*).

In animals, lipophilic compounds are usually metabolized to more hydrophilic compounds that are more easily excreted than the parent substances. Metabolites of persistent environmental contaminants, however, may also have hydrophobic properties and can thus be accumulated in the body, such as methyl sulfone metabolites of chlorinated biphenyls ($MeSO_2$-CBs) and of DDE ($MeSO_2$-DDE) (*6,7*), or they may have specific protein-binding properties, such as certain $MeSO_2$-CBs (*8–11*), hydroxy-CBs (*12*), and $MeSO_2$-DDE (*13,14*). These properties lead to the retention of certain $MeSO_2$-CBs in lung, kidney, and uterine fluid (*8–10*) and $MeSO_2$-DDE in adrenal tissue (*13,14*).

A major metabolic pathway of aromatic organochlorine compounds proceeds via P450-mediated formation of arene oxide intermediates, with subsequent formation of either hydroxylated metabolites or mercapturic acid pathway (MAP) metabolites after reaction between glutathione and the epoxide (*15–17*). The cysteine conjugates formed via MAP may form aryl thiols due to C–S lyase-induced cleavage of the C–S bond in the conjugate (*16*). The aryl thiol formed is enzymatically methylated and then subsequently oxidized to the corresponding aryl methyl sulfone (*15,16*). The structures of the formed compounds are exemplified by 3-$MeSO_2$-DDE, 4-$MeSO_2$-CB149, and 3-$MeSO_2$-CB149 (Fig. 1).

As environmental contaminants, $MeSO_2$-PCBs and $MeSO_2$-DDE were first identified in seal blubber from the Baltic (*6*). Since then such metabolites have been found in several species of animals from this and other parts of the world (*7,18–20*). In human samples, $MeSO_2$-PCBs were first reported in adipose tissue and milk from a woman exposed to PCBs in a capacitor factory in Japan (*19*). $MeSO_2$-PCBs and $MeSO_2$-DDE were also identified in adipose, liver, and lung tissue from Yusho patients as well as in a control person (*20–23*).

Considering these results and the fact that PCBs and DDE are major environmental contaminants present in human milk in Sweden, it is of interest to investigate the occurrence of methyl sulfone metabolites of PCBs and DDE in human milk. The aim of the present study was to develop a method for simultaneous analysis of PCB (and other chlorinated compounds) and its methylsulfonyl metabolites and to determine the concentrations of these compounds in human milk sampled during different time periods to visualize any trends over time for the PCB and DDE methyl sulfones.

The methods used to isolate aryl methyl sulfones include liquid–liquid partitioning (*6,18,24*) and various chromatographic methods (*7,18,20,25*). The final analyses are primarily performed by GC electron-capture detection (e.g., *7,18,20,24*), mass spectrometry in electron ionization (EI) (*25*), or negative ion chemical ionization mode (*27*), but recently detection by GC atomic emission detector was described (*28*). Our work is discussed here in relation to these analytical procedures.

Materials and Methods

Pooled samples of human milk from the Mothers' Milk Centre in Stockholm were analyzed. The milk was from native Swedish women living in the Stockholm area. Equal amounts of milk from 10–20 women were mixed and stored at -20°C.

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Several of the archived samples were pooled by mixing equal amounts of samples from the same time period. The average age of the women in the pools was 27–28 years, except for 1992 when the average age was 29 years. In each period, 55–60% of the women who supplied milk were nursing their first infant. The majority of the rest were nursing their second child.

Methanol, *n*-hexane, acetonitrile, dichloromethane, and trichloromethane were of HPLC-grade (Rathburn, Walkeburn, Scotland) and were redistilled before use. Formic acid was of *pro analysi* quality (Merck, Darmstadt, Germany). Water was deionized and purified with a Milli Q cartridge system (Millipore, Bedford, Massachusetts). The $MeSO_2$-CBs used as standard compounds are listed in Table 1. The synthesis of these compounds has been described elsewere (*29–30*). 3-$MeSO_2$-4,4′-DDE was synthesized as desribed by Bergman and Wachtmeister (*31*). 3-Methylsulfonyl-4-methyl-5,2′,3′,4′,5′-pentachlorobiphenyl was used as an internal standard. The Lipidex 5000 gel was from Packard Instruments (Downers Grove, Illinois). The Lipidex was washed and stored in methanol at 4°C (*32*). Immediately before use, the gel was rinsed with methanol in a separating funnel equipped with a sintered-glass disc and a polytetrafluoroethylene stopcock. For a 20-g portion of Lipidex, 2 × 25 ml of methanol was used. Most of the remaining methanol in the gel was removed with a gentle stream of nitrogen applied from the top of the funnel. The nitrogen, quality 5.5 from AGA (Stockholm, Sweden) was purified with moisture and oxygen filters (Chrompack, Middelburg, The Netherlands). Aluminum oxide 90 (activity grade II–III) from Merck was activated at 800°C for 4 hr and partly deactivated by adding water, corresponding to a concentration of 5% water (w/w). Bio-Beads S-X3, 200–400 mesh, was purchased from Bio-Rad Laboratories (Richmond, California).

All glassware was washed with detergents in an ultrasonic bath and rinsed thoroughly with tap water, deionized water and Milli Q water and then heated overnight at 280°C. The glassware was rinsed with hexane before use. The glass chromatographic columns used had ID of 2 and 1 cm.

GC/MS analyses were performed with a VG 70-250 mass spectrometer equipped with an HP 5890A gas chromatograph and a VG-250 data system (VG Analytical, Manchester, UK). Gas chromatography was performed using a fused silica SE-54 capillary column (25 m × 0.32 mm ID, 0.25-µm film thickness; Quadrex, New Haven, Connecticut) with helium as a carrier gas. An all-glass falling needle injector was used with an injector temperature at 270°C. The oven temperature was 190°C for 0.1 min, programmed to 230°C at 5°C/min, hold for 0.2 min, programmed to 235°C at 1°C/min, hold for 3 min, programmed to 270°C at 9°C/min, and hold for 8 min. EI was performed in an "EI-only" ion source at the electron energy of 31 eV and the trap current of 500 µA. The source temperature was 260°C. The acceleration voltage was 6 kV and the resolution at m/z 293 was 7000–9000. The MS was operated in a selected ion recording mode.

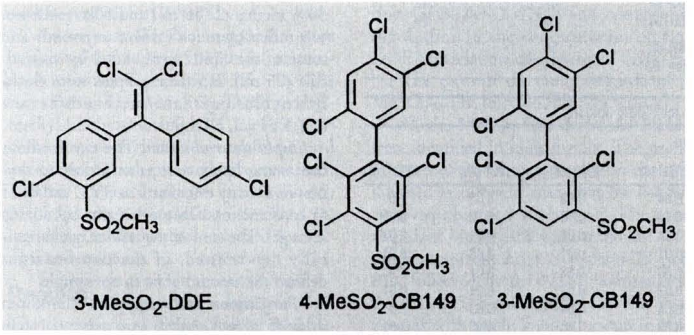

Figure 1. Structures of 3-$MeSO_2$-DDE, 4-$MeSO_2$-CB149, and 3-$MeSO_2$-CB149.

Table 1. Structures of the MeSO2-CBs analyzed and their parent compounds

Methyl sulfones	Parent compound
3-$MeSO_2$-2,5,2′,4′-tetraCB (3-49)[a]	2,4,2′,5′-tetraCB (CB-49)
4-$MeSO_2$-2,5,2′,4′-tetraCB (4-49)	2,4,2′,5′-tetraCB (CB-49)
3-$MeSO_2$-2,5,2′,5′-tetraCB (3-52)	2,5,2′,5′-tetraCB (CB-52)
4-$MeSO_2$-2,5,2′,5′-tetraCB (4-52)	2,5,2′,5′-tetraCB (CB-52)
3-$MeSO_2$-2,5,6,4′-tetraCB (3-64)	2,3,6,4′-tetraCB (CB-64)
4-$MeSO_2$-2,3,6,4′-tetraCB (4-64)	2,3,6,4′-tetraCB (CB-64)
3-$MeSO_2$-2,5,3′,4′-tetraCB (3-70)	2,5,3′,4′-tetraCB (CB-70)
4-$MeSO_2$-2,5,3′,4′-tetraCB (4-70)	2,5,3′,4′-tetraCB (CB-70)
3-$MeSO_2$-2,5,2′,3′,4′-pentaCB (3-87)	2,3,4,2′,5′-pentaCB (CB-87)
4-$MeSO_2$-2,5,2′,3′,4′-pentaCB (4-87)	2,3,4,2′,5′-pentaCB (CB-87)
3-$MeSO_2$-2,5,6,2′,4′-pentaCB (3-91)	2,3,6,2′,4′-pentaCB (CB-91)
4-$MeSO_2$-2,3,6,2′,4′-pentaCB (4-91)	2,3,6,2′,4′-pentaCB (CB-91)
3-$MeSO_2$-2,5,2′,4′,5′-pentaCB (3-101)	2,4,5,2′,5′-pentaCB (CB-101)
4-$MeSO_2$-2,5,2′,4′,5′-pentaCB (4-101)	2,4,5,2′,5′-pentaCB (CB-101)
3-$MeSO_2$-2,5,6,3′,4′-pentaCB (3-110)	2,3,6,3′,4′-pentaCB (CB-110)
3-$MeSO_2$-2,5,6,2′,3′,4′-hexaCB (3-132)	2,3,4,2′,3′,6′-hexaCB (CB-132)
4-$MeSO_2$-2,3,6,2′,3′,4′-hexaCB (4-132)	2,3,4,2′,3′,6′-hexaCB (CB-132)
3-$MeSO_2$-2,5,2′,3′,4′,5′-hexaCB (3-141)	2,3,4,5,2′,5′-hexaCB (CB-141)
4-$MeSO_2$-2,5,2′,3′,4′,5′-hexaCB (4-141)	2,3,4,5,2′,5′-hexaCB (CB-141)
3-$MeSO_2$-2,5,6,2′,4′,5′-hexaCB (3-149)	2,3,6,2′,4′,5′-hexaCB (CB-149)
4-$MeSO_2$-2,3,6,2′,4′,5′-hexaCB (4-149)	2,3,6,2′,4′,5′-hexaCB (CB-149)
3-$MeSO_2$-2,5,6,2′,3′,4′,5′-heptaCB (3-174)	2,3,4,5,2′,3′,6′-heptaCB (CB-174)
4-$MeSO_2$-2,3,6,2′,3′,4′,5′-heptaCB (4-174)	2,3,4,5,2′,3′,6′-heptaCB (CB-174)

[a]PCB congener numbers, according to Ballschmiter (*34*), are given in parentheses.

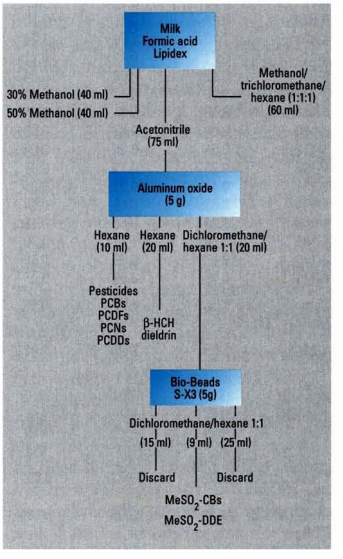

Figure 2. Scheme of method for analysis of aryl methyl sulfones.

Table 2. Recoveries of aryl methyl sulfones added to milk

$MeSO_2$-CB	Amount added (ng/ml)	n	Recovery Range (%)	Recovery Average (%)	Recovery Relative SD (%)
3-49	0.41–0.75	7	71–118	86	14
4-49	0.42–0.81	7	77–110	90	10
3-52	0.41	6	80–113	90	11
4-52	0.42	6	80–93	87	4
3-64	0.41	6	79–100	88	7
4-64	0.12–0.41	7	80–98	87	7
3-70	0.19–0.42	7	80–95	87	7
4-70	0.41	6	75–104	90	9
3-87	0.41–1.52	7	72–96	87	8
4-87	0.41–2.12	5	78–102	97	10
3-91	0.42–0.49	7	69–107	89	12
4-91	0.42	6	81–102	93	8
3-101	0.40–4.19	5	81–97	92	6
4-101	0.41–1.47	5	79–101	94	9
3-110	0.08–0.41	7	73–96	89	9
3-132	0.41	7	75–101	89	9
4-132	0.41–1.02	7	72–88	84	7
3-141	0.40	6	71–100	86	13
4-141	0.41	6	73–108	85	11
3-149	0.41–0.75	7	78–110	93	11
4-149	0.41–1.13	5	81–109	84	11
3-174	0.40	6	63–97	87	12
4-174	0.41	6	66–98	87	10
$MeSO_2$-DDE	0.44–1.34	7	69–89	80	7
Internal standard	0.42	7	74–100	84	8

Table 3. Levels of $MeSO_2$-CBs and $MeSO_2$-DDE in human milk (ng/g lipids)

Compound	Year 1972 (75)[a]	1976 (153)	1980 (199)	1984/85 (102)	1990 (40)	1991 (40)	1992 (20)
3-49	0.14	0.10	0.06	0.04	0.02	0.03	0.01
4-49	0.46	0.34	0.26	0.16	0.10	0.12	0.05
3-52	0.07	0.05	0.01	0.01	<0.01	0.01	0.01
4-52	0.06	0.06	0.02	0.01	0.01	0.03	0.01
3-64	0.03	0.08	0.06	0.03	0.03	0.01	0.01
4-64	0.10	0.10	0.05	0.02	0.02	0.01	0.01
3-70	0.12	0.09	0.07	0.04	0.04	0.03	0.01
4-70	0.09	0.11	0.07	0.03	0.15	0.05	0.01
3-87	0.15	0.12	0.10	0.06	0.05	0.02	0.04
4-87	2.13	2.09	1.20	0.70	0.94	0.67	0.33
3-91	0.21	0.17	0.11	0.08	0.06	0.04	0.04
4-91	0.12	0.08	0.04	0.02	0.03	0.02	0.02
3-101	0.20	0.17	0.16	0.15	0.12	0.09	0.06
4-101	0.78	0.64	0.52	0.32	0.33	0.28	0.13
3-110	0.04	0.07	0.03	0.02	0.02	0.01	0.01
3-132	0.08	0.06	0.04	0.03	0.04	0.01	0.03
4-132	0.25	0.15	0.07	0.05	0.06	0.04	0.04
3-141	0.11	0.09	0.04	0.12	0.06	0.01	0.02
4-141	0.63	0.56	0.34	0.19	0.25	0.14	0.11
3-149	1.23	0.80	0.48	0.34	0.41	0.20	0.24
4-149	2.00	1.87	0.70	0.64	1.00	0.59	0.35
3-174	0.09	0.09	0.08	0.03	<0.03	0.04	0.02
4-174	0.15	0.09	0.05	0.02	0.04	0.01	0.01
Sum of $MeSO_2$-CBs	9.24	7.98	4.56	3.11	3.66	2.46	1.57
3-$MeSO_2$-DDE	5.05	2.80	1.81	0.79	0.64	0.40	0.46
p,p'-DDE	2300	1500	1055	500	375	260	251
p,p'-DDT	630	340	185	61	46	36	32
PCB	1050	910	810	600	650	511	410
CB-153	208	197	152	103	116	106	96
$MeSO_2$-DDE/DDE	0.002	0.002	0.002	0.002	0.002	0.002	0.002
$MeSO_2$-PCB/PCB	0.009	0.009	0.006	0.005	0.006	0.005	0.004
$MeSO_2$-PCB/CB-153	0.04	0.04	0.03	0.03	0.03	0.02	0.02

[a]Number of women in parentheses.

