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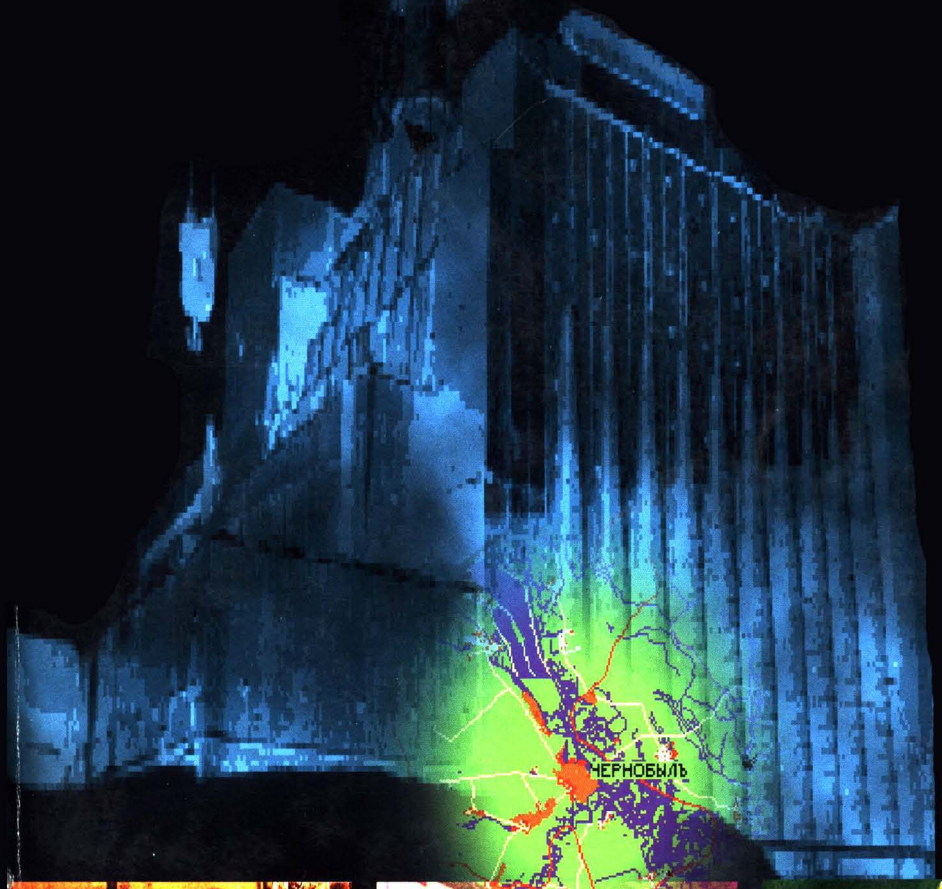


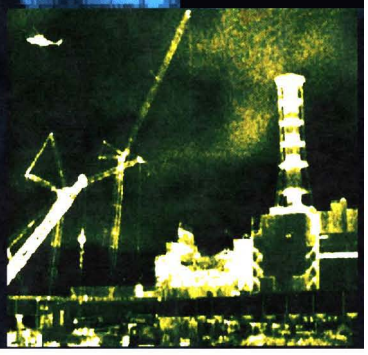

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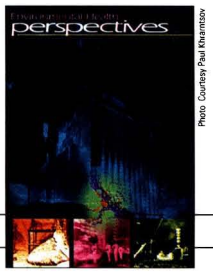
**On The Cover:** The Chernobyl meltdown and cleanup efforts exposed thousands to radiation. Kordysh et al. (p. 936) report nausea, hair loss, and depressed white blood cell counts as symptoms of radiation toxicity among exposed Ukranians.





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# In This Issue

## Environmental Signals

Populations of wildlife species exhibit anomalies in development, behavior, and reproduction shown to be caused by endocrine-disrupting chemicals in the environment. LeBlanc (p. 888) comments that scientists, regulators and legislators alike must not relax environmental regulations and should continue to catalogue cases of ecotoxicity, lest potential threats to human health signaled by indicator species be overlooked.

## Cleaning up Down Under

Because of their small population and geographic isolation, Australia and New Zealand have so far been spared many of the environmental health problems that plague other industrialized countries. However, these two nations recognize that problems such as ozone depletion, toxic waste disposal, and declining air and water quality are beginning to affect the environment, and they are working to halt the degradation and reverse the effects on human health. The first **Focus** article (p. 902) discusses the emerging environmental health problems in these countries and the programs they are implementing to ensure "a cleaner Australia" and a "clean and green" New Zealand.

## Canada Comes Clean

The second largest nation in the world, Canada has also been home to some very large environmental health problems, particularly those associated with chemical exposures to wildlife and humans from paper manufacturing, hydroelectric plants, and fossil fuel combustion. In recent years, however, Canadians have mounted a number of campaigns to clean up polluted air, water, and soil, and have succeeded in drastically reducing exposures to some chemicals. Programs such as Great Lakes 2000, Lawrence Vision 2000, and the recently enacted Toxic Substances Management Plan, discussed in the second **Focus** article (p. 908), will provide a plan of action for protecting Canada's environment and health into the next century.

## Pulling Answers out of Thin Air

Traditional air sampling by chemical and other manufacturers using hand-held "sniffers" provides only snapshot data on the type and amount of pollutants in the air. A new system, described in **Innovations** (p. 918), uses a technique called Remote Sensing Computed Tomography (RSCT) to give industrial hygienists the bigger picture. RSCT, developed by a team at the University of North Carolina at Chapel Hill led by Lori Todd, provides real-time measurements of multiple chemicals over large distances. The team hopes the more sophisticated technology will eventually help manufacturers ensure compliance with environmental health regulations and make better decisions regarding the safety of their employees.

## Exposure to Nuclear Fuels

Lang et al. (p. 920) reviews the toxicological effects of insoluble nuclear fuel compounds released into the environment by nuclear power plants, nuclear weapons tests, or nuclear accidents. The dosimetry, biological effects, and biokinetic properties of insoluble nuclear fuel compounds are discussed in relation to the various toxic reponses exhibited in the gastrointestinal tracts, lungs, and skin of animal models and in humans exposed during the Chernobyl accident.

## Chernobyl Radiation Affects Israeli Immigrants

About 1500 Russian immigrants to Israel were divided into groups based on levels of exposure to radiation from the Chernobyl nuclear power plant explosion. Kordysh et al. (p. 936) used questionnaires and physical examinations to document acute radiation exposure. They found that bronchial asthma and health complaints from children and hypertension in adults were among the nonmalignant effects from prolonged radiation exposure. These toxic responses were associated with the extent of radiation encountered by individuals as measured by body burdens of radioactive cesium-137.

## Use of Transgenic Mice to Identify Carcinogens and Risk

Tennant et al. (p. 942) used two transgenic mouse lines to accelerate testing of chemicals, prioritize chemicals for standard 2-year carcinogen tests, and to examine low-level dose responses for improved estimation of risk assessment. The p53+/- transgenic mouse and the TG.AC transgenic mouse carrying a v-Ha-*ras* construct were used in 20-week dosed-feed or skin paint assays, and results were compared with those from 2-year assays. The authors report a high degree of concordance between the short-term and long-term assays and suggest that that transgenic assays could be used to improve the extrapolation of data from rodents to humans.

## Lead Affects Children's Growth

Kim et al. (p. 952) report on a cohort of children exposed to lead that was examined at about 7 years of age for tooth lead and was reexamined at about 20 years of age for weight, height, and bone lead. The study revealed a positive association between childhood tooth lead and body mass index in cross-sectional and longitudinal analyses: tooth lead in 7 year old children compared to original body mass index or compared to change in body mass index between 7 and 20 years old. The data suggested that chronic lead exposure in childhood may result in obesity that persists into adulthood.

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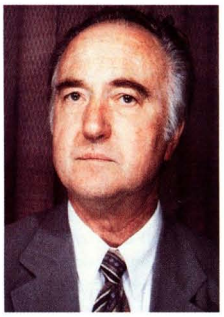
# Editorial

## Chemical Safety: A Global Challenge



Chemicals have become an indispensable part of human life, sustaining activities and development, preventing and controlling many diseases, and increasing agricultural productivity. The benefits are incalculable, yet countering these, chemicals may, especially when misused, exert adverse effects on human health and the integrity of the environment. The nature, number, and quantitites of chemicals used in countries vary widely according to factors such as the national economy, its industries, and agriculture. The growth of chemicals industries, in developing as well as developed countries, is predicted to go on increasing for the rest of this century. Chemical safety encompasses the prevention and management of adverse effects, both short and long term, to humans and the environment from the production, use, and disposal of chemicals. Chemical safety is essential if development is to be beneficial and not catastrophic for humans and the environment.

There are two main obstacles to the sound management of chemicals. First, there is a fundamental lack of knowledge of the risks that many chemicals pose to human health and the environment, an essential prerequisite for the sound management. Second, there is a lack of capability and capacity, particularly in developing countries, to manage chemical risks.

In 1992 the United Nations Conference on Environment and Development (UNCED) recognized that the use of chemicals is essential to meet social and economic goals, while also acknowledging that a great deal remains to be done to ensure the sound management of chemicals. It adopted Agenda 21, chapter 19, as an international strategy for action on chemical safety into the 21st century and named six major areas for this work: 1) expanding and accelerating the international assessment of chemical risks, 2) harmonization of classification and labeling of chemicals, 3) information exchange on toxic chemicals and chemical risks, 4) establishment of risk reduction programs, 5) strengthening of national capabilities and capacities for management of chemicals, and 6) prevention of illegal international traffic in toxic and dangerous products and wastes. Success in each will be facilitated by success in the others.

A number of international bodies, as well as many national chemical safety programs, are now involved in work on chemical safety. The International Programme on Chemical Safety (IPCS), formally launched in 1980, is a joint collaborative program of the International Labour Organization (ILO), the United Nations Environment Program (UNEP), and the World Health Organization (WHO). WHO is the executing agency of the program, and a great proportion of IPCS activities are implemented by the WHO Programme for the Promotion of Chemical Safety on behalf of the three cooperating organizations.

The two main roles of the IPCS are to establish the scientific health and environmental risk assessment basis for safe use of chemicals and to strengthen national capabilities for chemical safety. Among the primary activities of IPCS are evaluations of the risk to human health and the environment from exposure to chemicals and the promotion of the development, improvement, validation, and use of methods suitable for the evaluation of health and environmental risks and hazards from chemicals.

UNCED called for improved coordination and enhanced cooperation among international chemical safety activities and for the establishment of an intergovernmental mechanism for chemical risk assessment and management. In response to that request, two international entities have been formed. These new entities, the Intergovernmental Forum on Chemical Safety (Forum) and the Inter-Organization Program for the Sound Management of Chemicals (IOMC) have different but interrelated roles and responsibilities to that of IPCS in the area of chemical safety. The three complement each other in the effort to develop cooperative and coordinated activities in chemical safety at the international level.

The Forum is a new mechanism for cooperation among governments for promotion of chemical risk assessment and management of chemicals. It is a noninstitutional arrangement whereby representatives of governments meet with intergovernment and nongovernmental organizations with the aim to integrate and consolidate national and international efforts to promote chemical safety. The purpose of the Forum is to provide policy guidance with emphasis on regional and subregional issues, develop strategies in a coordinated and integrated manner, foster understanding of the issues, and promote the required policy support needed to discharge these functions.

The first meeting of the Forum was in 1994; the next meeting will take place in 1997. At its first meeting the Forum established priorities for action including targets for key items.

There is a Intersessional Group (ISG) of the Forum composed of 31 governments which meets between sessions of the Forum. The first meeting of the ISG was held in March 1995 in Bruges, Belgium. The participants discussed a number of priority activities under each of the program areas of chapter 19 of Agenda 21, identifying key items and issues for action and input into its second meeting to be hosted by Australia in March 1996.