For each compound, two ions of the molecular ion cluster were monitored. Ions from perfluorokerosene were used as reference masses for correction of mass spectrometer drift (lock mass).

A scheme of the analytical method is shown in Figure 2.

Extraction. The extraction was performed as previously described for multicomponent analysis of organochlorine contaminants in human milk (*33*). A sample of milk (10 ml) was weighed into a flask with a Teflon-lined screw cap. Internal standard (100 µl of 41.4 pg 3-methylsulfonyl-4-methyl-5,2′,3′,4′,5′-pentachlorobiphenyl/µl) was added and thoroughly mixed with the milk. Then, formic acid (10 ml) was added and finally Lipidex 5000 (5.0 g). The mixture was shaken at 35°C for 2.5 hr and then transferred to a glass column (2 cm ID). The solvent was drained and, in consecutive steps, was washed with 30% methanol (40 ml) and 50% methanol (40 ml). Organochlorine compounds and some of the lipids were eluted by acetonitrile (75 ml). Remaining lipids were eluted by trichloromethane/methanol/hexane (1:1:1 by vol, 60 ml).

Lipid determination. The two fractions containing lipids were taken nearly to dryness in a rotary evaporator at 35°C and dried to constant mass in a desiccator containing silica gel. The sum of the residue, gravimetrically determined, of the two fractions defined the amount of fat in the sample.

Purification and separation. Aluminum oxide (5 g) was packed in a column (1 cm ID) and washed with hexane (10 ml). Then the stopcock was closed and the residue from acetonitrile fraction was quantitatively transferred to the column with small portions of hexane. The sample on the column was concentrated by evaporation of the solvent with a gentle stream of nitrogen. Organochlorine compounds, such as pesticides, PCBs, polychlorinated naphthalenes, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, were eluted by hexane (10 ml) and β-hexachlorocyclohexane (β-HCH) and dieldrin in an additional fraction of hexane (20 ml). All $MeSO_2$-CBs and $MeSO_2$-DDE were obtained in the third fraction, collected when 50% dichloromethane in hexane (20 ml) was used as a mobile phase.

Bio-Beads S-X3 (5 g) were transferred to a beaker and dichloromethane/hexane (1:1, v/v) was added to cover the gel. The beaker was placed in an ultrasonic bath for a few seconds and then let equilibrate for 2 hr. The mixture was subsequently transferred to a column (1 cm ID). The solvent was drained and the gel was washed with dichloromethane/hexane (1:1, v/v, 20 ml). The vol-

ume of $MeSO_2$-CBs and $MeSO_2$-DDE-containing fraction was adjusted by evaporation to about 0.5 ml, transferred to a small, tapered centrifuge tube, concentrated to about 50 µl with nitrogen, and then quantitatively transferred with small volumes of dichloromethane/hexane (1:1) to the GPC column (Bio-Beads S-X3). The mobile phase, dichloromethane/ hexane (1:1), was collected at a rate of 20 drops/min. The first 15 ml were discarded. The following 9 ml contained $MeSO_2$-CBs and $MeSO_2$-DDE. To purify the column from contaminants, it was washed with an additional 25 ml of the solvent mixture and discarded. The fraction containing $MeSO_2$-CBs and $MeSO_2$-DDE was concentrated to 50 µl and transferred quantitatively to the same column, and the procedure was repeated. The fraction containing $MeSO_2$-CBs and $MeSO_2$-DDE (9 ml) from this second fractionation was concentrated and about half of the sample was used for analysis by GC–MS.

Recovery studies. Recovery studies were performed by adding 50–100 µl of standard mixtures in hexane of $MeSO_2$-CBs (see Table 1), 3-$MeSO_2$-DDE and 3-methylsulfonyl-4-methyl-5,2′,3′,4′,5′-pentachlorobiphenyl (the internal standard used) to the sample before extraction. Before determination by GC–MS, 2,3,4,5,2′,3′,4′-heptaCB (CB-170) (*34*) was added as an internal standard for volume correction.

Results

The congener-specific analyses of $MeSO_2$-CBs and $MeSO_2$-DDE were made by GC–MS using an "EI-only" ion source. At a resolution of 7000 and the electron energy of 31 eV, the detection limits of the compounds listed in Table 1 were 0.5–2 pg at S/N 2.5. Considerably lower sensitivity was obtained at 70 eV (detection limit 2–10 pg). In the investigation of milk, the detection limits of $MeSO_2$-CBs and $MeSO_2$-DDE were 0.01-0.05 ng/g lipid.

The mean recoveries of $MeSO_2$-CBs and $MeSO_2$-DDE added to the samples before extraction were 80–97% and of internal standard were 84% (Table 2). Congener-specific analyses of $MeSO_2$-CBs and $MeSO_2$-DDE in human milk collected in different years, starting from 1972, were performed. The concentrations of $MeSO_2$-CBs and $MeSO_2$-DDE are shown in Table 3. Of the metabolites studied, 3-$MeSO_2$-DDE had the highest concentration. The level of 3-$MeSO_2$-DDE was about 5 ng/g fat in milk from 1972 and declined successively with time to about 0.4 ng/g fat in 1991–1992. A decline in the concentrations of *p,p′*-DDT and *p,p*'-DDE in Swedish human milk has been demonstrated previously (*35,36*). The concentrations of *p,p′*-DDT and p,p'-DDE in the present samples are shown in Table 3. There is a good correlation between the concentrations of 3-$MeSO_2$-DDE and corresponding decline in the concentration of *p,p′*-DDE (correlation coefficient of 0.99; Fig. 3). The ratio of the concentrations of 3-$MeSO_2$-DDE to *p,p′*-DDE was 0.002.

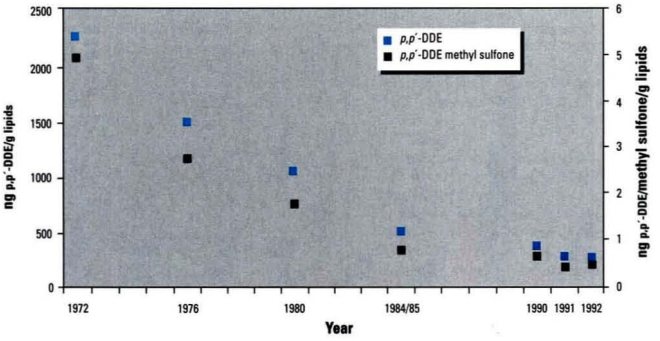

Figure 3. Concentrations of *p,p′*-DDE and *p,p′*-DDE methyl sulfone in human milk, 1972–1992.

All the $MeSO_2$-CBs shown in Table 1 were identified in human milk. The profile of the $MeSO_2$-CBs was similar in the milk sampled in different years, but the concentrations changed with time. The most abundant $MeSO_2$-CB was compound 4-87, originating from 2,3,4,2′,5′-pentaCB (CB-87), followed by 4-149, originating from 2,3,6,2′,4′,5′-hexaCB (CB-149). The compounds with methylsulfonyl group in position 4 (*para*) occur at higher levels than the corresponding compounds with methylsulfonyl group in position 3 (*meta*) (Fig. 4).

The $MeSO_2$-CBs in human milk originate from CBs that do not occur at high levels in milk. Of the precursors, only 2,5,2′,5′-tetraCB (CB-52) and 2,4,5,2′,5′-pentaCB (CB-101) were found in milk (to be published). The concentrations of $MeSO_2$-CBs decreased from 9.2 ng/g lipids in 1972 to 1.6 ng/g lipids in 1992 (Table 3) and correlated to the decline in the levels of total CBs by a correlation coefficient of 0.95 (Fig. 5). The total CB levels were determined by EC-GC using a packed column (*35,36*; in preparation). The ratios of the sum of $MeSO_2$-CBs ($MeSO_2$-PCB) to total CB (PCB) concentration decreased from 0.009 to 0.004 during the time course studied (Table 3). Comparisons were also made to 2,4,5,2′,4′,5′-hexaCB (CB-153), determined by congener specific analysis. This PCB congener does not contain adjacent, unsubstituted carbon atoms susceptible to metabolic reactions. Accordingly, it has a long half-life (*37*) and is the most abundant PCB congener in the human milk. The decline of $MeSO_2$-CBs relative to CB-153 indicates a somewhat more rapid decline of $MeSO_2$-CBs in milk than of PCB over the course of this time period.

Discussion

The analytical methods used in previously reported investigations of $MeSO_2$-CBs and $MeSO_2$-DDE involve several steps of liquid–liquid partitioning and column chromatography (*6,7,18,20,24,25*). The aryl methyl sulfones were extracted from tissue samples by nonpolar solvents or medium-polarity mixtures of solvents (*6,24,25,38*). Different combinations of methods have been used for purification and separation from other organochlorine compounds, e.g., chromatography (Bio-Beads, silica gel, aluminum oxide) and partitioning between solvents of different polarity, e.g., between hexane and aqueous acetonitrile (*24*) and between hexane and dimethyl sulfoxide with subsequent reextraction of the analytes with methyl *tert*-butyl ether:hexane, after dilution of the dimethyl sulfoxide phase with water (*7*). Because $MeSO_2$-CBs and $MeSO_2$-DDE possess the character of a Lewis base, partitioning between hexane and concentrated sulfuric acid has frequently been used as a method for purification of these compounds. However, a method using exclusively chromatographic fractionations (gel permeation, silica gel modified with KOH, Florisil, and basic aluminum oxide) for separation of the methyl sulfone derivatives was recently reported (*39*).

In the present study the extraction procedure for $MeSO_2$-CBs and $MeSO_2$-DDE from milk differs from the previously decribed methods. Instead of partitioning between solvents, the extraction was accomplished by partitioning between the milk–formic acid mixture and a lipophilic gel, Lipidex 5000. The extraction between an aqueous solution and Lipidex resembles the extraction with a solvent of medium

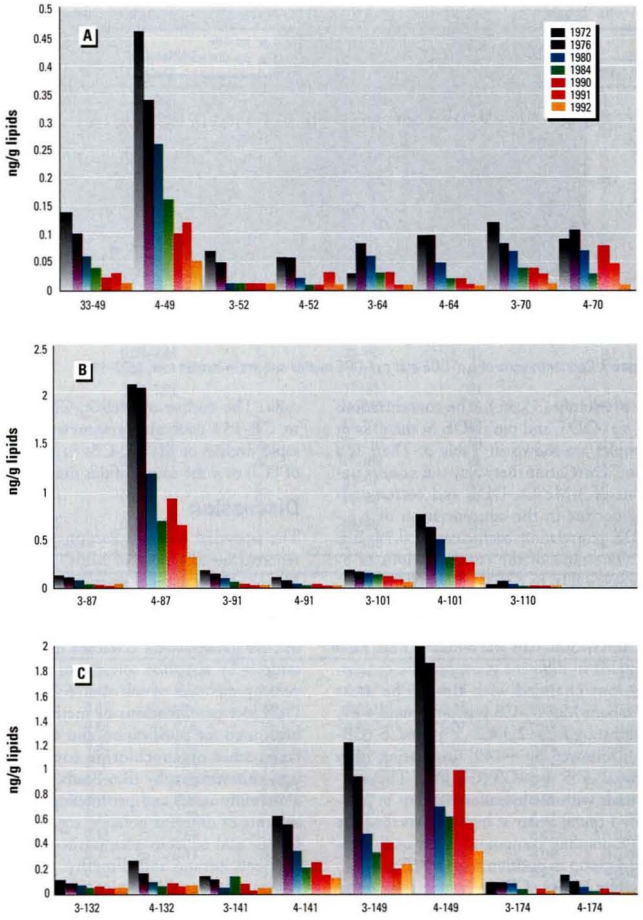

Figure 4. Concentrations of individual $MeSO_2$-CBs in human milk from 1972–1992. (A) Compounds with four chlorine atoms in the molecule, (B) compounds with five chlorine atoms, and (C) compounds with six to seven chlorine atoms.

polarity. The advantage is that the procedure can be done in one step. The column bed can subsequently be eluted with solvents of different polarity, facilitating partial separation of lipids and an initial purification of the analyte fraction. By this procedure about 60% of the lipids in the milk sample were separated from the analytes. In addition two chromatographic systems, aluminum oxide and Bio-Beads S-X3 were used. By repeating the gel permeation step once, sufficiently clean extracts were obtained for GC–MS analysis.

The recoveries of added $MeSO_2$-CBs and $MeSO_2$-DDE (mean 80–97%) with a relative standard deviation of 4–14% (Table 2) were considered satisfactory for the analysis using a small sample size (10 ml milk).

In the milk samples, 3-$MeSO_2$-DDE was the major aryl methyl sulfone compound (about 5–0.4 ng/g lipids), whereas only traces could be discerned of 2-$MeSO_2$-DDE. The ratio of $MeSO_2$-DDE/DDE (0.002) was constant in the samples from different years. In Japanese human samples (adipose, lung and liver tissue) the ratios were somewhat higher (0.007–0.009). A high species-specific organ selectivity has been demonstrated for $MeSO_2$-DDE in wild animals (*7*), but no concentrations in milk from these animals or in human milk have previously been reported. The present results are in accordance with the observations that $MeSO_2$-DDE is the major aryl methyl sulfone in Swedish human adipose tissue (*40*). However, high concentration of 2-$MeSO_2$-DDE and 3-$MeSO_2$-DDE (about 0.9 and 0.4 µg/g fat, respectively) were reported in the liver of polar bear (Canada). In grey seal the levels were higher in the liver than in adipose tissue, and the levels of 2-$MeSO_2$- and 3-$MeSO_2$-DDE were equal. In otter and mink, no 2-$MeSO_2$-DDE was detected and the levels of 3-$MeSO_2$-DDE were about equal in liver and muscle, calculated on lipid weight basis (*7*). The results indicate differences in exposure and/or metabolism.

$MeSO_2$-DDE is a potent toxicant for the adrenal cortex in mouse (*13*) and also in the human adrenal glands as observed as *in vitro* bioactivation of this compound in human adrenal gland (*14*). The irreversible binding of $MeSO_2$-DDE in the zona fasciculata of the adrenals led to the formation of necrotic cells and inhibition of glucocorticoid hormone synthesis (*13,41*). Also, *p,p′*-DDE was recently reported to be a strong androgen receptor antagonist (*42*); the potency of the corresponding $MeSO_2$-DDE is not yet known. Due to the potential toxicity of DDE and $MeSO_2$-DDE, it is necessary to reduce the exposure to DDT/DDE, especially in areas where DDT is still used.

Generally, the precursors to the $MeSO_2$-CBs were not found in the milk, indicating an effective metabolism of the precursor CBs. The $MeSO_2$-CBs originate from CBs with chlorine atoms in 2,5- or 2,3,6-positions of at least one of the phenyl rings of the PCB congener (*8*). In these compounds there are unsubstituted *meta*/*para* positions adjacent to two chlorine atoms. This strongly facilitates the reaction between the PCB arene oxide and glutathione and formation of the two isomeric 3- and 4-$MeSO_2$-PCBs, as observed in minks dosed with a technical preparation of the PCB Clophen A50 (*43*).

In milk, the PCB metabolites with the $MeSO_2$ group in the 4-position are present at higher concentrations than the corresponding compounds with the $MeSO_2$ group in 3-position, except for 3-91, which is present at a higher concentration than 4-91 (see Table 3). The major $MeSO_2$-CBs were 4-87 and 4-149. Both 4-87 and 4-149 were the predominant $MeSO_2$-CBs in human adipose tissue (*40*) and thus consistent with the results of the present study. In adipose and lung tissue from Yusho patients, $MeSO_2$-CB 4-87 was also the predominant PCB methyl sulfone, whereas a quite different profile was reported in the

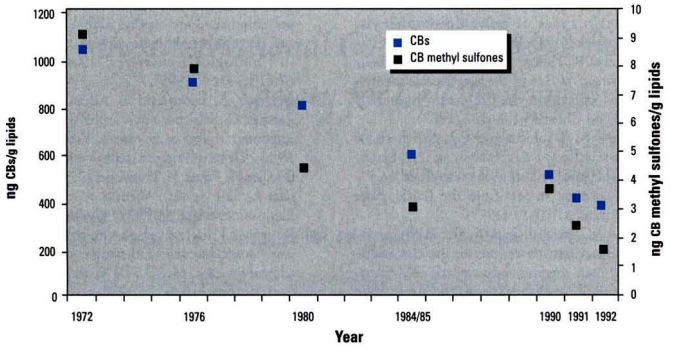

Figure 5. Concentrations of total chlorinated biphenyls (CBs) and sum of CB methyl sulfones in human milk sampled 1972–1992.

control sample with 4-$MeSO_2$-2,5,4′-triCB as the dominating and 4-$MeSO_2$-2,5,2′,4′-CB (4-49) occurring in second highest level (*20*). A different profile of $MeSO_2$-CBs is found in tissues of grey seal, otter, and mink (*7,18*). In these species 3-$MeSO_2$-2,5,2′,4′,5′-pentaCB, originating from CB-101, is the predominant PCB methyl sulfone. The results indicated differences in exposure and/or metabolism of DDE and PCBs, which may be of importance in possible toxic effects of these compounds.

The concentrations of $MeSO_2$-DDE and $MeSO_2$-PCB in milk decreased during the time course studied, in accordance with the previously reported decline of DDT, DDE and PCB in Swedish human milk (*35,36*; in preparation). In a study of milk from an occupationally exposed mother, a decline in the concentration of PCB, from 14,000 to 3700 ng/g lipids, and of $MeSO_2$-PCB, from 590 to 150 ng/g lipids, during 16 months of milk excretion was reported (*44*). At the time of that study, $MeSO_2$-PCB congener-specific analysis was not possible. However, the concentration of $MeSO_2$-PCB was estimated to 0.05 of the PCB concentration. In the present investigation the concentration ratios of $MeSO_2$-PCB relative to PCB were 0.009–0.004 and declined during the 20 years studied. In a Japanese study, correponding ratios in adipose tissues were 0.02 and 0.04 from two control persons and 0.004 and 0.006 from two Yoshu patients (*23*). The profile of the $MeSO_2$-PCB in these samples differed from the profile in Swedish human milk. In the present study the congeners with 5 and 6 chlorine in the molecule dominated, while in Japanese adipose tissue the lower chlorinated congeners were the dominating $MeSO_2$-CBs. In Swedish blood plasma, the profile of $MeSO_2$-PCB is similar to that in the milk (Weistrand and Norén, in preparation). Approximately twice as much 4-$MeSO_2$-CBs are present in the milk as are 3-$MeSO_2$-CBs. The latter type of metabolites have recently been reported as potent inducers of several hepatic microsomal drug-metabolizing enzymes while the 4-$MeSO_2$-CBs congeners tested were inactive (*45*). On the other hand, certain 4-$MeSO_2$-CBs are strongly retained in lung bronchial mucosa due to their binding to uteroglobin-like macromolecules in the Clara cells (*9*). It is also supposed that the chronic lung dysfunction symptoms in Yusho patients may have been caused by $MeSO_2$-PCB (*46*). It may thus be emphasized that the $MeSO_2$-CBs are also of potential toxicological importance and the presence of these PCB metabolites in human milk cannot be neglected. Further studies of their toxicological role are needed.

The data show that restrictions on the use of DDT and PCBs have led to decreased levels of these compounds and their methyl sulfone metabolites in human milk. The present study is a first attempt at congener-specific analysis of $MeSO_2$-DDE and $MeSO_2$-PCB in milk, and it should be followed up by analysis of other matrices of human tissue to investigate specific retention.

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OPENING ADDRESS
In Praise of Reductionist, Adaptationist, Progressivist, Gradualist Neo-Darwinist, Richard Dawkins

PLENARY SYMPOSIA

Saturday, December 7
Regulation of Cell Division and Genomic Instability, M. Kirschner, S. Elledge, P. Nurse, and T. Tlsty

Sunday, December 8
Cytoskeleton & Disease, D. Louvard, D. Cleveland, U. Francke, and J. Seidman
Chromatin Structure and Gene Expression, G. Hager, S. Gasser, M. Grunstein, and D. Spector

Monday, December 9
Phosphorylation and Dephosphorylation in Regulatory Pathways, J. Brugge, A. Pawson, T. Taniguchi, and N. Tonks
Adhesion and Signalling, Z. Werb, P. Sternberg, S. Tsukita, and F. Watt

Tuesday, December 10
Vesicular Traffic and Organelle Assembly, J. Rothman, G. Schatz, M. Zerial, and V. Malhotra
Protein Glycosylation in Sorting and Trafficking, P. Stanley, A. Helenius, K. Simons, and A. Varki

Wednesday, December 11
Master Genes and Early Development, W. Gehring, R. Beddington, E. Meyerowitz, and E. Olson
Regulation of Cell Death, G. Evan, S. Nagata, C. Thompson, and E. White

CONCURRENT SYMPOSIA

Sunday, December 8
Genetic Approaches to Human Disease, K. Davies, J. Friedman, Y. Shiloh, and R. Tanzi
Small G Proteins and Trafficking, Y. Goda, S. Pfeffer, J. Gerst, A. Hall, and L. Lim
The Cytoskeleton and Its Associated Proteins, A. Ephrussi, E. Wieschaus, B. Gumbiner, M. Peifer, and P. Polakis
Nuclear Architecture and Higher Order Controls of Gene Transcription, W. Brinkley, J. Lawrence, B. Emerson, and T. Kowhi-Shigematsu
Senescence, J. Campisi, O. Pereira-Smith, L. Guarente, S.M. Jazwinski, and W. Wright
Methylation and Imprinting in Mammalian Cells, W. Doeffler, R. Jaenisch, T. Bestor, and A. Surani

Monday, December 9
Extracellular Matrix: Regulation and Cell Behavior, R. Chiquet-Ehrismann, E. Ruoslahti, D.M. Bissell, A. Kornblihtt, and B. Olsen
Endocytosis, I. Mellman, M. Robinson, E. Rodriguez-Boulan, K. Sandvig, and S. Schmid
Molecular Studies of Neuron Target Interactions, S. McConnell, J. Sanes, C. Bargmann, J. Raper, and F. Walsh
Structural Biology of Membrane Pumps, Channels, and Receptors, R. Glaeser, H. Saibil, G. Schertler, and W. Kuhlbrandt, Telomeres and Telomerases, E. Blackburn, T. de Lange, Y. Hiraoka, D. Shippen, and V. Zakian
Developmental Biology of Gene Expression, L. Shapiro, J. Smith, J. Rossant, and C. Wylie

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Alterations of Cytochrome P450-dependent Monooxygenase Activities in *Eriocheir japonicus* in Response to Water Pollution

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Eriocheir japonicus, fresh-water crabs inhabiting rivers and estuaries in Japan, were investigated for cytochrome P450 (CYP)-dependent drug-metabolizing enzyme activities to see if these activities reflect the river pollution gradient. From the laboratory dose–response experiments, we found that the polycyclic aromatic hydrocarbon (PAH) 3-methylcholanthrene induced total CYP contents, ethoxycoumarin *O*-deethylase activity, and bunitrolol 4-hydroxylase activity in crab hepatopancreas. In the field studies, crabs collected from the river with the highest concentration of PAHs exhibited the highest levels of CYP, the highest activities of benzo[*a*]pyrene 3-hydroxylase, imipramine 2-hydroxylase, bunitrolol 4-hydroxylase, ethoxycoumarin *O*-deethylase, and the ability to metabolically activate benzo[*a*]pyrene, but erythromycin *N*-demethylase activity was not induced. The correlation between PAH levels and drug-metabolizing enzyme activities in female crabs were not as marked as in male crabs. The levels and acitivities of CYP did not appear to reflect the concentrations of organochlorines and polychlorinated biphenyl congeners (PCBs) studied in the fat of crab hepatopancreas. *Key words*: crabs, cytochrome P450, environmental monitoring, *Eriocheir japonicus*, PCBs, polycyclic aromatic hydrocarbons. *Environ Health Perspect* 104:774–778 (1996)

The monooxygenase system, which metabolizes a number of foreign compounds, is widely distributed among organisms. The presence of cytochrome P450 (CYP), an important component of the drug-metabolizing enzyme system, has been reported in several aquatic species (*1–5*). Rivers and marine environments are contaminated with many lipophilic chemicals. Due to the low solubility of these compounds in water, ingestion of food on to which pollutants have been solubilized could be a major route of exposure to environmental contaminants in aquatic species. Fresh-water crabs are at the top of the food chain in the aquatic environment and are among the commonest animals in rivers and estuaries. In crabs, the hepatopancreas—a large, fatty gland—is the major organ of metabolism and digestion (*6*). The induction of some forms of CYP by many foreign chemicals has been reported in several crab species. Therefore, we investigated this induction phenomenon in crabs as a way to monitor water quality.

Eriocheir japonicus is a common crab species in Japan. We investigated alterations of CYP concentrations and drug-metabolizing activities in hepatopancreatic microsomes after injecting these crabs with a polycyclic aromatic hydrocarbon (PAH). 3-methylcholanthrene (3-MC) was selected as the inducing agent because in other species it is a potent and effective inducer of the forms of CYP that metabolize PAHs. Several studies have shown that the administration of 3-MC causes induction of CYP in the hepatopancreatic microsomes of crustaceans.

A field investigation was undertaken to test if the levels of pollution correlate with drug-metabolizing enzyme activities in crabs. The level of environmental pollution, including PAHs in the areas inhabited by crabs and the concentrations of organochlorines and polychlorinated biphenyl congeners (PCBs) in the crabs, was determined.

Materials and Methods

Animals and preparation of microsomes. We collected adult male *Eriocheir japonicus* (230.0 ± 77.95 g) from the Barato River between August and September. The crabs were kept in glass aquariums and fed commercial pet food for 2 weeks before treatment with inducer. The crabs were given three oral intubations with different doses of 3-MC in corn oil 3 days before analysis (0.2, 1.0, 5.0, or 40.0 mg/kg/day). Control animals received three oral intubations of corn oil only (1.0 ml/kg/day). Crabs were sacrificed on the fourth day and microsomes were prepared.

For the field investigation, we collected crabs from the Ishikari Bay at the mouths of the Ishikari River, the Barato River, the Shiribetsu River, and the Tone River (Fig. 1). The Ishikari River has been contaminated with the waste of the paper mills. The Barato River is a branch of the Ishikari River and runs through a suburban agricultural area. The Shiribetsu River runs through a rural area and a mountain village. The Tone River flows through industrial, agricultural, residential, and urban areas and has been polluted with the wastes from these areas. Crabs of both sexes were caught between July and August. Only adults in their intermoult stage were selected for this study. Animals were sacrificed immediately after collection. Male (94.14 ± 51.95 g) and female (85.49 ± 28.39 g) crabs were anesthetized by cooling in ice for 10–20 min and dissected as rapidly as possible.