While the Forum is the mechanism for cooperation among governments, the IOMC is designed to serve as a mechanism for coordinating efforts of intergovernmental organizations. This program is designed to be a cooperative undertaking among six intergovernmental organizations that, within the framework of their own respective constitutional mandates, work together as partners to promote international work. The six participating organizations are the three cooperating organizations of IPCS (WHO, UNEP, and ILO), the Food and Agriculture Organization of the United Nations (FAO), the United Nations Industrial Development Organization (UNIDO), and the Organisation for Economic Development. Scientific and technical work of the IOMC will be carried out through the existing structures of the participating organizations, either individually or

Judy A. Stober provided technical assistance with this editorial.

jointly. IPCS is an example of joint work.

Specific coordinating mechanisms under the framework of the IOMC have been or are in the process of being established in relation to some program or subprogram areas, such as harmonization of classification of chemicals and information exchange on toxic chemicals and chemical risks. These mechanisms provide a regular means for all interested bodies working in the respective areas to consult with each other on program plans and activities and to discuss ways and means of ensuring that the activities are mutually supportive.

Each country has a stake in the success of chemical safety programs, national and international, as chemicals risks do not respect national boundaries. To respond to the challenge to the health of present and future generations and to the quality of the environment made by the increased use of chemicals and to more effectively use scarce resources, it is important that considerable efforts be made to work together at all levels to achieve globally sound management of chemicals. It is hoped that the recent events and developments will continue to lead to progress in the prevention and control of the harmful effects of chemicals to which humans and the environment are being increasingly exposed.

**Michel J. Mercier**
International Programme on Chemical Safety

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

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

Robert J. Kavlock, Ph.D.
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# Commentary

## Are Environmental Sentinels Signaling?

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There is an increasing perception that environmental contamination by chemicals no longer poses a significant health threat and that relaxation of environmental regulations is warranted. However, many wildlife populations are showing signs of developmental, behavioral, and reproductive dysfunction due to environmental contamination by endocrine-disrupting chemicals. Scientists, regulators, and legislators must mobilize to identify current health threats posed by environmental pollutants, develop testing protocols that will detect such properties of new chemicals, and strengthen legislation designed to protect environmental health. *Key words*: chronic toxicity, endocrine disrupters, environmental sentinels, pesticides, reproductive toxicity. *Environ Health Perspect* 103:888–890 (1995)

The year 1995 marked the 25th anniversary of both the U.S. Environmental Protection Agency and Earth Day. The inception of both institutions signified the need to temper anthropogenic stresses on the environment or face unsettling consequences. Decades of environmental abuse culminated in the 1960s when public perception of the repercussion of unabated environmental pollution was heightened by Rachel Carson's graphic depictions (*1*). The pressing environmental problems of 25 years ago were blatant. Among the most significant of problems were chemical and sewage discharges making aquatic resources unsuitable for human use and habitation by aquatic organisms, and the use of pesticides, which posed a significant threat to nontarget species. In response, the Clean Water Act was instituted in 1972 to regulate waste discharge and to ensure that high water-quality standards were maintained. The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) was amended three times during the 1970s to provide safeguards against pesticide-mediated harm to human and environmental health. Such legislation provided the foundation upon which a sound and reasonable national environmental policy was established. This policy has resulted in significant improvement in environmental quality concurrent with population and economic growth. The success of the environmental protection policies of the United States is best exemplified when environmental quality of the United States is compared to that of industrialized countries of the former Soviet block and other countries where such policies were never significantly instituted (*2–4*).

With the current movement toward the reduction of government size and spending, the issue is often raised as to whether environmental legislation and supporting research programs could be relaxed without intolerable consequences. Major fish and wildlife kills due to chemical waste discharge and improper pesticide usage are now largely relegated to distant memory. If one accepts the thesis that fish and wildlife species serve as sentinels for the protection of human health from environmental contaminants, then human health must also be adequately protected from the adverse health effects of pollution. Such logic supports the contention that environmental legislation and research need not be expanded and could perhaps be relaxed. However, not factored into this argument is that, while the flagrant environmental problems of 25 years ago have been addressed, more subtle, though no less beguiling, environmental threats may persist. Central to this issue is the question, are environmental sentinels currently signaling the existence of such environmental hazards?

Toxicant-mediated endocrine disruption is one example of a toxicological hazard currently presenting itself in the environment. Endocrine-disrupting effects of environmental pollutants were first recognized while investigating mechanisms responsible for reproductive failure among some bird species exposed to organochlorine pesticides (*5*). The observation that exposure to some chemicals can lead to reproductive failure led to the promulgation of regulations under FIFRA and subsequently expanded to nonpesticide chemicals under the Toxic Substances Control Act requiring that the effects of chemical exposure on the production of viable offspring be determined. Such tests, conducted in standard test species of birds, fish, mammals, and invertebrates, involve chronically exposing the parent organisms to various concentrations of a chemical, then assessing the number of viable offspring produced (*6*). With fish, only subchronic testing, involving the assessment of the effects of the chemical on survival and growth of larval fish, is initially required. Assessments of reproductive toxicity are mandated only if the no-observed-effect level established during the subchronic toxicity test is greater than 1/10 the expected environmental concentration of the chemical (*6*). Retrospective assessments have shown that such approaches will adequately protect the environment against most chemicals (*7*). However, unique toxicological properties of some chemicals can result in undetected toxicity using these protocols. Endocrine-disrupting chemicals can be among these undetected toxicants because they may 1) elicit effects on the developing fetus that are not manifested until the mature organism enters its reproductive stage, 2) elicit specific biochemical/physiological changes that affect an organism's reproductive capacity without affecting survival and growth as measured during subchronic testing, or 3) adversely affect endocrine processes characteristic of some species but absent in those surrogate species used in toxicity testing. Many pesticides, industrial chemicals, and wastes are among the toxicants that elicit such effects.

Shore birds such as gulls and terns typically produce broods of two or three eggs. Ornithologists began observing in the 1970s that broods of five or six eggs were not uncommon (*8*). This abnormal clutch size was found to be due to multiple females sharing a nest (*9*). This female–female pairing appeared to be due to a deficiency in reproductively competent males (*10*). Laboratory investigations demonstrated that exposure to DDT feminized male gulls during embryonic development (*11*). Further, incidence of female–female pairing was higher in environments with significant DDT contamination (*10*). Thus, abnormal breeding behavior in these birds appeared to be due to reproductive deficiency in males caused by embryonic exposure to environmental pollutants. This observation is not only of historical relevance, as female–female pairing of terns has been noted recently in areas contaminated with polychlorinated biphenyls (PCBs) (*12*).

Female Poeciliidae fish inhabiting areas receiving pulp mill effluent have been observed to undergo masculinization. Most obvious is the modification of the anal fin in

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affected females to a gonopodiumlike structure (used by males for sperm transmission) (*13*). Exposure to exogenous androgens has been shown to cause similar masculinization (*14*), and androgens generated by the action of bacteria on phytosterols present in the effluent are presumed to be responsible for this effect. Fish exposed to paper and pulp mill effluent can also experience altered steroid hormone titers (*15*), impaired gonad development (*16*), and reduced fecundity (*17*). Such effects, specific to reproduction, would not be detected in subchronic toxicity tests.

Propiconazole is a member of the imidazole-derivative class of fungicides. A common characteristic of these chemicals is their ability to inhibit enzymes responsible for steroid hormone biosynthesis and induce enzymes involved in steroid hormone metabolism (*18,19*). A consequence of this effect is severe reductions in some steroid hormone levels (*18*). This specific and potent effect has led to the consideration of some imidazole-derivatives for use as a male contraceptive (*20*). Propiconazole, which is used as an agricultural fungicide, shares these properties and thus has the potential to compromise reproductive success of chronically exposed organisms. These effects would not be detected in a subchronic toxicity test that did not evaluate reproduction. The Ecuadorian shrimp industry has called for a moratorium on the use of propiconazole for fear that it is responsible for the demise of shrimp populations (*21*).

Tributyltin has been used extensively for more than 20 years as an antifoulant in marine paints. Tributyltin has been identified as the causative agent responsible for imposex in many marine mollusk populations. Imposex is the imposition of sex characteristics of one gender onto another (a form of pseudohermaphrodism). In the case of tributyltin-exposed mollusks, females develop a penis, vas deferens, and in severe cases, seminiferous tubules (*22*). Affected females can be rendered infertile because the vas deferens blocks the release of eggs from the oviduct. The mechanism responsible for this effect has not been conclusively established, but it seems to involve the neuroendocrine regulation of sexual differentiation (*23*). Tributyltin can cause imposex at low part per trillion concentrations and has caused the extinction of some affected populations (*22*). Certain mollusk species may be particularly sensitive to the effect of tributyltin owing to unique aspects of sexual differentiation in these organisms (*24*). Intersexuality also has been observed in some crustacean populations in the vicinity of sewage discharge, though causality has not been established (*25*). Peri- and neonatal exposure of rodent models to a variety of environmental chemicals including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (*26*), PCBs (*27*), mirex (*28*), chlordecone (kepone) (*29–31*), dieldrin (*28*), aldrin (*28*), chlordane (*32*), and atrazine (*33*) have shown that these chemicals are capable of eliciting a variety of perturbations in the sexual differentiation of mammals.

Thus, it would appear that environmental sentinels are indeed signaling us that all is not well. Although the major environmental problems of the 1960s may have been successfully dealt with, we are faced in the 1990s with new problems to surmount. Speculation remains as to whether human heath issues such as increased incidence of breast cancer, prostate cancer, testicular cancer, endometriosis, birth defects in the male reproductive tract, and reductions in sperm count may be associated with the existence of endocrine-disrupting chemicals in the environment (*12*). Toxicity testing requirements for environmental chemicals must be expanded to consider effects that may go undetected using current guidelines. Existing toxicity testing requirements should be complemented with *in vitro* diagnostic tests designed to detect specific biological properties such as hormone agonistic or antagonistic activities. As discussed by McLachlan (*34*), the establishment of cell lines that have been transfected with specific receptor-reporter gene constructs would greatly facilitate the screening of chemicals for such properties. In addition, biomarkers must be identified that can be used as part of standard toxicity tests to identify chemicals that may pose risk of endocrine-disrupting effects. For example, a significant correlation has been shown between the percentage of males present in a litter of mice and the average anogenital distance in females in that litter (*35*). The intrauterine position of a female rodent with respect to the number of adjacent male siblings affects reproductive physiology and behavior (*36*) as well as anogenital distance (*37*). These observations suggest that the intrauterine hormonal environment affects the developmental and reproductive capacity of the offspring. Analyses of anogenital distance during conventional toxicity tests may thus serve as a biomarker of reproductive effects and as an indicator of the need for multigenerational toxicity tests. Production of estrogen-regulated proteins such as vitellogenin (*38*) and lactoferrin (*39*) in chemically exposed males would signal estrogenicity of the chemical, which would warrant more definitive testing protocols to explicitly characterize toxicity.

Changes in steroid hormone levels can also be indicative of endocrine-disrupting chemical exposure. Several studies have suggested that toxicant-induced alterations in steroid hormone levels or metabolism may contribute to reproductive impairment (*40–42*). Our laboratory has been conducting comparative studies of the effects of chemicals on reproductive capacity and steroid metabolism using the freshwater crustacean *Daphnia magna* in an attempt to validate this putative relationship. Experiments thus far indicate that concentrations of the toxicants that impair reproduction also perturb steroid metabolism (*43*). Furthermore, this effect on steroid metabolism can be detected after short-term exposure to the toxicant (*44*). These results suggest that for some reproductive toxicants, effects on steroid metabolism may be predictive of reproductive toxicity, and thus metabolic effects can serve as a biomarker of reproductive toxicity.

Clearly, many strategies exist that could improve our ability to detect endocrine-disrupting chemicals and identify exposure dosages at which effects are elicited. Further research is needed to better define such experimental approaches and validate their utility. Ultimately, testing requirements will need to be expanded to ensure the detection of endocrine-disrupting effects of environmental chemicals; environmental legislation must be strengthened to ensure protection against these and other chemicals that elicit subtle, yet devastating, effects. Legislators must be made aware that the absence of dead fish and wildlife is not justification for the relaxation of environmental legislation and supporting research. The deleterious consequences of chemicals in the environment continue. You just have to look a little harder to see them.