Hepatopancreas was washed in buffer (0.05 M Tris, 1 mM EDTA, 2.5 mM dithiothreitol, 0.5 mM diisopropyl fluorophosphate, 20% glycerol, 50 µg/ml aprotinine, pH 7.4) and homogenized (*7*). Particular attention was paid to keeping the homogenate cold and keeping the protease inactive by freshly preparing protease inhibitor at each experiment. Microsomes were prepared from postmitochondrial supernatants by centrifugation at 105,000*g*. The microsomal pellet resulting from the centrifugation was resuspended in the same buffer and centrifuged again for 60 min at 105,000*g*. The washed microsomes were then suspended in the buffer. The pools of microsomal fraction, derived from three crabs, were stored at -80°C.

Fat fractions were prepared from supernatants by centrifugation at 800*g* and cleaned up using the method of Kannan et al. (*8,9*) with some modifications.

Chemical analyses. The PAHs were trapped with blue rayon using the method of Hayatsu et al. (*10*). Blue rayon, bearing copper phthalocyanine trisulfonate as a covalently linked ligand, is an adsorbent specific for compounds with three or more fused rings. Blue rayon (3 g) in plastic-mesh bags, 30 cm length × 5 cm diameter, were placed at the crab collection sites for three days during the time period crabs were collected.

We dissolved the extracts in acetonitrile, and determined PAHs by UV-fluorescence spectroscopy. The fluorescence of the acetonitrile extract was assayed with an excita-

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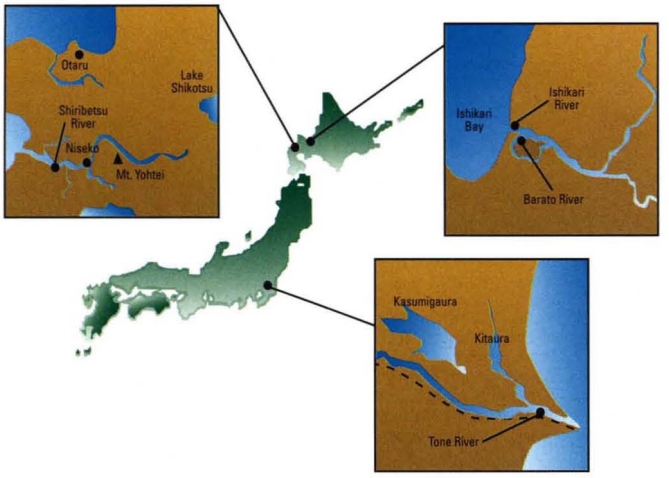

Figure 1. Map of Japan showing sampling locations.

tion wavelength of 384 nm and an emission wavelength of 406 nm, using benzo[*a*]pyrene as the standard.

Polychlorinated biphenyl (PCB) derivatives and insecticides in single-pool fat fraction from nine crabs were measured according to the method of Kannan et al. (*8,9*). PCB- and insecticide-containing fractions were analyzed using a gas chromatograph equipped with an ^{63}Ni electron capture detector. The injector and the detector temperature were kept at 220°C and 320°C, respectively. The column temperature was programmed to rise from 160°C to 260°C at the rate of 2°C/min, maintaining the final temperature for 20 min.

Biochemical assay. We determined microsomal protein concentrations using the method of Lowry et al. (*11*). Levels of CYP were determined according to the method of Omura and Sato (*12*). Enzyme activities in microsomes were measured at an incubation temperature of 20°C in the presence of an NADPH-generating system. Reactions were carried out under optimal conditions of incubation temperature, protein concentration, and incubation time.

Ethoxycoumarin *O*-deethylase activity which is CYP1A1 and CYP1A2 dependent in rats, was assayed according to Greenlee and Poland (*13*). The 1-ml reaction mixture contained 2 mg microsomal protein. We assayed the fluorescence of 7-hydroxycoumarin with excitation wavelength at 368 nm and emission wavelength at 456 nm using a JASCO FP-777 spectrofluorometer (JASCO Ltd., Tokyo).

Imipramine 2-hydroxylase activity, which is CYP2D dependent in rats, was measured by HPLC as described previously (*14*). The assay mixture, containing a final concentration of 1 mg/ml microsomal protein and 1 mM substrate, was incubated for 5 min.

Bunitrolol 4-hydroxylase activity, which is also CYP2D dependent in rats, was determined by HPLC equipped with a fluorescence spectrophotometric detector (wavelength 325 nm and emission wavelength 365 nm) according to the method described by Ishida et al. (*15*). The incubation mixture for the assay contained a final concentration of 1 mg/ml microsomal protein and 1 mM substrate in a final volume of 1.0 ml.

Benzo[*a*]pyrene 3-hydroxylase activity, which is CYP1A1 and CYP1A2 dependent in rats, was measured using the method of Nebert and Gelboin (*16*). The fluorescence of the alkaline extract was assayed with an excitation wavelength of 396 nm and an emission wavelength of 522 nm. The concentrations of 3-hydroxybenzo[*a*]-pyrene were calibrated with a quinine sulfate, and the values were converted using the factor given by Uemura and Chiesara (*17*).

Erythromycin *N*-demethylase activity, which is CYP3A dependent in rats, was measured by the method of Nash (*19*), with a final substrate concentration of 1 mM and a microsomal concentration of 160 µg/ml.

The mutagenesis assay was performed according to the method of Ames et al. (*20*) with modifications, using *Salmonella typhimurium* strains TA98 as the test strain and benzo[*a*]pyrene as the mutagen. The bacteria were preincubated with microsomes of crab hepatopancreas at 20°C.

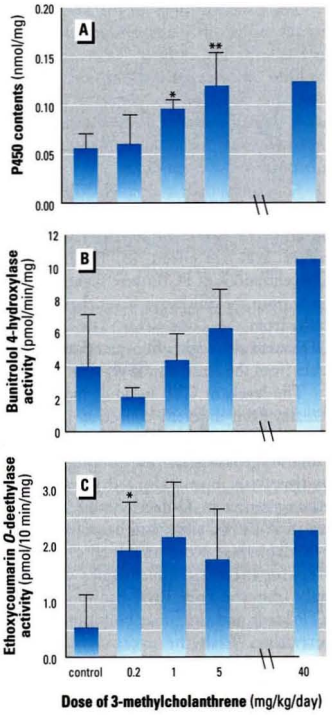

Figure 2. Cytochrome P450 levels and metabolic activities in hepatopancreatic microsomes from male crabs treated with 3-methylcholanthrene (3-MC). Five groups of crabs, three per group (except two in 40 mg/kg/day treatment group), were given three oral intubations with different doses of 3-MC in corn oil 3 days before analysis (0.2, 1.0, 5.0, 40.0 mg/kg/day). Control animals received three oral intubations of corn oil only (1. 0 ml/kg/day). *p<0. 1, **p<0.05, control vs. treated crabs. (A) Cytochrome P450 levels, (B) bunitrolol 4-hydroxylase activity, (C) ethoxycoumarin *O*-deethylase activity.

Results

Treatment of crabs with different doses of 3-MC resulted in induction of CYP related to drug metabolism (Fig. 2). CYP in hepatopancreatic microsomes showed a graded response to doses of 3-MC (Fig. 2A). Although statistically significant differences were not observed in comparing bunitrolol 4-hydroxylase activities among treated and control crabs (Fig. 2B), there were dose-dependent increases in treated animals. Ethoxycoumarin *O*-deethylase activity was significantly increased in treated animals (Fig. 2C). The level of activity was threefold high in crabs treated with 0.2 mg/kg/day, the lowest dose of 3-MC. The level of activity did not increase even at the 40 mg/kg/day, indicating that crab CYP was maximally induced at this lowest dose.

The concentrations of the blue rayon extract in the different rivers are presented in Figure 3. The extracts dissolved in acetonitrile were assayed with the same excitation and emission wavelengths used to assay benzo[*a*]pyrene. The fluorescence intensity was the highest in blue rayon extract from the Tone River.

The concentrations of total PCBs in the fatty fraction of hepatopancreas of crabs obtained from the Tone and Shiribetsu rivers are presented in Figure 4A. Concentrations of PCBs were higher in the crabs from the Shiribetsu River than in the crabs from the Tone River. The levels of DDE were also higher in hepatopancreas of crabs from the Shiribetsu River (Fig. 4B).

The levels of CYP in adult males and females from different collection sites are shown in Figure 5A. CYP-mediated monooxygenase activities, imipramine 2-hydroxylase, bunitrolol 4-hydroxylase, ethoxycoumarin *O*-deethylase, erythromycin *N*-demethylase, and benzo[*a*]pyrene 3-hydroxylase were analyzed (Fig. 5). Hepatopancreatic microsomes of male crabs from the Tone River had the highest CYP levels and drug-metabolizing enzyme activities. With the exception of erythromycin *N*-demethylase activity, CYP levels and activities were not detected or were very low in male crabs from the Shiribetsu River. High activities of benzo[*a*]pyrene 3-hydroxylase were not observed for crabs from the Ishikari Bay, even though these crabs had relatively high CYP content. Except for bunitrolol 4-hydroxylation and imipramine 2-hydroxylation, we have not observed remarkably high drug-metabolizing activities in female crabs from the Tone River. Significant variations were not observed in the erythromycin *N*-demethylase activities in hepatopancreas of male or female crabs inhabiting different areas.

The abilities of metabolic activation of benzo[*a*]pyrene were investigated in hepatopancreatic microsomes from male crabs (Fig. 6). Crabs inhabiting the Tone River had the highest rates of activation. The lowest rates of mutagenic activation were observed in crabs from the Ishikari Bay.

Discussion

CYP levels were significantly higher in hepatopancreas from 3-MC-treated crabs. Ethoxycoumarin *O*-deethylase activities were significantly increased in treated animals. Bunitrolol 4-hydroxylase activities tended to increase in treated animals. Possible explanations for the lack of greater increases in ethoxycoumarin *O*-deethylase activities in crabs treated with 5.0 or 40.0 mg 3-MC/kg/day are the toxicity of the inducer or that these doses are greater than the amount needed to cause maximal induction. The form of CYP corresponding to bunitrolol 4-hydroxylase activity may be differentially affected.

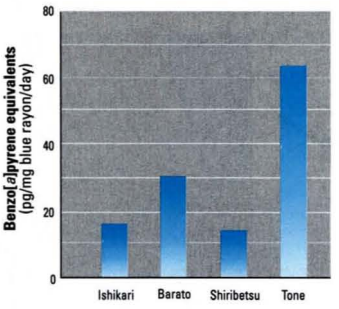

Figure 3. Levels of polycyclic aromatic hydrocarbons (PAHs) trapped with blue rayon. Three grams of blue rayon was suspended for 3 days in the rivers. The extracts were dissolved in acetonitrile, then PAHs were determined by UV-fluorescence spectroscopy, using benzo[*a*]pyrene as the standard. See Materials and Methods for details.

We investigated the levels of PAHs in rivers to assess a possible correlation between contaminant levels in water and CYP enzyme activity of crabs inhabiting the same water. The work by Lee et al. (*6*) with blue crabs (*Callinectes sapidius*) demonstrated that crabs should not retain petroleum hydrocarbons due to the high rate of hydrocarbon metabolism and excretion. Therefore, we did not measure tissue concentrations of PAHs in the crabs but measured PAH concentrations in river water. The highest rates of metabolic activation, enzyme levels, and drug-metabolizing enzyme activities were observed in hepatopancreatic microsomes of crabs from the Tone River, which contained elevated levels of contaminants.

Recent studies have demonstrated the usefulness of ethoxyresorufin *O*-deethylase activity as an indicator of the level of environmental pollution (*21,22*). Our study showed that levels of male hepatopancreatic CYP enzymes, activities of benzo[*a*]pyrene 3-hydroxylase, ethoxycoumarin *O*-deethylase, imipramine 2-hydroxylase, bunitrolol 4-hydroxylase, and metabolic activation of benzo[*a*]pyrene were the highest in hepatopancreatic microsomes of crabs from the Tone River. Activities of these enzymes appear to also be useful indicators of levels of PAHs in environment. Erythromycin *N*-demethylase activity did not reflect the levels of contaminants. Hexobarbital hydroxylase activity and aniline 4-hydroxylase activity could not be detected in hepatopancreatic microsomes from *E. japonicus* (data not shown). Bunitrolol 4-hydroxylase and

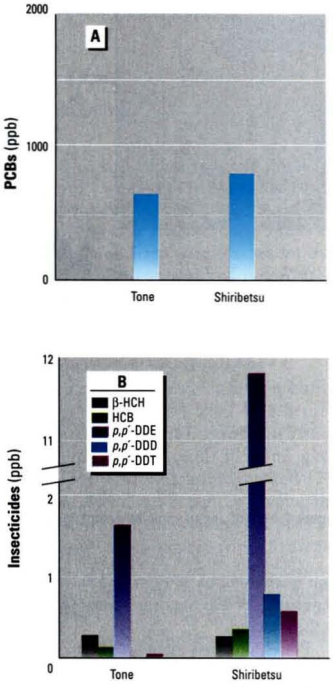

Figure 4. Levels of polychlorinated biphenyls (PCBs) and insecticides from the fat fraction of crab hepatopancreas. PCB- and insecticides-containing fractions were injected into a gas chromatograph equipped with an electron capture detector. See Materials and Methods for details. (A) Concentration (ng/g fat) of PCB compounds in fat fraction, (B) concentration (ng/g fat) of DDT compounds, hexachlorocyclohexane (HCH), and hexachlorobenzene (HCB) in fat fraction.

imipramine 2-hydroxylase are mainly catalyzed by CYP2D1 in rat liver microsomes, and benzo[*a*]pyrene hydroxylase is catalyzed by CYP1A1 and CYP1A2 in microsomes from the induced liver and by CYP2C11 in microsomes from the noninduced liver. Ethoxycoumarin *O*-deethylase is catalyzed by CYP1A1 and CYP1A2 and erythromycin *N*-demethylase by CYP3A. In crabs, however, there is no information on multiple types of CYP. The classification of CYP types based on the enzymatic activities using different substrates in the hepatopancreas of crabs may not follow the rule applicable to vertebrates.

Porte et al. (*23*) reported correlations between CYP levels and tissue concentrations of PCBs in *Mytilus sp.* In highly polluted areas, ethoxyresorufin *O*-deethylase activity and CYP1A1 protein were induced in flatfish (*24*). Induction studies in several aquatic species suggest that increases can be produced by exposure to Aroclor 1254 and

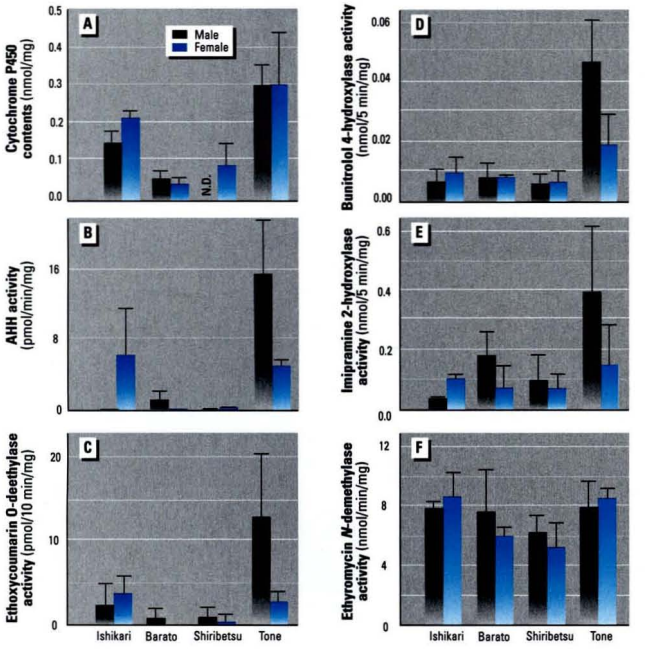

Figure 5. Cytochrome P450 levels and metabolic activities in microsomes from hepatopancreas of male and female *E. japonicus*. The cytochrome P450 content was calculated from the carbon monoxide-difference spectra of reduced microsomes (*12*). Each value represents the means ± SD from three animals. ND, not detectable. (A) Cytochrome P450 level, (B) benzo[*a*]pyrene 3-hydroxylase activity, (C) ethoxycoumarin *O*-deethylase activity, (D) bunitrolol 4-hydroxylase activity, (E) imipramine 2-hydroxylase activities, and (F) erythromycin *N*-demethylase activity.

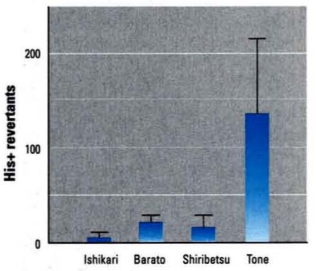

Figure 6. Metabolic activation of benzo[*a*]pyrene by microsomes from hepatopancreas of male *E. japonicus*. Mutagenicity was assayed using *S. typhimurium* TA98 (*20*).

DDTs (*25–27*). However, in the present investigation, the levels of CYP and enzyme activities did not reflect the tissue levels of PCBs and insecticides. It is possible that highly induced crabs have lower levels of environmental contaminants in their tissues because the contaminants are rapidly metabolized by induced enzymes. Another possibility is that the PCB isomers and congeners, which were accumulated in the crabs, did not induce CYP and drug-metabolizing enzymes. PCBs consist of many isomers and congeners which have a variety of induction potencies toward CYP (*28*). Isomer-specific analysis of PCBs was not conducted in this investigation.

CYP levels in female crabs paralleled those in male crabs. In females, however, the fluorescence intensity of extracts from the water the crabs inhabited showed high correlation only with imipramine 2-hydroxylase activity. The enzyme activities of the hepatopancreatic microsomes of crabs from Tone River appear to indicate the presence of sex-specific differences in these activities. However, clear sex differences were observed only in crabs from Tone River, the most polluted river studied. This may indicate that female crabs are less responsive to the induction of CYP; females may not be as sensitive as males to foreign chemicals in the induction of drug-metabolizing enzymes tested in this study. Female crabs may not accumulate pollutant chemicals to a high enough concentration to induce CYP because they lay large numbers of eggs, and the pollutants may be transferred to eggs. The male crabs collected from the Tone River, with high PAH concentrations, exhibited high CYP levels and drug-metabolizing activities.

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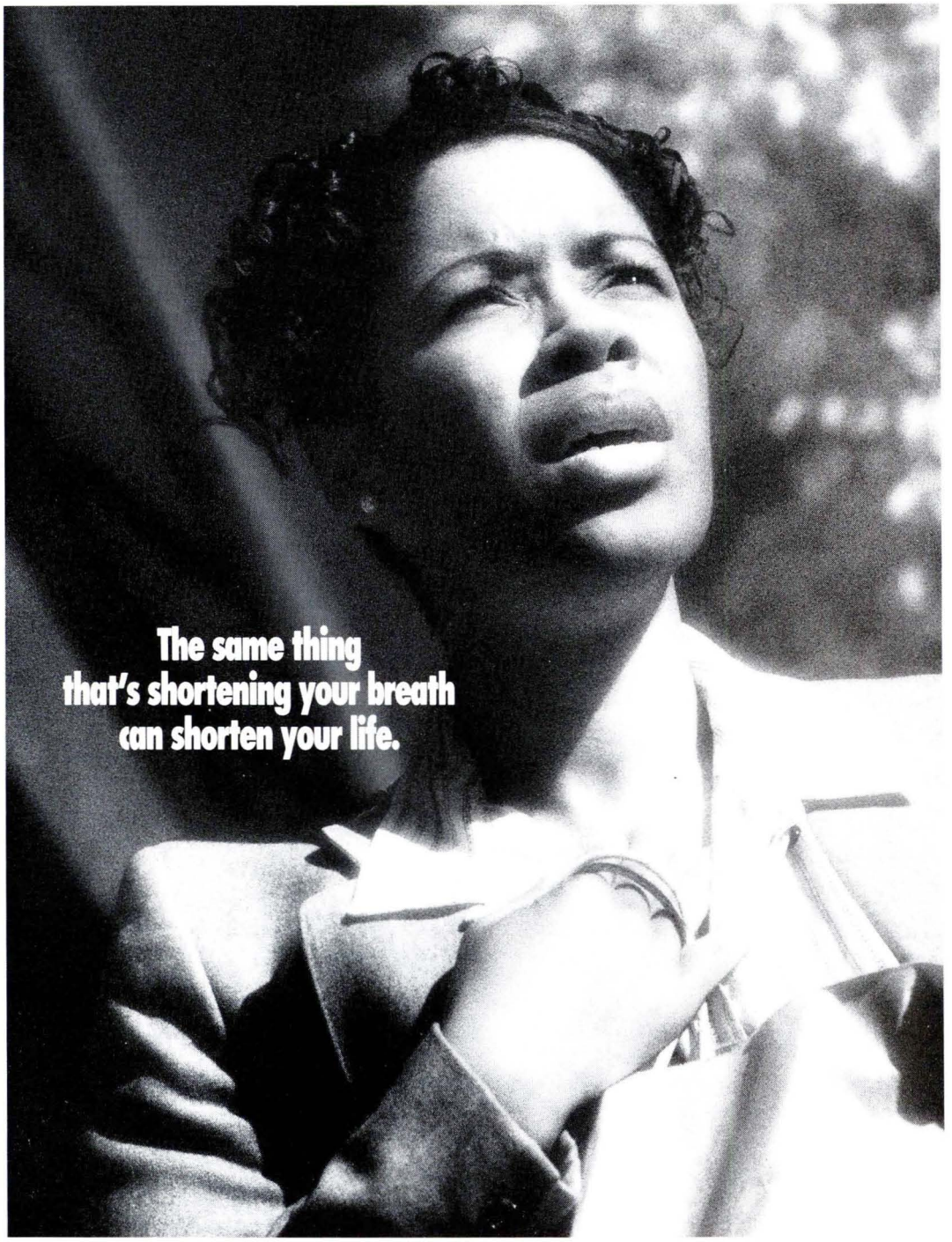

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ISBN: 0387948090, $79.

Biologic Markers in Urinary Toxicology
Subcommittee on Biologic Markers in Urinary Toxicology, Committee on Biologic Markers, Board on Environmental Studies and Toxicology, Commisson on Life Sciences, National Research Council
Washington, DC: National Academy Press, 1995, 309 pp. ISBN: 0309052289, $34.

The Case Against the Global Economy; And For a Turn Toward the Local
Jerry Mander, Edward Goldsmith, eds.
San Francisco: Sierra Club Books, 1996.
ISBN: 0871563525 (alk. paper), $27.

Chemistry of Water Treatment
Samuel D. Faust, Osman M. Alay
Chelsea, MI: Ann Arbor Press, Inc. 1996, 750 pp.
ISBN: 1575040115, $47.96.

The Ecological Basics of Conservation; Heterogeneity, Ecosystems, and Biodiversity
STA Pickett, ed
New York: Chapman & Hall, 1996.
ISBN: 0412098512 (alk. paper), $59.95.

Ecopolitics; The Environment in Poststructuralist Thought
Verena Andermatt Conley
New York: Routledge, 1996. ISBN: 0415102847 (cloth), $59.95 0415103061 (paper), $17.95.

Environmental and Health Atlas of Russia
Murray Feshbach, ed.
Moscow, Russia: PAIMS Publishing House, 1995, 100 pp. ISBN: 5876640530, $95.

Environmental Issues and Business; Implications of a Changing Agenda
Sally Eden
New York: John Wiley, 1996, 224 pp.
ISBN: 0471948721, $59.95.

Environmental Studies; Mathematical, Computational, and Statistical Analysis
Mary Fanett Wheeler, ed.
New York: Springer , 1996, 424 pp. ISBN: 0387947655 (alk. paper), $59.95.

Essential Environmental Science; Methods and Techniques
Simon Watts, Lyndsay Halliwell, eds.
New York: Routledge, 1996, 416 pp.
ISBN: 0415132479, $24.95.

Global Environmental Security; From Protection to Prevention
Y. Suzuki, K. Ueta, S. Mori
New York: Springer, 1996, 272 pp. ISBN: 354060944X (acid-free paper), $74.95.

Health Effects of Hazardous Materials
Neal K. Ostler, Thomas E. Byrne, Michael Malachowski
Upper Saddle River, NJ: Prentice Hall, 1996, 288 pp. ISBN: 0023895519, $46.95.

Healthy Living in a Toxic World; Simple Ways to Protect Yourself and Your Family from Hidden Health Risks
Cynthia E. Fincher
Colorado Springs, CO: Pinon Press, 1996.
ISBN: 0891099786, no price available.

The Home Environmental Checklist; 50 Environmental Hazards to Avoid when Buying Selling, or Maintaining a Home
Andrew N. Davis, Paul E. Schaffman
New York: Henry Holt and Company, 1996, 256 pp. ISBN: 080504177X (alk. paper), $14.95.

Life in the Cold; an Introduction to Winter Ecology
Peter J. Marchand
Hanover, NH: University of Press of New England, 1996, 256 pp. ISBN: 0874517850 (alk. paper), $17.95.

Mechanisms and Concepts in Toxicology
W. Norman Aldridge
Bristol, PA: Taylor and Francis, 1996, 254 pp. ISBN: 0748404139 (cloth), $89.50. 0748404147 (paper) $39.95.

Nature and the Idea of a Man-Made World; An Investigation Into the Evolutionary Roots of Form and Order in the Build Environment
Norman Crowe
Cambridge, MA: MIT Press, 1995, 270 pp.
ISBN: 0262032228, $29.95.

Naturally-Occurring Radioactive Materials: Principles and Practices
Philip T. Underhill
Delray, Beach, FL: St. Lucie Press, 1996, 220 pp.
ISBN: 1574440098, $49.95.

Science Under Seige; How the Environmental Misinformation Campaign is Affecting Our Laws, Taxes, and our Daily Lives
Michael Fumento
New York: W. Morrow, 1996. ISBN: 0688147666, $16.

Statistics in the Health Sciences: Survival Analysis A Self-Learning Text
David G. Kleinbaum
Brooklyn, NY: Springer-Verlag, 1996, 324 pp.
ISBN: 0387945431, $44.95.

Surface and Dermal Monitoring for Toxic Exposures
Shirley A. Ness
Florence, KY: Van Nostrand Reinhold, 1996, 556 pp. ISBN: 0442014651, $74.95.

Using Federalism to Improve Environmental Policy
Jonathan R. Macey
Washington, DC: AEI Press, 1996, 150 pp. ISBN: 0844739626 (cloth, alk. paper), $29.95. 0844739634 (paper, alk. paper), $12.95.

Volterra-Hamilton Models in the Ecology and Evolution of Colonial Organisms
PL Antonelli, RH Bradbury
Singapore River Edge, NJ: World Scientific, 1996, 250 pp. ISBN: 9810224508, $38.

Wiley Encyclopedia of Energy and the Environment
Attilio Bisio, Sharon Boots, eds.
New York: John Wiley, 1996, 2964 pp.
ISBN: 047114827X (alk. paper), $900.

YUSHO: A Human Disaster Caused by PCBs and Related Compounds
Masanori Kuratsune, Hidetoshi Yoshimura, Yoshiaki Hori, Makoto Okumura, Yoshito Masuda
Fukuoka, Japan: Kyushu University Press, 1996, 390 pp. ISBN: 487378431X, ¥ 12360.

VTH INTERNATIONAL CONFERENCE ON HORMONES, BRAIN AND BEHAVIOR

TORINO ITALY AUGUST 25-30, 1996

This international conference will bring together behavioral endocrinologists interested in brain mechanisms in relation to the control of reproductive and social behavior. A series of 34 invited lectures are organized on the following themes:

Melatonin and circadian/circannual rhythms

Central control of reproductive and endocrinology

Sex differentiation of brain and behavior

Brain steroids metabolism

Neurochemistry of behavior

Sensory inputs and behavior

Chemical neuroanatomy of brain areas implicated in behavior

Hormone induced brain plasticity

The program will also include, contributed oral and poster communications on these and related topics.