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**There is nothing in which the birds differ more from man than the way in which they can build yet leave a landscape as it was before.**

Robert Straughton Lynd, American sociologist (1892-1970)

# Forum

## New Mouse Is a Knockout

Scientists have a new tool to help them unravel the mysteries of the toxicity of dioxin. The development of the aryl hydrocarbon receptor-deficient mouse was reported by Frank Gonzalez and colleagues of the National Cancer Institute this May in *Science*.

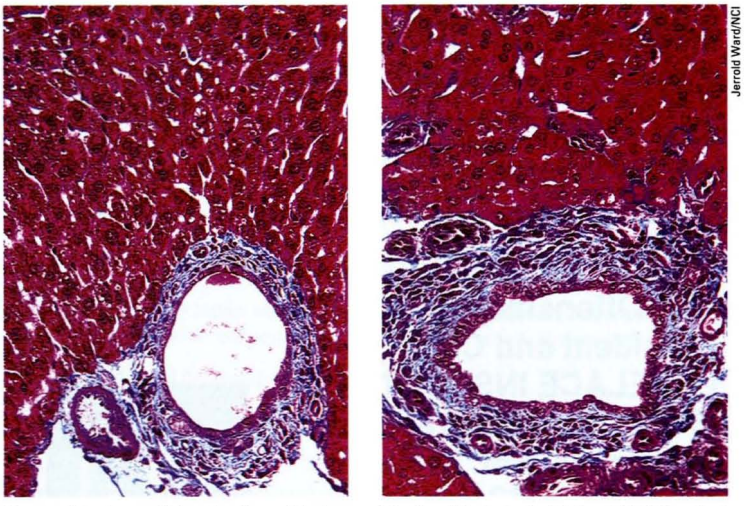
The controversy about the health effects of dioxin partly involves questions about how its toxicity is mediated. Most scientists agree that dioxin exerts its effects by binding to the aryl hydrocarbon receptor (AhR). What is not clear is how this binding relates to the particular cell types that are affected.

The AhR-deficient mouse will help answer questions about the mechanism of dioxin and similar compounds such as benzo[*a*]pyrene, PCBs, and PBBs. Gonzalez and his team produced the mouse by "knocking out" the gene that encodes the AhR. It is known that the AhR detoxifies poisons, but the NCI researchers found evidence that it has other important functions as well.

Half of the AhR-deficient mice die within a week after birth due to a lack of lymphocytes that leaves them susceptible to opportunistic infections. The mice that do survive have massive liver scars and only slowly build up the normal number of lymphocytes. At 10 weeks of age, the animals begin to lose the lymphocytes they built up and eventually become sick due to an incompetent immune system and liver problems. The livers of these mice are 50% smaller than normal, and they have bile duct fibrosis.

The AhR is obviously vital to immune function and liver health. But the depression of the immune system of AhR-deficient mice is a puzzle because the thymus, where T-lymphocytes mature, is normal in these animals. Gonzalez and co-workers hypothesize that AhR-deficient mice may lack a specific lymphoid population or have a systemic defect in the ability of lymphocytes to reside in the peripheral immune system (which includes the reticuloendothelial system, of which the liver is a component). Loss of the AhR may affect thymic processes or affect the migration of cells from the bone marrow (where precursor lymphocytes originate) to the thymus or to peripheral lymphoid organs. Alternatively, the normal life span of peripheral lymphocytes may be shortened in these animals.

Previous research has shown that the AhR may also play a role in brain development. Levels of AhR are high in the fetal neural tube, which gives rise to the central nervous system. Levels decrease after birth. In addition, AhR is found in the kidneys, lungs, and hearts of adults. It is hoped that the AhR-deficient mouse will help clarify the functions of the receptor. However, Gonzalez cautions that experiments with these mice may be difficult because of their poor health. Further genetic engineering may have to be done to turn on the AhR gene only in liver tissue so that the mice will be hardy enough to withstand testing.



Jerrold Ward/NCI

**Liver trouble.** Accumulation of collagen (blue) around the liver bile duct of a 30-day-old AhR knockout mouse (right) shows the beginning of fibrosis. (Left) Liver bile duct of a normal mouse.

## More Muddy Water

In the wake of a report that healthy people can be infected with infinitesimal exposures to *Cryptosporidium* comes the Natural Resources Defense Council's assertion that at least 45 million Americans are at risk of imbibing the diarrhea-causing protozoan in what appears to be clean drinking water.

At the University of Texas in Houston, infectious disease expert Herbert L. DuPont gave 112 healthy volunteers preparations containing between 30 and 1 million *Cryptosporidium* oocysts (the form in which the microbes are found in water). Monitoring enteric symptoms and analyzing stool samples for excreted oocysts, DuPont found that, for the strain he used, the median infective dose was only 132 oocysts. The study suggested the size of the dose did not affect the microbes' incubation period or the severity of the infection.

Just how many people may become infected from their drinking water is impossible to estimate. The NRDC figure of 45 million is based on a survey mailed to 100 of the nation's 61,000 water suppliers, says the organization's president, Eric Olson. The systems who responded serve only a fraction of Americans.

Cryptosporidia give rise to dormant oocysts that remain viable for months in sewage, runoff from feedlots, or groundwater until they find a new host. Unlike other waterborne organisms, the oocysts are neither killed by chlorine nor screened by standard filters, says DuPont. When one member of a household is infected, secondary spread can occur.

Once thought to infect only animals, especially young cattle, *Cryptosporidium* came to the attention of health authorities

in the 1980s, when it was found to cause life-threatening, chronic diarrhea in AIDS patients. In immunocompetent hosts the microbe typically causes a day or two of discomfort, with symptoms including nausea, vomiting, diarrhea, and cramps. Most sufferers need only oral rehydration until they recover.

"Crypto is a nasty nuisance, but not a large-scale public health menace," says DuPont, now at Baylor College of Medicine. Serologic evidence of past infection is found in 15% or more of Americans and nearly 100% of people in tropical areas with poor sanitation. "Here [in the United States] it probably causes many outbreaks of diarrhea in children at day care centers," Dupont said.

But to anyone with impaired immunity, the normally self-limiting illness can be fatal. At risk are patients taking immunosuppressive drugs (to treat cancer or prevent organ transplant rejection), anyone taking steroids, and older people.

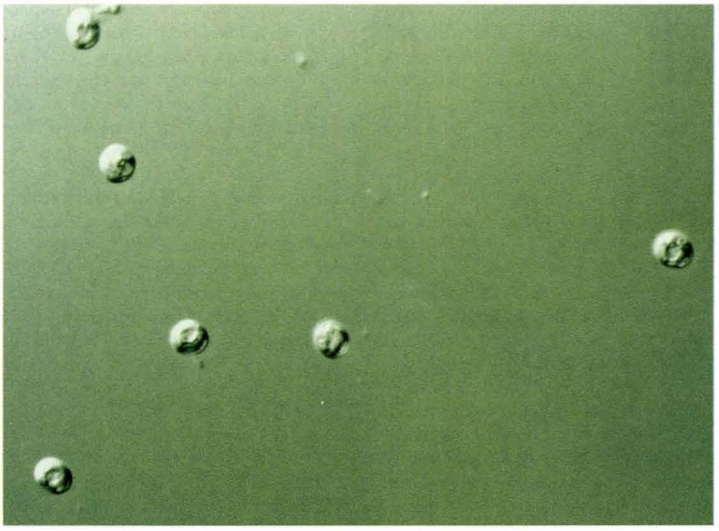
Recent guidelines from the CDC and the EPA say waterborne *Cryptosporidium* can be eliminated by boiling water for one minute or by using a home filtering device to screen particles less than one micron in diameter. Suitable are reverse-osmosis devices, those certified for "cyst removal" by the National Sanitary Foundation, and those labeled as "absolute" for one micrometer.

Point-of-use treatment doesn't satisfy NRDC, however. "Water suppliers shouldn't throw this problem in the lap of those at risk—the sick, the poor, and the elderly. Is it right to ask society's weakest members to boil their drinking water or buy a purifier?" Olson asks.

And expensive bottled water can't be assumed to be oocyte-free unless it's been distilled, properly filtered, or came from a protected spring or other pristine source, something consumers are relatively powerless to determine.

The cost of *Cryptosporidium*-free tap water will vary according to the size of the system, says environmental engineer Stig Regli of the EPA. For an existing major municipal system to add an additional filtering step, or more effective but costlier ozone disinfection would add $10 to $15 per year to a residential water bill, while patrons of small public or private systems might have to pay $100 or more per year, says Regli.

The CDC/EPA guidelines urge individuals who may be worried about *Cryptosporidium* to ask their health care provider about appropriate risk-reducing measures. Current data don't justify telling immunocompromised people to boil or avoid drinking tap water unless there's an outbreak, guidelines say, which warn that narrowly focusing on *Cryptosporidium* (or other single health risk) could draw attention from other potential opportunistic infections.



Frank W. Schaefer/EPA

**Drinkable danger.** New research shows that relatively small numbers of *Cryptosporidium* oocysts are enough to cause potentially severe infections.

Even the most motivated individual will find absolute safety from *Cryptosporidium* hard to attain. The CDC/EPA guidelines suggest, "Individuals who contact bottlers or filter manufacturers for information should request data supporting claims that a brand of bottled water or filter can meet the above criteria." No agency lists the brands of either safe bottled water or effective home water filter systems, though a list of filters meeting CDC/EPA criteria is available from National Science Foundation (1-800-NSF-8010).

This fall, water systems can enter a voluntary quality control program sponsored by the EPA and the American Waterworks Association, which will certify that they are doing everything feasible to keep the water safe. By participating, systems may shield themselves somewhat from liability if there's a *Cryptosporidium* outbreak similar to the one in 1993 in Milwaukee which incapacitated thousands and led to the deaths of several immunocompromised individuals. "Everybody wants to avoid another Milwaukee," says Regli.

But until and unless water systems eliminate *Cryptosporidium*, DuPont urges anyone with compromised immune function, including anyone over the age of 80, to boil their drinking water, invest in a certified filter, or seek out a reliable brand of bottled water.

## Reviving Hemp

Many people know hemp (*Cannabis sativa*), which contains the psychoactive drug tetrahydrocannabinol, as the marijuana plant. But for centuries the Asian herb has been used to make rope or cord, especially large-diameter ropes for ships. Now attention is turning to the use of hemp to make paper.

"It's one of the best fiber sources [for paper] around," says John Ralph, professor of forestry at the University of Wisconsin-Madison. Hemp has long fibers which increase the strength of paper made from it.

Because cultivating marijuana plants is illegal, hemp cannot be grown in the United States. Hemp is widely grown in Hungary and China, and several firms import hemp paper into the United States. Etienne Fontan, a sales manager of the Virginia-based firm Ecolution, touts hemp's environmental advantages. Fontan says that producing hemp paper is more environmentally benign than producing paper from wood. Hemp doesn't require the chlorine bleach and acids used to make paper from wood pulp.

In April of this year, Tree Free EcoPaper of Portland, Oregon, made what it said is the first commercial U.S.-produced hemp-containing paper in 40 years. The paper, made at the company's Massachusetts paper mill, is composed of 10% hemp grown in Europe, plus other nonwoody and recycled materials. According to firm President Paul Stanford, the paper is a high-quality bond paper. Stanford said EcoPaper also plans to use imported hemp to make a lower-grade paper for copiers.

Hemp can be combined with other materials to add strength. Roger Rowell, a researcher at the U.S. Forest Products Laboratory in Madison, Wisconsin, said hemp can be added to plastic products, such as fan blades, to help stiffen them. Hemp fibers are also used in clothing.

The idea of using hemp is being taken seriously at governmental levels as well. Erwin Scholts, in charge of developing and diversifying agriculture at the Wisconsin Agriculture Department, called a conference last spring to explore the commercial possibilities of hemp. And the governor of Kentucky set up a task force with a similar goal.

But the law banning the growth of hemp is a major barrier to commercialization. "It's killing it," says Rowell about the law's impact on any potential hemp industry. Rowell gave up plans to grow the plant for research purposes when he learned about the strict security and record-keeping measures he would have to take.