Information:
GianCarlo Panzica
Department of Human Anatomy and Physiology
c.so. M.D'Azeglio 52.1 10126 Torino Italy
Fax: 39 11 6707732 E-mail: Panzica@Albert.unito.it

Calendar

August

4–14 August, Sun–Wed. International Geological Congress, Beijing, China. Information: Z. Xun, Deputy Secretary General, 30th International Congress, PO Box 823, Beijing 100037, P.R. China, 86-1-8327772, FAX 86-1-8328928

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

12–15 August, Mon–Thu. International Symposium on Representation of the Cryosphere in Climate and Hydrological Models, Victoria, British Columbia, Canada. Information: Secretary General, International Glaciological Society, Lensfield Road, Cambridge, CB2 1ER, UK, +44-1223-355974, FAX +44-1223-336543

17–22 August, Sat–Thu. Institute on Economics for Journalists, Jackson Lake Lodge, Grand Teton National Park, Wyoming. Information: Doug Ramsey, Senior Vice President, Foundation for American Communications, 3800 Barham Boulevard, Suite 409, Los Angeles, CA 90068

24–29 August, Sat–Thu. Seventeenth International Congress of Biochemistry and Molecular Biology In Conjunction with 1997 Annual Meeting of the American Society for Biochemistry and Molecular Biology, San Francisco, California. Information: Congress Secretariat, 9650 Rockville Pike, Bethesda, MD 20814-3996, e-mail: 171UBMB@asbmb.faseb.org

26–28 August, Mon–Wed. American Chemical Society Fall National Exposition, Orlando, Florida. Information: American Chemical Society, ACS Expositions, 1155 Sixteenth Street, NW, Washington, DC 20036, (202) 872-4553, FAX (202) 872-4410

29–30 August, Thu–Fri. Dietary Fat and Cancer: Genetic and Molecular Interactions, Loews L'Enfant Plaza Hotel, Washington, DC. Information: Judith Cohn, (202) 328-7744, FAX (202) 328-7226, e-mail: jcohn@capcon.net

September

1–7 September, Sun–Sat. Cellular and Molecular Biology, Second World Congress, Ottawa, Canada. Information: Second World Congress Secretariat, Suite 353, 2660 Southvale Crescent, Ottawa, Ontario, Canada K1B 4W5, (613) 247-1344, FAX (613) 247-2187, e-mail: mhamelin@ottawa.net.

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 358-0-47-47548

12–15 September, Thu–Sun. The Extracellular Matrix: Its Synthesis, Function, and Degradation, Holiday Inn, Lake Placid, NY. Information: The Organizing Committee, W. Alton Jones Cell Science Center, Inc. 10 Old Barn Road, Lake Placid, NY 12946 (518) 523-1252, FAX (518) 523-1849

12–21 September, Thu–Sat. XVIII Quadrennial Ozone Symposium-96, Rome, Italy. Information: R.D. Bojkov, c/o World Meteorological Organization, C.P. 2300, Geneva-2, CH-1201 Switzerland, FAX +41 22 7400984

15–20 September, Sun–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

22–27 September, Sun–Fri. Third USA/CIS Joint Conference on Environmental Hydrology and Hydrogeology, Tashkent, Uzbekistan. Information: American Institute of Hydrology, 3416 University Avenue SE, Minneapolis, MN 55414-3328, (612) 379-1030, FAX (612) 375-0169

24–29 September, Tue–Sun. 42nd Annual Eastern Pacific Oceanic Conference, Stanford, California. Information: M. Korso, College of Oceanic and Atmospheric Sciences, Oregon State University, Ocean Administration, Building 104, Corvallis, OR 97331-5503, (503) 737-3079, FAX (503) 737-2064, e-mail: kosro@oce.orst.edu

29 September–3 October, Sun–Thu. Trefoil/P-Domain Peptides: From Basic Research to Molecular Medicine, Aix-les-Bains (Savoy), France. Information: INSERM Institut National De La Sante, Conferences Philippe Laudat, 101 rue de Tolbiac, 75654 Paris, Cedex 13 France, 33 (1) 44 23 60 89/87, FAX 33 (1) 44 23 60 89, e-mail: laudat@tolbiac.inserm.fr

October

12–15 October, Sat–Tue. Fourteenth International Neurotoxicology Conference. Arlington Hotel, Hot Springs, Arkansas. Information: Joan Cranmer, Department of Pediatrics, #512, University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, AR 72205, (501) 320-2986, FAX (501) 320-4978.

13–17 October, Sun–Thu. V(D)J Recombination and Other Models of DNA Repair and Mutagenesis, Aix-les-Bains (Savoy), France. Information: INSERM Institut National De La Sante, Conferences Philippe Laudat, 101 rue de Tolbiac, 75654 Paris, Cedex 13 France, 33 (1) 44 23 60 89/87, FAX 33 (1) 44 23 60 89, e-mail: laudat@tolbiac.inserm.fr

16–19 October, Wed–Sat. Conference on the Integrative Biology of Exercise, Vancouver, British Columbia, Canada. Information: Conference Management, 9650 Rockville Pike, Bethesda, MD 20814, (301) 530-7010, FAX (301) 530-7014, e-mail: vancouver96@faseb.org

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

20–24 October, Sun–Thu. Seventh North American ISSX Meeting, San Diego, California. Information: International Society for the Study of Xenobiotics, PO Box 3, Cabin John, MD 20818, FAX (301) 983-5357

25–31 October, Fri–Thu. Molecular Genetic Approaches to the Treatment of Genetic Disease, Hyatt Regency, Lake Tahoe, Nevada. Information: Cambridge Symposia, 1037 Chestnut Street, Newton Upper Falls, MA 02164, (617) 630-1399, FAX (617) 630-1395, e-mail: symposia@xensei.com

27–29 October, Sun–Tue. Nutritional Implications of Macronutrient Substitutes, Crystal City Gateway Marriott, Arlington, Virginia. Information: New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021, (212) 838-0230, FAX (212) 838-5810, e-mail: nyas@nyas.org

29–31 October, Tue–Thu. Water Resources & Environmental Research: Towards the 21st Century, Kyoto, Japan. Information: S. Ikebuchi, Water Resources Research Center, Kyoto University, Gokasho, Uji, Kyoto 611 Japan +81-774-32-3093, e-mail: conf@wrcn2.-dpri.kyotou.ac.jp

31 October–5 November, Thu–Tue. Molecular Genetic Approaches to the Treatment of Genetic Disease, Hyatt Regency, Lake Tahoe, Nevada. Information: Cambridge Symposia, 1037 Chestnut Street, Newton Upper Falls, MA 02164, (617) 630-1399, FAX (617) 630-1395, e-mail: symposia@xensei.com

November

3–7 November, Sun–Thu. CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) Protein: Traffic-King, Expression and Cellular Functions, Aix-les-Bains (Savoy), France. Information: INSERM Institut National De La Sante, Conferences Philippe Laudat, 101 rue de Tolbiac, 75654 Paris, Cedex 13 France, 33 (1) 44 23 60 89/87, FAX 33 (1) 44 23 60 89, e-mail: laudat@tolbiac.inserm.fr

3–7 November, Sun–Thu. International Conference on Radiation and Health, Ben Gurion University of the Negev, Beer Sheva, Israel. Information: Conference Secretariat, Ortra Ltd., 2 Kaufman Street, PO Box 50432, Tel Aviv 61500, Israel, 972-3 517-7888, FAX 972-3-517-4433

10–14 November, Sun–Thu. Environmental Impact on Male Reproductive Function, Aix-les-Bains (Savoy)

How to Reach Us

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

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

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

Public Service Advertising—On a space-available basis, we will accept your public service announcements in a variety of formats. We can accept full, half, and one- or two-column camera-ready ads. If you do not have a formal ad but can provide an electronic version of your text via electronic mail or on a computer disk, we will include it for a limited time in the journal.

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

France. Information: INSERM Institut National De La Sante, Conferences Philippe Laudat, 101 rue de Tolbiac, 75654 Paris, Cedex 13 France, 33 (1) 44 23 60 89/87, FAX 33 (1) 44 23 60 89, e-mail: laudat@tolbiac.-inserm.fr

December

1–5 December, Sun–Thu. The American Society of Tropical Medicine and Hygiene 45th Annual Meeting, Hyatt Regency, Baltimore, Maryland. Information: The American Society of Tropical Medicine and Hygiene, 60 Revere Drive, Suite 500, Northbrook, IL 60062, (847) 480-9592, FAX (847) 480-9282

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

1997

August

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

Fellowships, Grants & Awards

Postdoctoral Fellowships in Toxicology/Epidemiology

Postdoctoral fellowships are available in a unique NIH-sponsored training program in toxicology/epidemiology of respiratory tract disease caused by environmental agents. Conducted jointly by the Inhalation Toxicology Research Institute (ITRI) and the Department of Medicine, University of New Mexico (UNM), the program provides training focus in either laboratory or epidemiology-based research with cross-training in the other disciplines. 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 who are studying pulmonary epidemiology. Programs are tailored to individuals. Training can lead to an MPH degree from UNM. 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. Participants must be US citizens or permanent resident aliens.

Contact: Dr. David Coultas, MD, The Mexico Tumor Registry, University of New Mexico, School of Medicine, 900 Camino de Salud NE, Albuquerque, NM 87185, (505) 277-5541 or Marcy Wood, Education Specialist, Inhalation Toxicology Research Institute, PO Box 5890, Albuquerque, NM 87185, (505) 845-1257. We are an Equal Opportunity/Affirmative Action 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.

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

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

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

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.

Forest History Society Offers 1996 Travel Grants

The Forest History Society announces the availability of Alfred D. Bell, Jr. travel grants for 1996. Those wishing to study at the Society's library and archives may receive up to $750 in support of travel and lodging expenses. Five Bell grants were awarded during 1995. For information on the Society's holdings and application procedures, write: Bell Travel Grants, Forest History Society, 701 Vickers Avenue, Durham, NC 27701 or call: 919 682-9319.

Forest Ecosystem

The U.S. Forest Service is soliciting proposals to develop a quality assurance and scientific assessment program within forest ecosystem research, monitoring and socioeconomic projects under its Southern Global Change Program. Applicant evaluation factors include: 1. experience in developing a quality assurance program for biological research , especially for field research; and 2. knowledge, educational background and experience regarding air pollution and potential climate change impacts to forest resources. For solicitation copy, immediately write: USFS, PO Box 2750, Attn: Nancy H. Meadows, Asheville, NC 28802. or Fax (704) 257-4876. Telephone for more information only: (704) 257-4297 or Alan Moore at (704) 257-4291. Reference: RFP SE-96-28.

Environmental Restoration

Applied research and development of technologies for environmental restoration and waste management are sought by the U.S. Department of Energy. Areas of interest include: 1.) contaminant plume containment and remediation; 2.) mixed waste characterization, treatment and disposal; 3.) landfill stabilization; 4.)sensor technology; and 5.) robotic technology. Proposals should address new concepts, long-term technology needs, and barriers and gaps in current technology. For solicitation copy, write: DOE, Morgantown Energy Technology Center, PO Box 880, Attn: Crystal A. Sharp, Morgantown, WV 26507-0880. Phone for more information only: (304) 285-4442. e-mail: csharp@metc.doe.gov

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 36th Annual Meeting of the Society of Toxicology to be held March 9–13, 1997 in Cincinnati, Ohio. General areas of research may include male or female reproductive toxicology, reproductive endocrine toxicology, teratology/developmental toxicology, and/or postnatal development. By November 8, 1996, candidates for these awards should send to the address below 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 in their evaluation. The abstracts and posters should describe the original research which may include applied studies, investigations of mechanisms of toxic response, or studies of basic mechanisms of action. Interested individuals may request Society information and abstract forms from the Society of Toxicology in Reston, Virginia (703) 438-3115 or sothq@toxicology.org). All submitted material will be treated as confidential. The Winning presentations will be announced at the Annual Meeting of the Specialty Subsection in Cincinnati. For further information, contact: Betsy D. Carlton, DABT Rhône-Poulenc, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

Breast Cancer in the Elderly

Research on the unique problems of older women with breast cancer is sought by the National Cancer Institute and the National Institutes of Aging and of Nursing Research. This initiative encourages research from the behavioral and social sciences, as well as from biology, clinical medicine, and epidemiology. Although the focus is on women 65 and older, studies may involve younger women for comparison. Deadlines under this ongoing program include October 1 and February 1 Contact: Claudette G. Varicchio, NCI, Executive Plaza North, Suite 300, Bethesda, MD 20892, (301) 496-8541, FAX (301) 496-8667, e-mail: Varricci@DOPCEPN. nci.nih.gov. Reference PA-96-034

Child Health Research

Seven new and competing continuation grants are available from the National Institute of Child Health and Human Development to operate centers that will provide resources to help speed the transfer of basic science knowledge to clinical applications that benefit the health of children. "This will be accomplished," NICHD noted, "by increasing the number of pediatric medical centers that can facilitate the application of research findings to pressing pediatric problems, as well as by increasing the number and effectiveness of pediatric investigators who have a grounding in basic science and research skills that can be applied to the clinical problems of children." Eligible applicants include children's hospitals and the departments of pediatrics of approved medical schools that have as a primary teaching site either a general children's hospital or a children's program. Approximately $2.8-million is available for first-year funding of these awards. Letters of intent are requested by August 15; full applications are due November 13. Contact: Ephraim Y. Levin, Center for Research on Mothers and Children, NICHD, 6100 Executive Blvd., Room 4B11 MSC7510, Bethesda, MD 20892-7510. (301) 496-5593, FAX: (301) 402-2085, e-mail: fjt@cu.nih.gov. Reference RFA HD-96-003.

Waste Management

Proposals for applied research are invited by the U.S. Environmental Protection Agency under its Environmental Restoration and Waste Management Program. Proposals may be submitted anytime through approximately May 2, 1997. This program seeks the development of technologies (a device, process, material, or method) that improves DOE's capabilities in the following areas: 1) subsurface containment; 2) mixed waste characterization, treatment and disposal; 3) robotics technology development; 4) efficient separations and processing; 5) tank waste remediation; 6) decontamination and decommissioning; and 7) characterization, monitoring, and sensor technology. Proposals are not sought for basic research, demonstrations, conferences, or training. Proposals should address only one of the need areas, but multiple proposals may be submitted. Contact: DOE, Crystal S. Sharp, M.S. I07, Morgantown Energy Technology Center, PO Box 880, 3610 Collins Ferry Rd., Morgantown, W.V. 26507-0880. (304) 285-4634, FAX (304) 285-4683, e-mail: csharp@metc.doe.gov. The information package is also available on the web at: http://www.metc.doe.gov/business/solicita.html. Reference: ROA DE-R021-96MC33204.