Scholts said that it may be possible to develop hemp commercially by breeding in genetic markers, such as color, that would identify the crop. And scientists say that hemp lacking the psychoactive ingredient tetrahydrocannabinol can easily be bred.

The governor's task force in Kentucky, which studied hemp's commercial possibilities over six months, concluded in June that the crop had no value for the state's farmers. According to Scott Smith, a University of Kentucky agronomy professor who worked with that task force, hemp's gross return would be $200 an acre. "That's not enough to interest many farmers," he said. But Gale Glenn, a farmer on the task force, disagreed, saying the question of profit hasn't been completely answered.

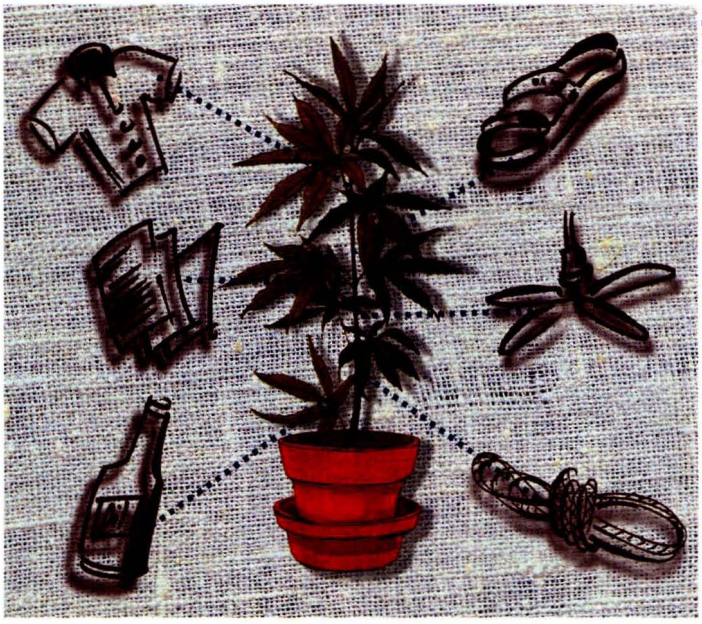
Hemp isn't the only plant that can be used to make paper and fiber-containing products. Jute, kenaf, and flax are also used, as is bagasse, the waste from sugar cane.

And the cost of hemp may simply be too high, compared to those plants and to wood, to make it very attractive in the United States, according to Rowell. For example, kenaf, which is used in specialty papers, sells for $.15 a pound, he said, and wood fiber sells at $.03–.05 a pound. Rowell estimates that hemp would sell for about $.50 and $.75 a pound.

William Lopatin, a project manager at the Ohio Hempery, which sells hemp-containing paper, acknowledged that hemp is expensive and that the price of other grasses would have to "go up dramatically" for hemp to be economical. Others say that hemp prices would decrease as markets for it increase.

Hemp advocates boast of the plant's environmental virtues, arguing that using hemp saves trees, that its pesticide demands are minimal, and that it helps hold soil, thus preventing erosion. However, should hemp be intensively cultivated as a cash crop, cautioned Smith, it would require nitrogen fertilizer, much the same as other cash crops. Another potential drawback is that hemp plants contain silica, which can damage paper-making machines.

Even though hemp may not be a problem-free crop, it's attractive enough for Scholts to explore. He is planning a second forum on hemp. "If this can be a valuable crop for American agriculture, we have to keep moving forward and investigating it," he says.



Joseph Tart

**Hemportunities?** Researchers, manufacturers, and government officials are all looking at possible uses for hemp including paper, clothing, and other products.

## Mother's Milk

A study released in the April 1995 *American Journal of Public Health*, authored by NIEHS statistician Beth Gladen and epidemiologist Walter Rogan, shows evidence for a correlation between DDE levels in milk and shortened lactation in 229 Mexican women. DDE is the most stable derivative of the pesticide DDT, which is banned in most of the world, including the United States and Mexico. Since DDT is persistent in the environment, women, and consequently breast-fed babies, may still be subject to its effects.

Infants face serious health risks if their mothers suffer from shortened or failed lactation. Decreased lactation has been associated with increased infant mortality, especially in developing countries, but effects are also detectable in the developed world, including the United States. In developing areas where water may be contaminated, feeding babies with powdered milk instead of breastfeeding may leave them vulnerable to diarrheal diseases and other waterborne pathogens, leading to infant mortality. Even where the water is clean and does not present a hazard, many researchers believe that bottle-fed babies may be missing out on important health benefits of breastfeeding, including stronger immune function.

In a 1987 study, the North Carolina Breast Milk and Formula Project, researchers found that while DDE in milk did not show any direct effect on infants' health, those children whose mothers' milk carried high levels of DDE were breast fed for markedly shorter times than those with lower levels. To replicate the study, the authors chose an agricultural region of Mexico where DDE levels were likely to be high due to previous pesticide use—the town of Tlahualilo, in the northern state of Durango. The researchers used local medical personnel to administer questionnaires about the demographic and socioeconomic status of the mother, her medical and reproductive history, the pregnancy, the delivery, attitudes toward breast feeding, and the baby's feeding pattern since birth.

The study followed 229 women every 2 months from childbirth until weaning or until the infant was 18 months old. Samples of breast milk were collected just after birth and when the infants were 6 months old.

The fat content of the milk samples was analyzed for *p,p′*-DDE, the most common isomer of DDE. The correlation between *p,p′*-DDE and shortened lactation was confirmed, even when factors such as previous lactation and other reasons for weaning were considered. Among reasons for weaning were illness of the mother, use of oral or injected contraceptives, and the mother's perception that the child was old enough to be weaned. The most common reason for weaning was insufficient milk.

Since infant illnesses showed no correlation with DDE levels, the researchers reasoned that DDE was somehow tampering with the process of lactation itself. According to the report, "The most plausible explanation of a relationship between DDE and duration of lactation is estrogenicity." While the *p,p′*-DDE isomer which the researchers measured is nonestrogenic and shows no effect on lactation in rats, it is assumed to occur in proportion to another isomer, *o,p*-DDE, a weak but persistent estrogen in several animal models. DDE and other pesticide residues may mimic the activity of natural estrogens, which occur at high levels during pregnancy but fall just after birth to allow lactation. Medically administered estrogens can also decrease or halt lactation.

Gladen emphasizes that although this epidemiological study established a correlation between DDE and shortened lactation, the mechanism for DDE's action is still unknown. Gladen would like to see a good animal model for lactation developed that could be used to test chemicals including DDE. Says Gladen, "We need to understand the underlying mechanism more before we can block DDE's action."

## EHPnet

Scientists around the world are piecing together the puzzle of what causes cancer and how it may be treated. OncoLink (URL: http://cancer.med.upenn.edu/), an award-winning World Wide Web site created at the University of Pennsylvania, offers researchers, clinicians, and patients the latest clues to the puzzle. The goal of Oncolink, the first comprehensive multimedia cancer information resource ever placed on the Internet, is to communicate cancer information worldwide.

OncoLink's homepage contains hyperlinks to information on the latest cancer news, cancer-related meetings, peer-reviewed journal articles on cancer, site statistics, and a search function. OncoLink also has a menu hyperlinked to a variety of submenus. Items on the main Oncolink menu include disease-oriented menus, psychosocial support and personal experiences, cancer causes, screening, and prevention; clinical trials, global resources for cancer information, book reviews, and more.The disease-oriented menu features general cancer groupings for hyperlinks to more specific information on particular types of cancer. For example, under the heading "Adult Cancers," there is a heading for leukemia. Under the leukemia heading there are specific hyperlinks for acute lymphocytic leukemia and acute myeloid leukemia. These links then lead to patient information, transplant information, treatment information, diagnosis, and current research information. Users may also search a particular topic or field of interest.


The bulk of research information is found under the cancer causes, screening, and prevention menu and the global resources menu. From the cancer causes menu, users may link to an environmental factors and causes submenu. This submenu offers hyperlinks to information on topics such as lung cancer and radon, DDT and breast cancer, electromagnetic fields and cancer, and risk assessment. The global resources menu offers links including hospitals, universities, institutes, associations, and government sites related to cancer including the National Cancer Institute's international Cancer Information Center and the EPA.

Since its inception, OncoLink has been accessed over three and a half million times from around the world. OncoLink was awarded the International "Best of the Web '94" Award and was a 1995 finalist in the National Information Infrastructure Awards.

## Mining the Radon Studies

An extensive analysis of 11 studies of radon-exposed miners shows that radon exposure in U.S. homes may account for as many as 14,400 lung cancer deaths a year, about 10% of American lung cancer victims. Residential exposure to radon, an odorless, invisible gas emitted by the decay of uranium in the earth's crust which can accumulate in enclosed areas, has been identified as the second leading cause of lung cancer in the United States. Because radon occurs naturally, exposure cannot be totally eliminated. However, some 2,000–4,000 U.S. lung cancer deaths per year may be prevented if all homes with radon levels exceeding the EPA's action level were repaired, the analysis estimates. A summary of the analysis was published in the June 7 issue of the *Journal of the National Cancer Institute.*

The joint analysis, which pooled original data on some 65,000 miners in China, Canada, Europe, and the United States, confirms the findings of an earlier National Academy of Sciences BEIR (Biological Effects of Ionizing Radon) IV report based on 360 lung cancer deaths among 2,700 miners. The BEIR IV report helped form the basis for the EPA's recommendations that every U.S. home be tested for radon and that homes with levels above 4 picocuries per liter (pCi/l) be repaired. The EPA estimates that some 6 million homes will have radon levels at or above the 4 pCi/l action level.

"This study confirms that radon is a serious public health problem, as EPA, the Surgeon General, [the Department of] Health and Human Services, and many others have been saying," says David Rowson, director of the EPA's radon division. "It adds robustness to the data we have supporting our recommendations.

We now have data on nearly 70,000 humans who have been exposed to radon."

The joint analysis, based on more than eight times the data of the earlier study, represents the most comprehensive look at radon exposure risk to date. The study's authors used a measurement called a "working-level month," or WLM, to compare exposures to underground radon of miners to residential exposures. A working level as defined by the authors is any combination of radon progeny in 1 liter of air that results in the emission of 130,000 MeV (megaelectron volts) of energy from alpha particles. The study found that in an average home, yearly exposure results in approximately 0.2 WLM, or an approximate lifetime cumulative exposure of 10–20 WLM. People who reside their entire lives in a home at the EPA action level of 4 pCi/l accumulate 40–80 WLM. The study also found that from 5–10% of American homes have radon levels that would produce a lifetime exposure equal to 40–50 WLMs.

The study concludes that in miners, about 40% of all lung cancer deaths may be due to radon exposure, accounting for 70% of lung cancer deaths among never-smokers and 39% of lung cancer deaths among smokers. Using a 1993 figure of 149,000 U.S. deaths from lung cancer, the authors estimate that 4,700 never-smokers and 9,700 smokers each year may die of lung cancer attributable to residential radon exposure.

Among the findings of the analysis were that the relative risk relationship for cumulative radon exposure was consistently linear in miners, suggesting that exposures at lower levels, such as in homes, would carry some risk, that the excess relative risk for never-smokers was threefold the trend for smokers, and that for equal total exposure, exposures of long duration and low levels were more harmful than exposures of short duration and high levels.

The study also evaluated in greater detail the effects of other factors on risk, and found, for example, that the relative risk from exposure diminished over time. "Even 25 years after exposure, the risk continued to decline," says NCI health statistician Jay Lubin, the lead author of the report. The analysis also suggests that children are not at any greater relative risk merely because they were exposed as children. And among miners, Lubin notes, "the suggestion is that the person who [is exposed] at low rates for long periods of time may be at a slightly higher risk, although what that means in terms of residential exposure is still unclear."

NCI

**Jay Lubin**

Estimates of miners' risks are consistent with the seven epidemiological case–control studies that seek to directly measure exposure in homes, says Lubin. But Lubin notes two difficulties with residential studies. Extrapolating from mines to homes, using a lifetime exposure at 4 pCi/l, gives a relative risk of 1.2–1.4, compared to a relative risk of 15–20 for smoking. "The risk is very small, which makes it difficult to detect," Lubin said. Second, measurements in one or two rooms of a home do not precisely characterize lifetime exposure, since people move and exposure outside the home is unknown. "The effect of the imprecision is to totally muddy the water. It becomes much more difficult to establish risk in that kind of setting," says Lubin.

In the face of ambiguous results of case–control studies and confounding factors, the authors urge caution in interpreting the results of the analysis, "because concomitant exposures of miners to agents such as arsenic or diesel exhaust may modify the radon effect and, when considered together with other differences between homes and mines, might reduce the generalizability of findings in miners." Still, the authors insist, the findings do suggest that for certain homes with high levels of radon, "there is no question that remedial action should be taken; as a result, lung cancer risk would be lowered."

*Editor's Note:* An article by Warner et al. in an upcoming issue of *EHP* discusses the effect of residential mobility on radon exposure (vol. 103, no. 12).

Joseph Tart

**Radon exposure in U.S. homes may account for as many as 14,400 lung cancer deaths per year.**

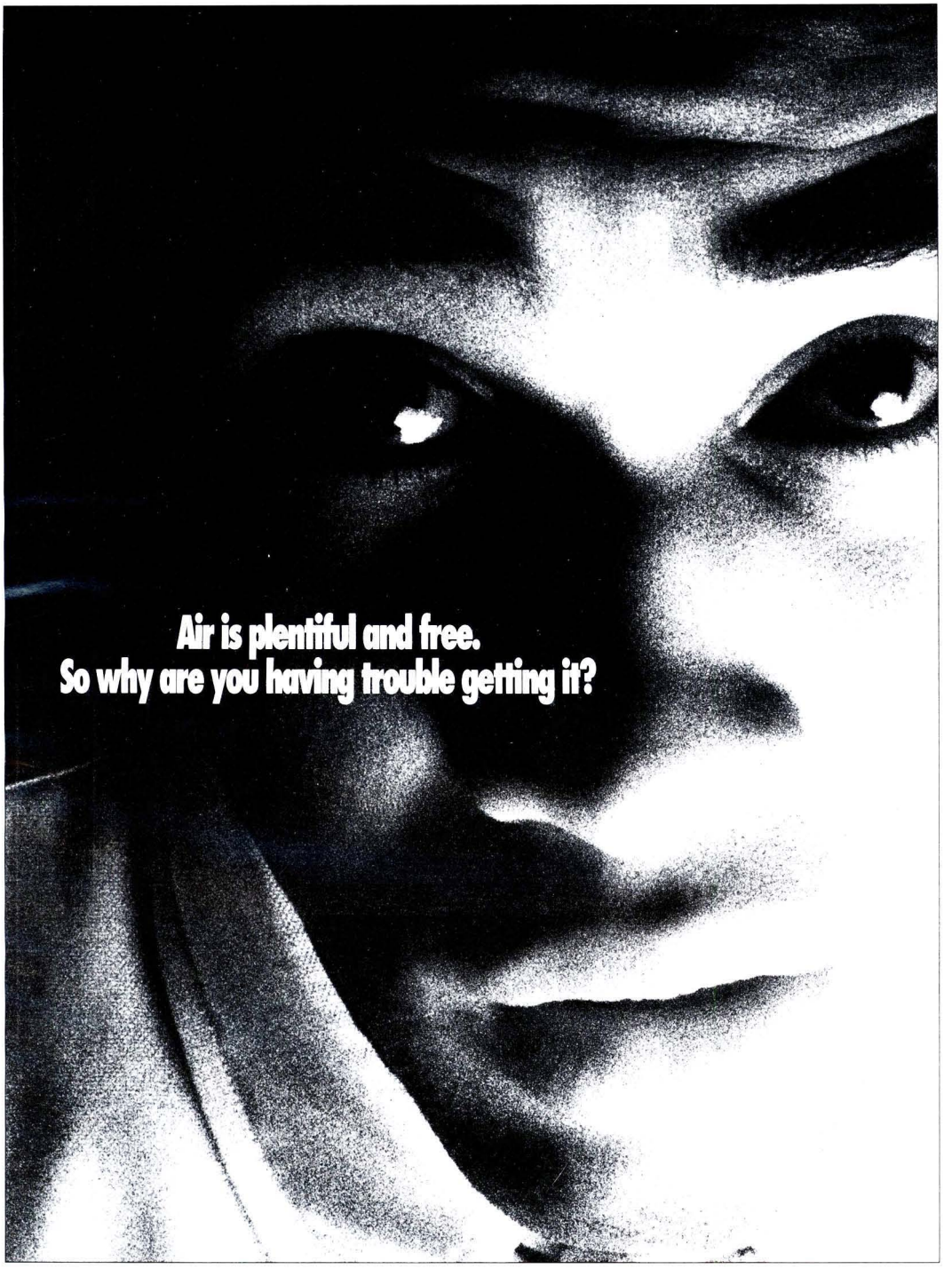
**Residential exposure to radon is the second leading cause of lung cancer in the United States, accounting for some 10% of lung cancers.**

**The EPA estimates that 6 million homes have radon levels above the 4 pCi/l action level.**

**Repairing homes with radon levels above the 4 pCi/l action level would prevent as many as 2,000–4,000 U.S. lung cancer deaths per year.**

## What's News to You?

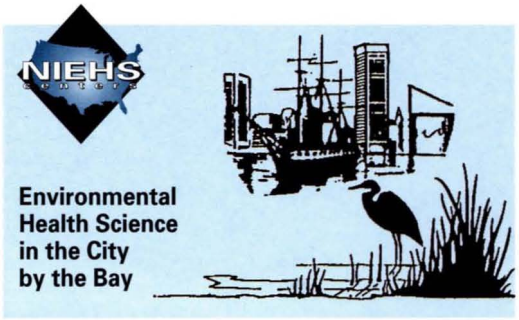
*Environmental Health Perspectives* welcomes topic suggestions for upcoming "Environews" articles. Send suggestions to: News Editor, *Environmental Health Perspectives*, National Institutes of Environmental Health Sciences, PO Box 12233, 111 Alexander Drive, Research Triangle Park, NC 27709 USA. Fax: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




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

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

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




On the Chesapeake Bay off the bustling shores of Baltimore, Maryland, "skip jacks," or "skippers" still fish the waters for the area's famous oysters and crabs, toiling six days a week, from sunup till sundown, much as their ancestors did centuries ago. Yet today, these fishermen are telling researchers at the Johns Hopkins University's Environmental Health Sciences Center (EHSC) a different fish tale from the one their colonial forefathers might have told. These fishermen have strikingly high rates of skin cancer. In an ongoing study of about 800 fishermen, center researchers determined that skippers have a 10-fold greater risk than the general population of developing skin cancer. Among Baltimore-based fishermen over the age of 70, the rate of nonmelanoma skin cancers is 40%, reports Paul Strickland, director of occupational health in the school's Department of Environmental Health Sciences. This compares to a 4% prevalence rate in the general population. Scientists believe the elevated numbers are mainly due to the dramatic increase in life expectancy over the past century: the fishermen are living longer, revealing the effects of a lifetime of prolonged sun exposure.

The project is just one of many center-sponsored studies that looks at various environmental and occupational exposures in populations in Baltimore. Work at the center focuses on exposures to agents—ranging from radon to chromium to semiconductor toxicants to dust mite antigens—in populations from firemen to female telephone workers.

In addition to its diverse industrial populations, Baltimore is a prime test area for everyday urban exposures. For example, Baltimore ranks sixth nationwide for ozone problems in cities. The Johns Hopkins center has one of only a handful of academic inhalation facilities in the nation. Lead exposure, in both inner city children and adults, is another serious problem and is among the new research directions of the Johns Hopkins center. In addition, Delaware, Maryland, and the District of Columbia have some of the highest overall cancer rates in the nation.

The Johns Hopkins center has made a name for itself for research in biomarkers and molecular epidemiology, mechanistic toxicology, and pulmonary pathophysiology. Center researchers seek to identify environmental and occupational risk factors, early biological indicators of disease, and biological and chemical markers of exposure, in addition to understanding the mechanisms of environmentally related disease, improving models of the effects of pollutants, and developing interventions for reducing exposure risks.

The center's leaders are also putting an increased emphasis on bringing the results of the center's research to the local community. "We're trying to move it out faster than we did before. Previously, we left it to the rest of the world to use research. Now outreach is a criteria for performance of our center," explains Director Morton Corn. Although the outreach program is still getting off the ground, Johns Hopkins researchers are already working on a project with Maryland Public Television to develop educational videos for elementary schoolchildren on the environment, health, and how to modify their lifestyles.

## Philosophy and Focus

What really makes the center unique is its location in the Johns Hopkins University School of Hygiene and Public Health, the oldest and the largest public health school in the world, says Corn, who became the center's director in 1991. Corn is carrying on the legacy of the center's first director, Gareth Green, who helped bring an EHS center to Johns Hopkins in the fall of 1985. The choice seemed to be a natural, as Johns Hopkins maintains the largest department of environmental health sciences in the country and the largest environmental health science training program as well. The Johns Hopkins center embraces a philosophical focus toward prevention and protection that is characteristic of the way researchers think at the school of public health, says Michael A. Trush, who became deputy director in 1991. At the same time, the center is critical to environmental health science at Johns Hopkins. "The idea of the center is to make the whole more effective than the sum of its parts," says Corn.

For example, the center interfaces with the NIOSH Educational Resource Center in Occupational Safety and Health, the nearby Kennedy-Krieger Institute, the Johns Hopkins Center for Alternatives to Animal Testing, and the Johns Hopkins Oncology Center. The School of Hygiene and Public Health is composed of 10 departments, which also adds to the opportunities for unusual collaborations and linkages. There is a close interrelation between university research and the NIEHS training program. The NIEHS training program is currently training 20 predoctoral and 4 postdoctoral students at the center through an NIEHS training grant.

JHU-EHSC

**Molecular markers in mold.** Center researchers are studying moldy corn and peanuts which contain aflatoxin, a major risk factor for liver cancer in China.

## Structure

The center has come a long way since Green founded it. In fact, the organization is completely different from when the center was started a decade ago, says James Yager, who coordinates the training program. And it continues to evolve. The structure has mainly been fine-tuned "to get greater synergy out of it," says Trush. "The center structure and its core facilities are very, very important to facilitating research and interactions."

The current structure of the center has evolved even during the past five years to reflect the shifting emphasis of the center and to better meet investigator needs and improve the quality and efficiency of the research. About 67% of the center's $800,000 annual budget goes into facilities to help attract scientists to the center. Center researchers bring in about $16 million in grants per year.

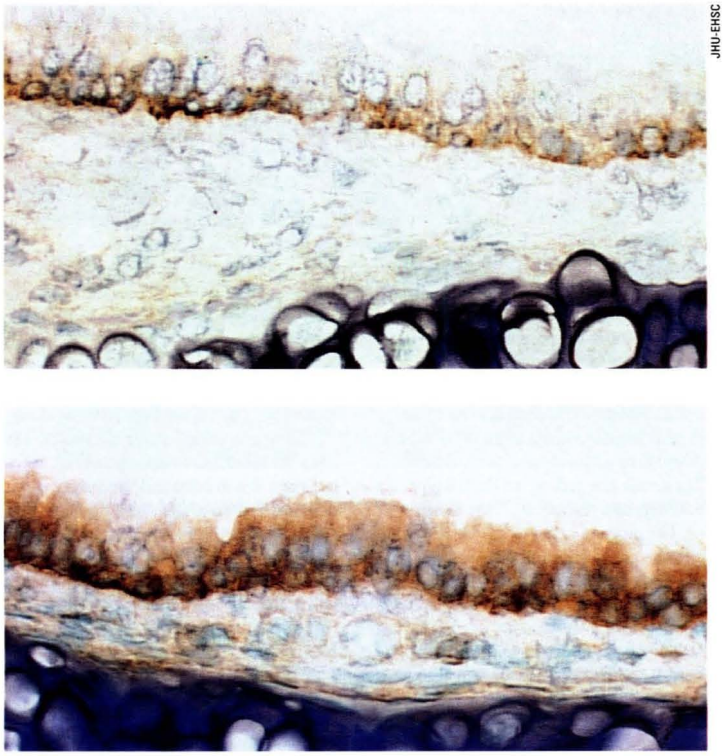
Pilot projects are another new venture to attract researchers. The Johns Hopkins center has $75,000 each year up for grabs, with maximum awards of $12,000 each. In the past 4 years, the center has awarded 28 pilot grants. Ten of these projects have been funded at the national level.