Mycobacterium Avium Infection

Research to quantitatively evaluate therapeutic agents for efficacy against mycobacterium avium in a mouse model is sought by the National Institute of Allergy and Infectious Diseases. One five-year award is available. Proposals are due August 15. Contact: Joyce U. Sagami, Contracts Management Branch, NIAID, 6003 Executive Blvd. Room 3C07/MSC 7610, Bethesda, MD 20892-7610. (301) 496-7118, FAX: (301) 402-0972, e-mail: js73b@nih.gov. The solicitation is available on the web at http://www.nih.gov. Reference RFP NIH-NIAD-DAIDS-97-01.

Position Announcements

Retinal Anatomy/Physiology
Postdoctoral Position availbable now to work on NIH-funded study of interactions between rod and cone signals in macaque monkey retina. Training is offered in physiology and anatomy of primate retina in the lab of Dr. Dennis Dacey (Department of Biological Structure). Background in electrophysiology, visual psychophysics, and/or color science would be helpful. Annual salary: $25,000 for one to three years. Please send curriculum vitae and names of three references to : Dr. Steven Buck, Department of Psychology, University of Washington, Box 351525, Seattle, WA 98195. FAX: 206-685-3157, e-mail: sbuck@u. washington.edu

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/MD with 5 or more years highly relevant experience is required. Candidates should be knowledgeable about cutting-edge toxics issues such as disproportionately impacted subpopulations, noncancer endpoints, and emerging issues regarding carcinogenesis. The salary is $50,000--$65,000, commensurate with experience. Send resume to: Public Health Program, NRDC, 1350 New York Avenue, NW, Suite 300, Washington, DC 20005. Equal Opportunity Employer.

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 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, e-mail: Hong3@niehs.nih.gov

Characterization of Receptor–Ligand Interactions (HNV96-1)
Mass spectrometry combined with protection assays is being used to probe structural motifs involved in molecular interactions, such as the interaction of HIV rgp120 and immunoglobin, relevant to an understanding of the basic processes occurring during HIV viral infection. Candidates should have expertise in protein and immunochemistry.
Contact: Kenneth Tomer, (919) 541-1966, Laboratory of Molecular Biophysics, Maildrop 6-01, e-mail: Tomer@niehs.nih.gov

Eicosanoids, Airway Inflammation and Asthma (HNV96-2)
The expression and/or activation of the enzymes that metabolize arachidonic acid to inflammatory lipids; prostaglandin H synthase, lipoxygenases and phospholipases as related to the inflammatory response of trachea-bronchial epithelium is being investigated. A rat or human culture system is used for these studies in which differentiation to mucociliary or squamous phenotypes is regulated by retinoids. Differentiation of the epithelium has profound effects on the arachideonic acid metabolism. Experience with molecular biology techniques required and an interest in pulmonary biology desirable.
Contact: Thomas Eling, (919) 541-3911, Laboratory of Molecular Biophysics, Maildrop B3-01, e-mail: Eling@niehs.nih.gov

ATM Function in Cell Cycle Checkpoints and Senescence (HNV96-3)
Signal transduction mechanisms regulating cell cycle checkpoints and cellular senescence are being investigated, focusing particulary on the role of the ataxia telangiectasis mutated (ATM) gene product. Studies focus on the regulation of certain cyclin/cyclin-dependent kinase complexes in response to DNA damage following exposure to selected environmental carcinogens and in response to the normal aging process. Candidates should have experience in molecular biology, cell biology, or biochemistry.
Contact: Richard S. Paules, (919) 541-3710 or Cynthia Afshari (919) 541-1310, Laboratory of Environmental Carcinogenesis and Mutagenesis, Maildrop C1-09, e-mail: Paules@niehs.nih.gov or Afshari@niehs.nih.gov

Signal Transduction/Protein Purification (HNV96-6)
Inhibition of calcium-dependent chloride secretion by $Ins(3,4,5,6)P_4$ represents a new field of signal transduction regulating salt and fluid secretion, osmoregulation and neurotransmission; pharmacological intervention is relevant to cystic fibrosis and cardiac hypertrophy. The successful applicant will contribute to purification and characterization of the $Ins(3,4,5,6)P_4$ receptor and the enzymes of $Ins(3,4,5,6)P_4$ receptor synthesis and metabolism.
Contact: Stephen Shears, (919) 541-0793, Laboratory of Cellular and Molecular Pharmacology, Maildrop 7-10, e-mail: Shears@niehs.nih.gov

X-ray Crystallography (HNV 96-8)
The structural basis for the broad substrate specificity in the drug-metabolizing enzymes including P450s and sulfotransferases is studied. Contact: Masahiko Negishi, (919) 541-2404, Laboratory of Reproductive and Developmental Toxicology, Maildrop E4-07, e-mail: Negishi@niehs.nih.gov

Xenobiotic Transport Mechanisms (HNV96-9)
We use fluorescent substrates, confocal microscopy, and image analysis to characterize xenobiotic transport mechanisms in renal and non-renal epithelial tissues. The focus is on understanding the specificities, energetics, and regulation of both plasma membrane transporters and intracellular mechanisms such as vesicle-mediated transcytosis. Experience in the biochemistry and physiology of membrane transport is expected. Position available October 1, 1996.
Contact: David S. Miller, (919) 541-3532, Laboratory of Cellular and Molecular Pharmacology, Maildrop 7-01, e-mail: Miller@niehs.nih.gov

Renal Transport Physiology (HNV96-10)
The transport mechanisms responsible for elimination of xenobiotics are studied in epithelial tissues including kidney and choroid plexus. Applicant will examine the mechanisms and energetics of organic anion transport using cultured monolayers and isolated membrane vesicles. Experience in the biochemistry or physiology of membrane function is expected. Position available August 1, 1996.
Contact: John Pritchard, (919) 541-4054, Laboratory of Cellular and Molecular Pharmacology, Maildrop 19-02, e-mail: Pritchard@niehs.nih.gov

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, e-mail: Jetten@niehs.nih.gov

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, e-mail: Schaaper@niehs.nih.gov

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, e-mail: Nettesheim@niehs.nih.gov

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. Regulation of the gene expression is studied using Northern analysis, RT-PCR, and protein immunoblotting, immunohistochemistry and *in situ* hybridization. Applicants should have a strong background in cell and molecular biology.
Contact: Darryl Zeldin, (919) 541-1169, Laboratory of Pulmonary Pathobiology, Maildrop D2-01, e-mail: Zeldin@niehs.nih.gov

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, e-mail: Yakel@niehs.nih.gov

Toxicokinetic Modeling (HNV121)
Toxicokinetic models are being studied as a means of relating the response of laboratory animals and humans to exposure to environmental toxins. Research relating to the utility of these models for the design and analysis of laboratory and epidemiology studies and for risk estimation is planned. Applicants should have a strong background in computer modeling and some experience in modeling biological systems.
Contact: Christopher J. Portier, 919-541-3519, Laboratory of Quantitative and Computational Biology, Maildrop A3-06, e-mail: Portier@niehs. nih.gov

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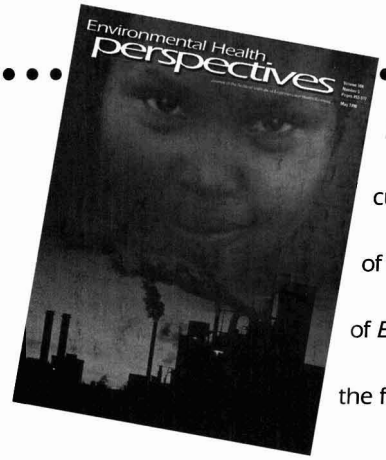

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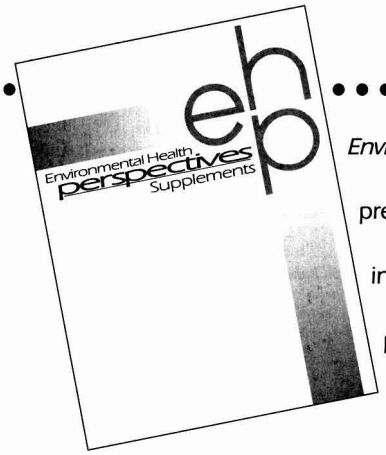

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Editorial Policy

Environmental Health Perspectives is intended to be a forum for the discussion of issues in environmental health, and several formats have been devised for that purpose. In addition, several formats are available for the publication of scientific articles and scientific discussion. All scientific articles are subject to rigorous peer review. The primary criteria for publication are environmental significance and scientific quality.

Environmental science is made up of many fields, and therefore we are prepared to consider scientific progress in all of them. Cross-fertilization and serendipity have proven to be extremely important processes in the advance of science in general, and this must hold true for the science of environmental health. We will consider for publication articles ranging from the most basic molecular biology to environmental engineering. We particularly encourage those researchers concerned with mechanisms of toxic action and new approaches for detecting and/or remedying environmental damage.

Opinions and ideas based on scientific observation and argument are welcome. While the expression of opinions may lead to debate and disagreement, such reactions are healthy and can lead to new research and discoveries. Presentations of ideas and opinions will be promoted, but our policy will be to strive for objectivity and balance.

In addition to scientific articles and discussion, we publish news of the environment. We will consider factual articles about issues that affect the environment and human health. We summarize legislative and regulatory developments, grant information from NIEHS and other granting agencies, new research areas, environmental problems, technological advances, and information about the National Toxicology Program and other important programs. Presentations of news strives for objectivity and balance and is based on the strength of scientific evidence.

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

Perspectives

The journal is a forum for the expression of ideas and opinions. Opinions and ideas should be carefully considered and based on scientific principles. Three formats are offered:

Editorial statements are published by our editors, members of our editorial boards, and occasional guest editors. These statements are intended to focus attention on important or neglected areas of environmental health, offer opinions and ideas, and stimulate discussion.

Commentaries are up-to-date articles that may present commentaries offering perspective and insight on a particular topic. Commentaries are subject to peer review.

Friendly Fire is a scientifically based debate between environmental experts with accompanying clarifications and rebutals. The debates focus on selected topics of broad interest to the environmental community that are of great importance for public health.

Correspondence is encouraged. Opinions, perspectives, and insight are welcome. Comments on articles published in *Environmental Health Perspectives* are also welcome, but criticism will always be balanced by the opportunity for defense and clarification.

Research

To ensure fairness in the review process, we routinely seek opinions from three reviewers. Suggestions for reviewers of manuscripts will be considered. The research portion of the journal consists of four formats:

Research Articles are original manuscripts reporting scientific research and discovery in the broad field of environmental health. Research articles may come from any field of scientific research, from the most basic molecular biology and biochemistry to atmospheric physics, ecology, and engineering. The criteria for publication are weighted toward scientific quality and environmental significance. The work will be assessed according to its originality, scientific merit, and experimental design; the manuscript will be evaluated based on its conciseness, clarity, and presentation. We also attempt to address certain ethical problems during the review process. We require assurances that all human and animal subjects have been treated humanely and with due regard for the alleviation of suffering. Manuscript review also considers scientific integrity as part of the process.

Research Advances are concise articles intended to address only the most recent developments in a scientific field. Clarity of presentation is of primary importance because these articles are intended to be educational though targeted to the expert audience.

Reviews are narrowly focused articles that emphasize recent developments in a particular field of research. Lengthy historical perspectives are not appropriate.

Meeting Reports are short summaries of conferences, symposia, or workshops in which the scientific objectives and achievements of a meeting are described.

Environews

The news section provides up-to-date information on important issues in environmental health covering a variety of areas including policy, legislative, and regulatory actions; innovative technological and conceptual research advances; conference and meeting summaries; and emerging environmental problems. The news section consists of several components:

Forum articles are brief reports on matters of potential environmental health significance such as chemical spills and contamination episodes. Brief reviews of recent scientific advances are also included.

NIEHS News summarizes significant activities or accomplishments at NIEHS and the National Toxicology Program.

Focus articles are substantive news items about important issues in environmental health. Examples include reports on risk assessment, risk management dilemmas, women's health initiatives, environmental equity, relevance of animal models to toxicity testing, and structure–activity approaches to toxicity evaluation.

Spheres of Influence is a legal/regulatory column that presents reports on significant events and decisions involving the executive branch, Congress, and regulatory agencies. Examples include new directions of White House policies, impact of Clean Air Act legislation, and coverage of congressional hearings on environmental health issues.

Innovations presents emerging opportunities in environmental health based on new discoveries or approaches in biology, chemistry, engineering, or information sciences. Examples include the use of transgenic animals in toxicity testing, new advances in molecular biology, development of more rapid and efficient methods for clean-up of hazardous wastes, and methods for early detection of environmental damage and environmentally mediated diseases.

Announcements includes a calendar of upcoming events such as conferences, workshops, and public hearings. Appropriate listings are made for industrial, academic, regulatory, and legal activities. This section also includes listings of fellowship and grant announcements and positions available.

Environmental Health Perspectives Supplements

During the last 20 years, we focused on the development of a series of monographs that have generally arisen from symposium or conference proceedings. Monographs are now published as supplements to the main journal. Six to eight supplements are published per year: four to seven of these consist of conference, workshop, or symposium proceedings, and one issue is dedicated to solicited and unsolicited comprehensive reviews on environmental health. Conference manuscripts must be of the highest scientific quality and are subject to rigorous peer review. Manuscripts that do not meet *EHP* standards are not published.

Each supplement resulting from a conference should address a specific area of concern, a research problem, or a particular scientific issue. Supplements are, in general, dedicated to scientific issues and not to programmatic themes. Each supplement should form a landmark statement for a particular subject and must be an up-to-date, balanced source of reference material for researchers, teachers, legislators, and the informed public. Publication of conference proceedings in *EHP Supplements* requires the submission of a proposal as described in Instructions to Authors.

Supplement Articles from conferences are generally the result of research investigations, reviews, or a combination of both; however, brief reports and commentaries are also appropriate.

Perspective Reviews are targeted to the one or two specific issues of *EHP Supplements* set aside for the publication of reviews in environmental health sciences. Perspective reviews are in-depth, comprehensive articles that address developments in specific areas. Perspective reviews must not be simply a compilation of the literature but should be scholarly, landmark statements offering a complete and balanced perspective as well as insight into the environmental significance of the research.

Instructions to Authors

Environmental Health Perspectives covers all disciplines engaged in the broad field of environmental health. Authors should therefore write in a clear and simple manner, avoiding unnecessary technical jargon, so that the article is understandable to readers in other disciplines.