There are four interlocking research programs: human exposure assessment and molecular epidemiology, mechanisms of toxicity and carcinogenesis, physiologic responses to inhaled pollutants, and neurotoxicology. In 1991 and through part of 1994, the center had six program areas. The human exposure assessment and molecular epidemiology program combines two programs, the former epidemiology and exposure assessment and the molecular dosimetry and biological monitoring programs. The merger was made to better integrate the research efforts in epidemiology, exposure assessment, and biomarkers. In turn, the former environmental carcinogenesis program was renamed the mechanisms of toxicity and carcinogenesis program to reflect its changing research interests. And while the name remains unchanged, the research focus of the neurotoxicology program has become more mechanistic.

## Molecular Epidemiology

The human exposure assessment and molecular epidemiology program, directed by John Groopman, brings together toxicologists, epidemiologists, and environmental engineers to assess individual risk from exposure to environmental and occupational agents.

One of the main thrusts of the program has been to develop interventions to reduce the incidence of liver cancer in China. Hepatocellular carcinoma, which is almost always fatal, causes more than 250,000 deaths a year worldwide. Groopman and his colleague Thomas Kensler have established the first clinical study of aflatoxin, which is now under way in China. Aflatoxin is a mold contaminant of food that has been implicated as a major risk factor for human liver cancer in sub-Saharan Africa and China. The dietary parent compound, called aflatoxin $B_1$, is found in many foods and is converted to its carcinogenic forms through metabolism by members of the cytochrome P450 enzyme superfamily. The scientists have studied the resulting metabolites, including two aflatoxin epoxides, to determine how aflatoxins contribute to causing this disease. The team found the first proof of aflatoxin's chemical–viral interaction. Now they are following up that work with a chemopreventive intervention for liver cancer with a drug called oltipraz. Later the team plans to study how hepatitis B virus and other risk factors affect aflatoxin biomarkers.



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**Ozone effects.** Detection of monoclonal antibodies in the tracheal epithelium of mouse lung by the Cell and Tissue Analysis facility allows comparison of normal tissue (top) and tissue following a three-hour exposure to ozone (bottom).

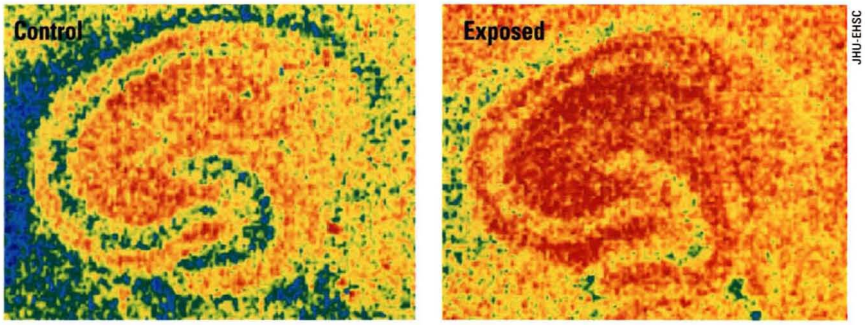
## Toxicity and Carcinogenesis

The mechanisms of toxicity and carcinogenesis program is composed of scientists whose research includes investigations on fundamental mechanisms of toxicity and cellular processes. Understanding these basic mechanisms is key for developing new exposure markers and identifying cellular targets and susceptibility indicators.

Through a pilot grant, Yager, the program coordinator, and his colleagues are looking into the effects of the altered metabolism of endogenous estrogens, caused by environmental exposures to dioxins and similar chemicals, on the oxidative DNA damage. The researchers plan to determine whether the oxidative damage is site specific, and possibly gene promoter specific, in the presence of the estrogen receptor. Johns Hopkins researchers are also breaking ground in the study of benzo[*a*]pyrene, benzene, dioxin, and dietary carcinogens.

## Physiologic Responses to Inhaled Pollutants

The center's program on physiologic responses to inhaled pollutants, directed by Wayne Mitzner, attempts to mimic real world breathing conditions—just like those experienced if one were to take a walk down a Baltimore street. For instance, people are not exposed to a single agent at a time. In an




**Brain lead.** Qualitative autoradiography shows low (blue) and high (red) levels of binding to the NMDA receptor of the hippocampus in rats exposed to lead during gestation and lactation.

urban setting there is heavy air pollution made up of all kinds of volatile compounds. In addition, the various agents may interact to produce additive or synergistic effects. "It's almost like sucking on the tail pipe of a bus," says core researcher George Jakab.

The program hopes to be able to investigate the entire spectrum of questions that pertain to specific substances, from the quantitative analysis of the exposure magnitude to a quantitative understanding of basic pathophysiologic responses of cells and tissues to these exposures. Plans are also in the works to expand the facility to study volatile organic compounds and indoor air pollutants. This global approach is fairly well developed for ozone, with studies spanning from molecular genetics to human exposures.

A prime example of work in inhalation toxicology at the center is Steven Kleeberger and Roy Levitt's work on the genetic influences on resistance and susceptibility to environmental exposures. Their current work involves comparing mice that are susceptible to inhalation of ozone to mice that are resistant to characterize the genetic control of the pulmonary response to ozone in mice. By studying first- and second-generation mice, the team has identified separate genes that regulate responses to acute and subchronic exposures and derived a map assignment for those genes. Finding human endpoints of ozone exposures is the next and ultimate step. The scientists plan to begin addressing this question in the next year.

## Neurotoxicology

The neurotoxicology program, headed by Tomas Guilarte, is evolving to focus more on mechanistic studies. Lead neurotoxicity is the program's primary area of research. A succimer intervention trial is being conducted in local children through the Kennedy-Krieger Institute. The multicenter clinical trial is the first human trial of succimer, a chemical shown to chelate lead and bring down blood lead levels in laboratory animals. Researchers are testing the efficacy of succimer in chelating or binding lead and removing it from the bodies of children who have high concentrations of lead in their bloodstreams.

Guilarte is studying the role of the NMDA (*N*-methyl-D-aspartate) receptor in learning and memory deficits found in experimental animals and humans who were exposed to lead during development. Scientists have demonstrated that NMDA receptor activation is essential for the induction of use-dependent physiologic processes such as long-term potentiation—a cellular model of learning and memory. Guilarte's work seeks to delineate how lead inactivates the NMDA receptor complex and to characterize the effects of lead exposures on the development and regulation of the NMDA receptor.

Paul Strickland and his colleague Brian Schwartz are trying to determine whether there is a link between plasma delta-aminolevulinic acid (ALA) and blood lead levels in children and whether ALA is a good biomarker of lead exposure.

Aside from state-of-the-art research and provocative new approaches to environmental health science, what really drives the center's work is the commitment that the center and the Johns Hopkins University School of Hygiene and Public Health have made to the citizens of Baltimore and the surrounding areas to help create a healthy living environment. "That's the way we think," Yager says.

**Julie Wakefield**

## Barrett Named Scientific Director


Steve McCaw, Image Associates

J. Carl Barrett

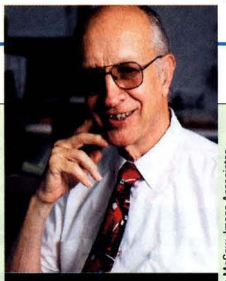
J. Carl Barrett, chief of the NIEHS Laboratory of Molecular Carcinogenesis that was part of the team that isolated the breast cancer susceptibility gene, has been named Scientific Director of the NIEHS. As Scientific Director, Barrett will assume leadership of 700 scientists and support personnel in 18 laboratories and branches ranging from molecular biology to applied toxicology and clinical research.

Barrett was chosen after a competitive national search, in part, because of his contributions to research on the multiple steps of the cancer process, the mechanisms of environmental carcinogens such as asbestos and hormones, the relationship between cellular aging and cancer, and the identification of the genes involved in human cancers. In May, Barrett and colleagues at the NIEHS identified a gene that suppresses the spread of prostate cancer and that may provide a marker for prostate cancers that metastasize.

In announcing the appointment, NIEHS Director Kenneth Olden noted, "Dr. Barrett has stimulated great excitement within the scientific community with his laboratory's achievements. Scientists within the institute cannot help but benefit from his example and leadership as we advance into the next century."

Barrett received a bachelor's degree in chemistry at The College of William and Mary in 1969 and a doctorate in biophysical chemistry from The Johns Hopkins University in 1974. After a three-year postdoctoral fellowship at Johns Hopkins, he joined NIEHS in 1977. Barrett has authored or co-authored 265 scientific publications and is an adjunct professor at the University of North Carolina at Chapel Hill in the departments of pathology, epidemiology, toxicology, and genetics and molecular biology. He is also an adjunct senior fellow in the Center for the Study of Aging and Human Development at Duke University Medical Center.

## Fouts Retires



James R. Fouts

Steve McCaw, Image Associates

James R. Fouts, a scientist who has spent more than 40 years at the frontier of the development of toxicology and environmental health as scientific disciplines, has retired as Senior Scientific Advisor to the director of the NIEHS. Fouts joined the NIEHS as a senior scientist in 1970 and served until his retirement on the institute's executive committee. "The science that Dr. Fouts did here, the scientists that he trained here and around the nation and the world, and his creative contributions to programs will have a lasting impact for the future," said NIEHS Director Kenneth Olden.

During his years of scientific research, Fouts's expansive range of interests have included mammalian and marine drug-metabolizing enzyme systems; comparative pharmacology and drug metabolism; the pharmacology of antimetabolites and antibiotics; drug–chemical interactions; preclinical drug testing; and pharmacogenetics. Fouts is 1 of 57 pharmacologists to be named to the "1000 Contemporary Scientists Most-Cited 1965–1978," by *Current Contents*, an indication of the fundamental impact of his research to the research of others.

Fouts received a bachelor's degree with highest honors in chemistry at Northwestern University, where he was appointed a tutorial fellow in biochemistry. He earned his doctorate in biochemistry and pharmacology at Northwestern University and later worked at Burroughs Wellcome Research Laboratories in Tuckahoe, New York, under Nobel Laureate George Hitchings. In 1957, Fouts joined the faculty of the Department of Pharmacology in the College of Medicine at the University of Iowa, where he also served as director of the Oakdale Toxicology Center.

A touchstone throughout his career has been Fouts's enthusiasm for training young scientists. Over the years he has trained 19 postdoctoral students and 17 graduate students, including senior scientists at the NIEHS, and has served as an informal mentor and teacher to many others.

## NTP Update

The National Toxicology Program presented eight technical reports at a public review by the NTP's Board of Scientific Counselors on June 20–21 at the NIEHS. These reports included a predictive analysis of potential noncarcinogenicity based on metabolism, six standard studies characterizing the toxicology and carcinogenicity of selected chemicals, and a comparison study of the effects of limiting food consumption or body weights on carcinogenesis evaluations.

***1,4-Butanediol.*** The NCI nominated 1,4-butanediol for study because of its high volume production as a chemical intermediate and potential for worker exposure. 1,4-Butanediol metabolizes rapidly to γ-hydroxybutyric acid, which is also the end metabolite of γ-butyrolactone. Because the NTP has already performed a full toxicology and carcinogenesis study of γ-butyrolactone and found no effects at any doses, it was possible to predict that 1,4-butanediol would similarly not be carcinogenic in animals.

***Codeine.*** Codeine is used in a variety of pharmaceuticals as an analgesic, sedative, and antitussive agent and is one of the most frequently prescribed therapeutic drugs in the United States. In two-year studies, rats and mice were given codeine in the feed at doses ranging from 10 to 100 times the human prescription doses (on a dose per body weight basis). There was no evidence of carcinogenicity of codeine in these studies.

***1,2-Dihydro-2,2,4-trimethylquinoline.*** 1,2-Dihydro-2,2,4-trimethylquinoline is used in the preparation of antioxidants for butadiene-based rubbers and latexes. In two-year studies, the chemical (in acetone solution) was applied five times per week to the skin of rats and mice. Male rats exhibited an increased incidence of kidney neoplasms, indicating some evidence of carcinogenic activity. There was no evidence of carcinogenicity in female rats or in either sex of mice.

***Butyl benzyl phthalate.*** Butyl benzyl phthalate is used as a plasticizer in a variety of synthetic polymers. In a mating study, exposure to butyl benzyl phthalate in the feed for 10 weeks produced marked effects on male reproductive parameters, including decreased testis, epididymis, and seminiferous tubule weights, and dramatically decreased sperm production. In two-year studies, male rats exhibited some evidence of carcinogenic activity based on an increase incidence of neoplasms of the pancreas. There was equivocal evidence of carcinogenic activity in female rats based on the occurrence of a few uncommon neoplasms in the pancreas and urinary bladder.

***Salicylazosulfapyridine.*** Salicylazosulfapyridine (SASP) is used in the treatment of ulcerative colitis and Crohn's disease. When given in the feed for two years, SASP caused liver tumors in male and female mice, which was interpreted as clear evidence of carcinogenic activity. In rats, SASP caused the formation of calculi and proteinaceous concretions in the urinary bladder, extensive urinary bladder hyperplasia, and some papillomas, which were interpreted as some evidence of carcinogenic activity.

***t-Butylhydroquinone.*** *t*-Butylhydroquinone is an antioxidant used in cosmetics and meat products. It was not carcinogenic in rats or mice when given in the feed for two years.

***Scopolamine hydrobromide trihydrate.*** Scopolamine hydrobromide trihydrate is the active ingredient in transdermal patches for motion sickness and is also used in ophthalmic preparations. It was not carcinogenic in rats or mice when given in water by oral gavage for two years.

### Diet Restriction Studies

Studies of butyl benzyl phthalate, SASP, *t*-butylhydroquinone, and scopolamine hydrobromide trihydrate each included additional groups of animals that were used to compare the standard NTP bioassay protocol, in which animals are given access to feed *ad libitum*, with protocols where the amount of food consumed was restricted. Comparisons were made between the tumor incidences in animals exposed to chemical with their normal controls and with controls that had diets restricted such that their body weights matched the exposed animals. Other comparisons were made where control animals and exposed animals received approximately 20% less feed than animals eating unrestricted amounts.

In general, animals with lower body weights or those receiving less food had lower incidences of neoplasms at several sites. The sensitivity of the bioassay to detect carcinogenic responses was affected by dietary restriction: when dosed animals in the *ad libitum* protocol were compared with controls that had similar body weights, the significance of the tumor incidences was greater. However, some effects observed in the normal *ad libitum* protocol were not reproduced when control and dosed animals were subject to dietary restriction. The relations between reduced body weights and lower incidences of neoplasms also illustrated the importance of dose selection in design of long-term animal studies, in which doses higher than the "minimally toxic dose" might result in lower body weights and complicate comparisons with concurrent control groups.

# Sustaining Health in the Southern Hemisphere

Australia still seems a wide-open frontier full of boundless possibilities. This is an image the country promotes, posting advertisements suggesting to readers that if they run a business, employ a certain number of people, and possess a certain amount of capital, they might consider moving there lock, stock, and assembly line. Agricultural New Zealand has become a popular travel destination and movie production center with its unusually wide range of scenic outdoors settings. Open space, fresh air, sunshine, and a slower pace are features these nations emphasize in promotions.

But, over the past two decades, both nations have come to realize that as reasonable as their self-billing might be, they have not escaped the environmental troubles that beset other countries. In Australia, ozone depletion has added to factors such as geographic location and population type (predominantly fair-skinned Anglo-Saxons prior to the post World War II migration boom) to increase skin cancer rates, particularly melanomas, to among the highest in the world. Mining industries, a major part of Australia's economy, continue to pollute air and water, particularly in certain regions. Toxic waste, particularly from urban industries, and sewage, which is still disposed of in major metropolitan areas through ocean outlets, threaten the quality of the country's drinking water and its renowned beaches. Although the magnitude of most environmentally related health problems still does not approach that of the United States or Europe, government, industry, and public interest organizations are joining forces to mitigate existing problems and prevent problems that could occur if environmental degradation continues.

New Zealand also experiences high skin cancer rates. Its main urban areas of Auckland and Hunt Valley are just beginning to experience air pollution levels of health concern caused by industrial sources and vehicle emissions. New Zealand has recently reorganized its environmental policies and now has a strategy designed to carry it to the year 2010 and beyond.

## Australia

When the first ships carrying English settlers arrived in 1788 in what was to become Sydney, they encountered essentially a virgin environment. Semi-nomadic Aboriginal peoples sustainably managed their land use by varying the areas in which they hunted and gathered food. Australia is now a country of more than 18 million people, most of whom live in a small number of coastal cities. The world's smallest continent and largest island, Australia has climate zones ranging from sub-Antarctic to tropical. It is also the second driest continent (Antarctica is first), with desert or near-desert stretching across the majority of the inland country. Primarily agricultural until the 1940s, Australia has experienced unprecedented industrial growth, primarily in mining, since World War II and while retaining a strong agricultural economy, now supports a thriving minerals, manufacturing, tourism, information technology, and export economy.

The result of industrial growth, aside from affluence, is that environmental resources, particularly those along the coastal fringe, have suffered damage, and health problems have resulted. Rivers, particularly some of those in the inland that are a source of agricultural and rural drinking water, have been choked with pollution-fed blue-green algae. While algal blooms were recorded in the Murray-Darling river system as early as the 1840s, the frequency of outbreaks has increased. In major population centers, particularly Sydney and Melbourne along the southeastern coast, air quality is declining because of vehicle emissions. Although in general, authorities say, the human health problems do not have a wide-ranging impact (the frequency of exceedences of the national guidelines of Australia's Health and Medical Research Council, which are comparable to those of the World Health Organization, is generally less than five per year in each population center), there is concern that they will if not curbed. "Australia's cities, in comparison with most cities around the world, are reasonably healthy. But not healthy enough," stated *A Cleaner Australia*, a 1992 federal government report on the environment.

### Who's in Charge

In Australia, unlike the United States, states and territories have the constitutional responsibility for environmental health. Keith Bentley, director of environmental health for the Department of Human Services and Health, explains that health and environment management is through joint commonwealth and state forums (usually ministerial councils), which operate in an advisory capacity, issuing guidelines for adoption into law by states and territories. State and territory health and environmental pollution control authorities have the power to issue and revoke consents to operate industrial facilities and implement programs for pollution abatement. In general, says Bentley, authorities try to encourage voluntary compliance by industries on a facility-by-facility approach, but powers exist for civil action through state-based land and environment judiciaries.

Each state and territory has its own environmental authority and environmental health organization. These bodies administer regulations and guidelines developed at the federal level or may adopt guidelines based on models developed by other national or international agencies. They generate their own data and regularly issue reports on environmental and health criteria. Data from each state and territory is currently being evaluated for inclusion in a National State of the Environment report, Bentley says. The national report will also serve to fulfill Australia's reporting obligations to the Organization for Economic Cooperation and Development and United Nations bodies such as the United Nations Environment Programme and the Economic and Social Commission for Asia and the Pacific.

As might be expected, there is a diversity of opinions about how well environmental measures are implemented and enforced.

Michael Moore, director of the National Centre for Environmental Toxicology, says, "In many cases state governments are led by their pockets. . . . Just because something is decided in Canberra doesn't mean it's going to get done. Each state jealously guards its rights within the federation."

Yet Ian Calder, director of the Environmental Health Branch of the South Australia Health Commission, insists that relations between the states and the federal government on environmental issues are "fairly cooperative." Says Calder, "The implementation is a little variable depending on what can be afforded and the perceived risk. Sometimes there are tensions but on the whole, there are times when it's been quite good."

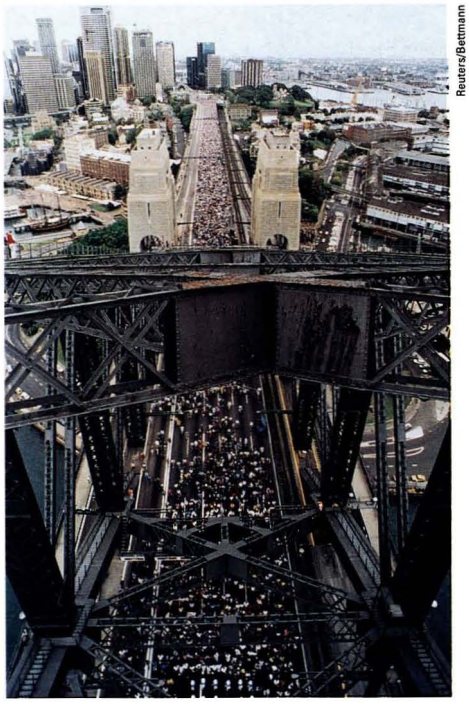
Bentley says that there is a much greater degree of voluntary compliance in Australia than in the United States. Industries share responsibility with the community for health and environmental management through a variety of activities and through extensive public consultations with the nongovernmental sector. The major nongovernmental groups, including World Wide Fund for Nature, the Australia Conservation Foundation, and the Consumer Health Forum, work closely with government on policy setting, says Bentley. Still, not every group is satisfied with its government interactions. Greenpeace spokesperson Jon Walter says that much of government is engaging in "a fair bit of lip service. They're more interested in solving a political problem than in solving the environmental problem."

The emphasis in environmental legislation seems to be shifting toward a more national scope. Legislation has been finalized to create a National Environmental Protection Council by the end of the year. Standards promulgated by the council will focus on health as well as on cultural and social aspects of the environment and will be implemented nationally in all jurisdictions, Bentley says. The council and the federal Environment Protection Agency, established three years ago to administer national policy, will report to the Department of Environment. In addition, the Intergovernmental Agreement on the Environment provides a legal structure for states to work across their boundaries to solve mutual environmental problems.

## In the Air

Drive across the Sydney Harbor bridge during a summer rush hour and except for the view, you could be in nearly any populous, industrial city. Because of Australia's open spaces, sulfur dioxide and nitrogen oxides that have contributed to heavy, throat-irritating smog in other cities are "presently not a huge problem," according to Steve McPhail of the New South Wales Environment Protection Authority. Nevertheless, to ensure that these pollutants do not cause trouble in the future, air quality standards are closely monitored in the urban centers. In addition, the federal government has set voluntary guidelines for acceptable limits on air pollutants. In Melbourne, the one-hour acceptable limit for nitrogen dioxide has been exceeded one or two times per year in recent years, according to a 1990 information bulletin from the Victoria authority, but local policy allows three such incidents annually before objectives are considered breached.

A more significant problem in cities now is lead from vehicle emissions and, to a lesser extent, from old paint. Lead began to accumulate in the environment with the rise of industry starting in the 1860s and accelerated through World War II, said Bentley. Australia is now the third largest producer of lead in the Organization for Economic Cooperation and Development. However, "since the early 1960s and particularly since the introduction of unleaded gasoline in 1986, the air concentrations [of lead] have been rapidly declining," said Bentley. "The present peak air concentrations, even in the most traffic-intense city intersections, seldom exceed 0.7 micrograms per cubic meter ($\mu g/m^3$) over a three-month average. This corresponds to less than 50 percent of the United States Environmental Protection Agency criteria value."



Reuters/Bettmann

**Rush hour.** During a city race, runners replace the usual heavy traffic across Sydney Harbor Bridge.

Lead accumulates gradually in tissues and may cause no noticeable symptoms in adults until the levels in blood reach 40 micrograms per deciliter (μg/dl), according to Barbara Nelson, program analyst in the lead prevention branch of the U.S. National Center for Environmental Health. Symptoms may begin with headache and anorexia. Children are far more vulnerable; levels as low as 10 μg/dl may subtly affect development, including mental acuity.