All submitted manuscripts are acknowledged upon receipt and subjected to three independent peer reviews. Submit four copies of the manuscript, along with three sets of publication-quality figures. Authors may suggest reviewers when submitting a manuscript, although suggested reviewers may not be chosen. Peer review is generally completed within four weeks and authors are notified of necessary revisions or rejection of the manuscript. Revisions are requested within three weeks of notification. Authors must submit two copies of the revised manuscript, a letter responding to reviewer's comments, and a diskette containing the revised manuscript. Articles are generally published three months after receipt of revisions. Corresponding authors are sent 200 free reprints of their article upon publication.

Manuscript Preparation

All manuscripts must be typed, double-spaced, in English, on only one side of the paper. 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). Number pages consecutively, beginning with the title page. Reference lists, tables, and figure legends should be on separate pages, and should also be double-spaced. If the manuscript is accepted for publication, a computer disk copy must be submitted along with two hard copies of the revised manuscript.

Titles should not exceed 20 words and should generally not contain abbreviations or numerical values. The title page should also list authors (first or second names spelled out in full), full address of the institution where the work was done, and affiliation of each author. Indicate author to whom galley proofs and reprints should be sent (include complete address for express mail service, telephone and FAX numbers).

Place a **running title**, not to exceed 50 characters and spaces, on the second page of the manuscript. Also on this page, list 5–10 **key words** for indexing purposes, list and define all abbreviations, and include **acknowledgments** and grant information, not to exceed 50 words. Nomenclature and symbols should conform to the recommendations of the American Chemical Society or the International Union of Pure and Applied Chemistry (IUPAC).

All articles except those for Friendly Fire and meeting reports must include an **abstract**, not to exceed 250 words, which should be placed on the third page of the manuscript. Do not include details of materials and methods or references in the abstract.

Text should begin on the fourth page. For research involving human subjects, include a statement that informed consent was obtained. For animal subjects, include a statement that care and treatment was conducted in accordance with established guidelines. Concise headings (not to exceed 8 words) may be used to designate major sections. Recommended headings, where appropriate, are "Materials and Methods," "Results," and "Discussion" or "Conclusion." **References** must be listed by number, in order of citation. Reference numbers should be italicized, if possible, and placed in parentheses in the text.

The **reference list** should begin on a separate page. Personal communications, unpublished observations, manuscripts in preparation, and submitted manuscripts should not be included in the reference list, nor should explanatory text (footnotes). Such references should be inserted at appropriate places in the text, in parentheses, without a reference number. "In press" articles should be included in the reference list. Abbreviate journal names according to *Index Medicus* or *Serial Sources for the BIOSIS Previews Database*. List all authors and editors; do not use et al. in the bibliography. Include the title of the journal article or book chapter and inclusive pagination. For reports, include the authoring organization, report number, "publisher" and location, and year of publication. Some examples are shown below:

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.

Report:

3. U.S. EPA. Status of pesticides in reregistration and special review. EPA 738-R-94-008. Washington, DC:Environmental Protection Agency, 1994.

Each **table** must be on a separate page. Tables should be numbered with Arabic numerals, followed by a brief title (not to exceed 25 words). General footnotes to tables should be indicated by lowercase superscript letters beginning with *a* for each table. Footnotes indicating statistical significance should be identified by asterisks (*, **) and daggers (†, ‡). Type footnotes directly after the table. Tables should contain no more than three layers of column headings, and the entire table should fit on one journal page.

Figure legends should be typed, double-spaced, on a separate page. Legends should be as brief as possible without compromising explanation of the figure. Use Arabic numerals to number figure legends. Define any abbreviations in the legend on first mention.

Three sets of publication-quality **figures** must be submitted. Electronic versions of figures are encouraged, but should be submitted in addition to, not in lieu of, hard copies of the figures. Dot matrix computer drawings are not acceptable as original art. The style of figures should be uniform throughout the paper. Identify all figures on the back with the authors' names and figure number and indicate orientation. Label axes of graphs clearly and define all symbols used.

Material suitable for inclusion as **on-line documentation**, such as kinetic studies, is welcome. Contact the *EHP* office for instructions regarding submission.

Electronic copies of accepted manuscripts are required. We prefer 3.5-inch diskettes, Macintosh platform, Microsoft Word, but IBM PC-compatible files are acceptable. The file should contain ***all*** parts of the manuscript in ***one*** file. Label the diskette with title, author, manuscript number, and software used. Diskettes are not returned to authors. Electronic files created by word processors or similar equipment are not acceptable.

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:

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In your covering letter please provide assurances that the manuscript is not being considered for publication elsewhere and that all animals used in the research have been treated humanely according to institutional guidelines, with due consideration to the alleviation of distress and discomfort. If the research involved human subjects then a statement must be made to the effect that participation by those subjects did not occur until after informed consent was obtained.

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:

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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.

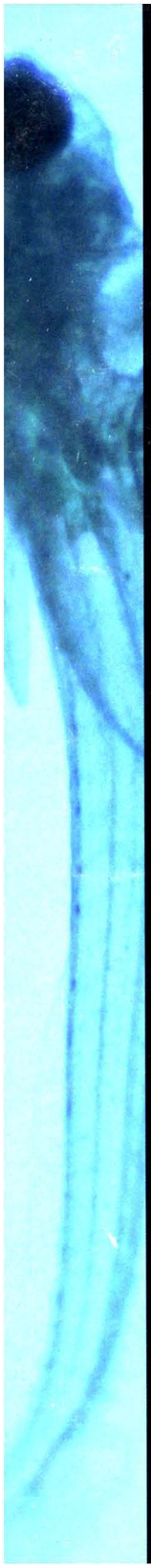
Public information advertisements will be run free-of-cost as space becomes available. All ads are run subject to their appropriateness to the editorial format of the journal. Submissions of advertisements should include full-page, half-page, and quarter-page formats if available. Ads should be camera-ready, black and white positives.

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

Published Volumes of EHP Supplements

Year Vol/No	Subject
1972	
1	Polychlorinated Biphenyls[1]
2	Review–Perspective Articles[1]
1973	
3	Phthalate Esters[1]
4	Review–Perspective Articles[1]
5	Chlorinated Dibenzodioxins and Dibenzofurans[1]
6	The Evaluation of Chemical Mutagenicity Data in Relation to Population Risk[1]
1974	
7	Low Level Lead Toxicity and the Environmental Impact of Cadmium[1]
8	Review–Perspective Articles[1]
9	Asbestos[1]
1975	
10	Mobile Air Emission; Biometerological Hazards; Abstracts on Heavy Metals in the Environment, Conference II[1]
11	Components of Plastics Manufacture[1]
12	Heavy Metals in the Environment[1]
1976	
13	US–USSR Environmental Health Conference[1]
14	Human Health Effects of New Approaches to Insect Pest Control[1]
15	Target Organ Toxicity: Liver and Kidney[1]
16	Target Organ Toxicity: Lung[1]
17	WHO/NIEHS Symposium on Plastics Manufacture[1]
18	Target Organ Toxicity: Development[1]
19	Arsenic and Lead[1]
1977	
20	Proceedings of the Second NIEHS Task Force; NIEHS Science Seminar[1]
21	Vinyl Chloride Related Compounds[1]
1978	
22	Air Pollution and Human Health: Extrapolation from Animal to Man[1]
23	Polybrominated Biphenyls[1]
24	Target Organ Toxicity: Gonads; PCBs[1]
25	Factors Influencing Metal Toxicity[1]
26	Target Organ Toxicity: Cardiovascular System, Nervous System[1]
27	Higher Plants as Monitors of Environmental Mutagens; Hazardous Solid Wastes and Their Disposal[1]
1979	
28	Cadmium[1]
29	Pollutants and High Risk Groups[1]
30	USA/USSR Cooperative Research[1]
31	Aneuploidy[1]
32	JAPAN/USA Biostatistics; Statistics and the Environment[1]
33	Target Organ Toxicity: Intestines; Effects of Increased Coal Utilization; NIEHS Science Seminar[1]
1980	
34	Aquatic Toxicology; Biological Effects of Mineral Fibers and Particulates[1]
35	Experimental Models for Pulmonary Research[1]
36	Application of Negative Ion Spectrometry[1]
1981	
37	Pollen Systems[1]
38	Target Organ Toxicity: Endocrine Systems[1]
39	Target Organ Toxicity: Blood[1]
40	Role of Metals in Carcinogenesis[1]
41	Polyvinyl Chloride[1]
42	Environmental Epidemiology[1]
1982	
43	Target Organ Toxicity: Immune System[1]
44	Target Organ Toxicity: Eye, Ear, and Other Special Senses; Author and Subject Indexes, Volumes 26–40[1]
45	Phthalate Esters[1]
46	Drinking Water Disinfectants[1]
1983	
47	Mutagenicity and Carcinogenicity of Air Pollutants[1]
48	Health Effects of Toxic Wastes[1]
49	*N*-Substituted Aryl Compounds[1]
50	Tumor Promotion[1]
51	*In Vitro* Effects of Mineral Dusts[1]
52	Environmental Epidemiology; Air Quality Control; NIEHS Science Seminar[1]
53	Asbestos[1]
1984	
54	Metallothionein and Cadmium Nephrotoxicity[1]
55	Monograph on Pulmonary Toxicology[1]
56	Methods in Pulmonary Toxicology; Proliferative Pancreatic Lesions[1]
57	Glycol Ethers; Mechanisms of Cell Injury[1]
58	Carcinogenic Potency Database; Formaldehyde[1]
1985	
59	PCBs: Japan–U.S. Symposium[1]
60	PCBs: U.S. Symposium; Finland–U.S. Symposium[1]
61	Monograph on Structure–Activity and Molecular Mechanisms[1]
62	DNA Adducts; Environmental Epidemiology[1]
63	Health Effects of Acid Precipitation; Environmental Risk Assessment[1]
64	Monograph on Free Radical Metabolites[1]
1986	
65	Metal-Binding Proteins; Phthalic Acid Esters[1]
66	Cotton and Grain Dusts[1]
67	Dietary Mutagens[1]
68	SENCAR Mouse; Author and Subject Indices, Volumes 41–60[1]
69	Drinking Water Disinfectants[1]
70	Airborne Pollutants and Respiratory Cancer; Reproductive and Developmental Toxicology[1]
1987	
71	Aquatic Toxicology[1]
72	Methyl Isocyanate[1]
73	Ovarian Pathology; Nitrogen Oxides[1]
74	Biomarkers in Reproductive and Developmental Toxicology[1]
75	NIEHS 20th Anniversary[1]
76	Basic Research in Risk Assessment[1]
1988	
77	Male Reproductive System; Extrapolation of Carcinogenicity Data[1]
78	Lead–Blood Pressure Relationships, Author and Subject Indices, Volumes 61–73[1]
1989	
79	Acid Aerosols[1]
80	Monograph on Regulation of Differentiation in Eukaryotic Cell Systems[1]
81	Scientific Advances in Environmental Health[1]
82	Benzene Metabolism, Toxicity and Carcinogenesis[1]
83	Monograph on Groundwater Quality[1]
1990	
84	Calcium Messenger Systems[2]
85	Chemicals and Lung Toxicity; Upper Respiratory System[1]
86	Butadiene; Environmental Health in the 21st Century[2]
87	Biostatistics in Human Cancer; Structure–Activity[1]
88	Risk Factors and Mechanisms in Carcinogenesis[1]
89	Advances in Lead Research[1]
1991	
90	Contaminated Aquatic Food Resources; Quantitative Risk Assessment[2]
91	Lead in Bone[2]
92	Chromium[2]
93	Target Genes in Chemical Carcinogenesis[2]
94	Environmental Epidemiology[2]
95	Indoor Air Quality[1]
96	Genotoxicity and Carcinogenicity Databases; Global Warming[2]
1992	
97	Particle Clearance by Alveolar Macrophages[2]
98	Biomarkers in Human Cancer–Part I: Predisposition and Use in Risk Assessment[2]
1993	
99	Biomarkers in Human Cancer–Part II: Exposure Monitoring and Molecular Dosimetry[1]
100	Twenty Years of Environmental Health Research[2]
101/1	NTP Abstracts, 1976–1992[2]
101/2	Impact of the Environment on Reproductive Health[2]
101/3	Environmental Mutagenesis in Human Populations at Risk[2]
101/4	Environmental Epidemiology[2]
101/5	Cell Proliferation and Chemical Carcinogenesis[2]
101/6	Health Effects of Gasoline[1]
1994	
102/1	Biostatistics and the Study of Toxicology[1]
102/2	Reviews in Environmental Health, 1994; Human Developmental Neurotoxicity[2]
102/3	Molecular Mechanisms of Metal Toxicity and Carcinogenicity[2]
102/4	Risk Assessment of Urban Air[2]
102/5	Biopersistence of Respirable Synthetic Fibers and Minerals[2]
102/6	Carcinogenic and Mutagenic *N*-Substituted Aryl Compounds[2]
102/7	Health Effects of Boron[2]
102/8	Biostatistics in the Study of Human Cancer[2]
102/9	Toxicological Evaluation of Chemical Interactions[2]
102/10	Oxygen Radicals and Lung Injury[2]
102/11	Dosimetry for Risk Assessment[1]
102/12	Genetic and Molecular Ecotoxicology[2]
1995	
103/1	Fate, Transport and Interactions of Metals[2]
103/2	Health Effects of Ozone; Environmental Epidemiology; Perinatal Exposure to Dioxins[2]
103/3	Human Tissue Monitoring and Specimen Banking[2]
103/4	Wildlife Development[2]
103/5	Biodegradation[2]
103/6	Child Health; Asthma[2]
103/7	Estrogens in the Environment[3]
103/8	Carcinogenic Potency Database; Avoidable Cancer Causes[3]
103/9	Great Lakes and Human Health[3]
1996	
104/1	Reviews in Environmental Health, 1996; Indices, Vol 103, Suppl 1–9 (1995)[3]
104/2	Neurobehavioral Toxicity[3]
104/3	Environmental Mutagens[3]

[1]Out of print. [2]Available free of charge from NIEHS-EHP, P.O. Box 12233, Research Triangle Park, NC 27709. [3]Available by subscription only from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.



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