In 1993, a study reported that as many as 630,000 Australian children less than four years old had blood lead levels over 10 μg/dl, said Geoff Duggin, associate professor of medicine and pharmacology at Sydney University. A 1995 survey involving all the states and territories shows that the incidence of lead in blood is substantially less than the earlier figures indicated. Although all data for the National Blood Lead Survey have not been reported, preliminary figures indicate that 7,000 out of 1.2 million children (based on 1986 census figures) under the age of four have blood lead levels over 15 μg/dl. By comparison, in the United States the Third National Health and Nutrition Examination Survey estimated that 500,000 of approximately 22 million children (based on 1995 census data) under age 6 have levels over 15 μg/dl, according to Nelson.

In 1993, federal and state governments adopted new blood lead goals of less than 10 μg/dl in all individuals. These goals are being implemented through state-based initiatives that focus on lead mining and smelting activities and urban lead abatement education programs that focus on reducing exposures from historical sources of lead, particularly from paint in old houses. (Australia reduced the acceptable level of lead in domestic paints almost a decade before the United States.) The federal government has commissioned a study to determine whether the new goals are lowering blood lead levels, and there is preliminary evidence that in some areas the levels have dropped.

Although industry improvements have also helped decrease the amount of lead emitted into the environment, auto exhaust from leaded gasoline remains a significant contributor to lead pollution in the air and

Reuters/Bettmann

**Toxic curl.** A surfer at a Sydney beach wears a gas mask to highlight dangers of sewage and industrial pollution.

soil. The maximum level of lead permissible in gasoline was reduced in 1994, and further reductions are set for 1995–1997. These reductions were accompanied by fiscal measures that favored unleaded fuel and by a community education program encouraging the use of unleaded fuel. "Australians retain their family cars for much longer than in the United States, reflecting our climate and environmental energy concerns," says Bentley. "It is anticipated that while about 60 percent of cars now use unleaded fuel, leaded vehicles will be on the road in Australia until about the year 2003."

Australian smelting towns are continuing their own clean-up efforts. One example is Port Pirie along the coast of South Australia, where airborne lead dust that settled in and around housing, together with lead-based paint, have caused elevated blood lead levels in children. According to Calder, the mean blood lead level in a total of 750 children between the ages of 1 and 4 was about 22.5 µg/dl in 1984. That year, the South Australian government began a 10-year decontamination program that enlisted the cooperation of Pasminco, the firm that operates the Port Pirie site. The 1995 data show that the mean blood level in the 1–4 age group has dropped to about 12 µg/dl.

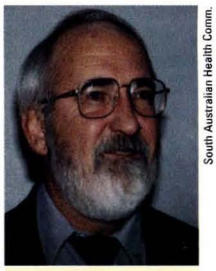
While the national goal in 1984 was to reduce children's blood lead levels to below 30 µg/dl, the new objective is to reach levels below 10 µg/dl. "It's quite a difficult job to get that extra level of clean-up to achieve lower levels," Calder said. In the New South Wales towns of Broken Hill (lead and zinc mining) and Boolaroo (lead smelting), state, industry, and community cooperative intervention programs are underway.

Australia is also instituting programs to monitor and reduce the emission of greenhouse gases that contribute to global atmosphere problems as part of its commitment to the Global Convention on Climate Change. Australia is a signatory of the Montreal Protocol to reduce stratospheric ozone depletion, and it has implemented the agreement's recommendations in relation to chlorinated fluorocarbons and other targeted substances. Australia has also provided resources to its neighbors, particularly the Pacific Island countries, to assist their implementation of the agreement.

Depletion of stratospheric ozone has led to increased levels of ultraviolet radiation across Australia. In Hobart, Tasmania, UV levels increased by 6.4% between 1980 and 1990 and are expected to rise another 7% by 2002, according to figures compiled by the Department of Human Services and Health. For the southeast of Australia, which has many heavily populated cities, UV levels may rise by as much as 5.4% by 2002, the department said.

Australia has one of the highest rates of skin cancer in the world, with about 40 cases of malignant melanoma per 100,000 women and 49 cases per 100,000 men, according to the U.S. National Cancer Institute. The mortality rate from cutaneous malignant melanoma approaches 25%. In comparison, rates in the United States are about 11 cases per 100,000 men and 9 per 100,000 women. A 1992 study by the University of Wollongong suggested that increased UV-B radiation may contribute an additional 400 cases of malignant melanomas in Australia by the year 2000.

The report also suggested that UV-related cataracts may be a more severe problem, with an extra 71,000 cases expected by 2005 because of increases in UV exposure. The Australian Cancer Society, in cooperation with federal and state health agencies, has conducted a number of education programs encouraging modification of behavior to reduce risks. One such campaign, popularly called "Slip, Slap, Slop" for "Slip on a shirt, slap on a hat, slop on some sunscreen," has been very successful, according to Bentley. Bentley adds that children in school are not allowed to play outside between 11 a.m. and 2 p.m. unless they are wearing hats.



South Australian Health Comm.

**Ian Calder**—State and federal interactions are fairly cooperative.

Australia's children are also in danger of another rising environmental health problem: asthma. Although definitions of asthma differ from country to country, most include symptoms ranging from intermittent wheezing to full-blown, regularly occurring asthma attacks. There are currently about 800 fatalities from asthma each year in Australia. *Asthma and the Indoor Environment*, a report from the New Zealand Ministry of Health, notes that wheezing in 8- to 11-year-olds in Australia has roughly doubled between 1982 and 1991 to 25%. In the United States, this figure went from 4.8% to 7.6% during the same period, according to the New Zealand report. An extensive education campaign targeted at parents is encouraging asthma management.

## In the Water

On a dry continent, water is a particularly precious resource. Australia relies on a network of groundwater aquifiers and rivers for its domestic, agricultural, and industrial supplies. Pollution, agricultural runoff, and salinity have damaged many of these sources. "One of Australia's most pressing environmental problems is declining water quality," said the federal government's 1993 Statement on the Environment.

Drinking water quality is usually good in urban centers. In rural and remote regions, disease outbreaks are minimal, but there are problems, particularly with turbidity and total dissolved solids. In Adelaide and other parts of South Australia, the water contains high levels of dissolved minerals. In the river systems, nitrates from fertilizers used in high-intensity farming, animal wastes, phosphates from detergents, and salinity from extensive irrigation have contributed to stream degradation. Blue-green algae blooms seasonally strike rivers; the algae have been a particularly severe problem along the Darling, and to a somewhat lesser extent, along the Murray River, said Moore. The rivers' catchment area is huge, extending over four states and providing domestic water for several industrial cities.

By decreasing water flow in rivers, drought leads to the development of algae blooms that cut off oxygen, causing fish to die. Although the algae can be filtered out, they secrete toxins that remain behind and have been fatal to livestock and wildlife. One outbreak along the Darling River in 1991 cost at least $1.3 billion, according to the 1993 statement. The toxins

cause a number of animal deaths annually, but there have been no recorded human fatalities from drinking the contaminated water. Skin irritations are common, though, following recreational use of some of the waters. The effects of algal toxins on humans have yet to be adequately explored, Moore says, but there is evidence they have the potential to cause liver damage. One concern is the effect of low-level, long-term exposure to the toxins. The federal Department of Primary Industry is trying to induce agriculture to lower its use of nitrate-containing fertilizers, said Moore.

The federal government is funding biological and water quality monitoring to be added to routine monitoring of rivers at 1,000 sites across the country, according to the 1993 statement. Since there is little information about groundwater on a national basis, the federal government is instituting a baseline survey at key areas.

Working with a model provided by the World Health Organization in 1984, Australia revised its drinking water guidelines in 1987. Following the latest review of the WHO guidelines in 1994, Australia's Environment Protection Agency has drafted new guidelines, according to Brian Hobsbawn of the EPA Environment Standards Branch. The guidelines, according to their introduction, "are intended to provide the Australian community and the water supply industry with guidance on what constitutes good quality drinking water, as distinct from water that is acceptable." The guidelines consider aesthetics as well as health and safety. Acceptable ranges for substances that may affect water quality such as waterborne, disease-causing microorganisms and pesticides, as well as procedures for developing water monitoring programs, assessing performance of water supply systems, and reporting such performance to the public and health authorities are included. The guidelines are expected to be in place by the end of this year. Once the guidelines are adopted, says David Gregory, acting project manager for the National Water Quality Management Strategy, which is advising the federal government on water problems, it will be up to states, territories, towns, cities, and other water delivery services to implement them.

In addition, governments are trying to improve the treatment of wastewater returned to rivers. Although guidelines exist for wastewater recycling, the 1993 statement on the environment states that the federal government is providing $8 million for demonstration of innovative wastewater treatment technologies and systems in the Perth region. The goal is to encourage the use of innovative methods nationwide.

If Australians treasure inland water, they also love their beaches. When sewage and refuse began floating in on the waves to land on some of Sydney's best shorelines in the late 1980s, there was a "huge outcry," says Walter. The government was forced to take action: the result was that waste was pumped out to sea in long pipelines. That permitted floating refuse in the sewage discharge to disperse, but Greenpeace is still dissatisfied. This waste cannot be biologically treated because it contains chlorine-containing chemicals that kill the organisms used for treatment, and the effects of these chemicals on the sea environment and people is unknown. "We advocate getting waste treated on site," Walter said. "The government doesn't really advocate that." However, the new governor of New South Wales has pledged to eliminate the offshore sewage problem over the next few years.

## In the Soil

The extensive catalog of Australian flora and fauna includes the bothersome cattle tick (*Boophilus microplus*) that is responsible for certain diseases in livestock and was controlled in the past with arsenic salts. Until about 1945, sheep and cattle would be herded into "dips," vats containing arsenic salts dissolved in water. As this practice was abandoned, the dips were bulldozed over. Increasingly, these former grazing lands are being developed for housing. With the alarm sounded by the death of household pets, public health authorities have found that the dusty topsoil in the area of the former dips sometimes contains 1,000 times the acceptable limits of arsenic, according to Moore. There are some 560 such abandoned sites in New South Wales alone, according to Jack Ng, laboratory manager at the National Research Centre for Environmental Toxicology. "A recent survey of urinary arsenic levels of people who live in the area were in the normal range," said Ng. However, he noted, "people, especially young children, who live on old dip sites could still be at risk." In each case where arsenic contamination has been found, the affected soil has been carted away and dumped in a secured storage site, Moore said.

Australia, with its heavy stores of brown coal, has no need for nuclear power plants. It does, however, have a small nuclear facility for research and the production of medical isotopes. The total accumulated spent fuel rods from this reactor contain an amount of radioactive material so small "they wouldn't make one week's output from one of the United States' major power stations," said Bentley.

Australia's only major radioactive waste problem is the result of weapons testing. Between 1952 and 1958, the United Kingdom conducted nine nuclear test explosions and smaller trials involving either burning or explosive dispersal of uranium, plutonium, and short-lived radionuclides in Emu and Maralinga, South Australia, according to the Australian Radiation Laboratory in Victoria, which provides national oversight for the use of radiation in Australia. Some 300 Aboriginal people who had lived in the area were moved 200 kilometers south to Yalata before the testing began, a laboratory statement said.

Most of the contamination has been removed, but further rehabilitation is planned to allow the aboriginal Maralinga Tjarutja people, who are the traditional owners of the area, to return. Studies designed to estimate the radiological hazards from dust inhalation, as well as other preparatory studies, are underway to help form a basis for the cleanup. A report by the Parliamentary Public Works Committee estimates that $74.27 million will be spent on the Maralinga Rehabilitation Project.

## A Cleaner Australia

"Clean production techniques, environmental management services, water treatment, air pollution control, solid waste management, and recycling are likely to continue to be high-growth areas as Australia moves towards the next century," the federal government stated in *A Cleaner Australia.*

Acording to Walter, larger industries are now more prepared to support environmental initiatives, some realizing that their continued financial viability will depend on their environmental outlook. Industry–university partnerships on environmental issues are springing up, and, said Moore, the federal government is providing grants to companies for

Reuters/Bettmann

**Aborigine activists.** Hughie Winlass (left) and Barka Bryant have campaigned for the cleanup and return of the Maralinga site to their people.

demonstration projects aimed at developing cleaner production technologies. Business is also developing alternative energy production methods. For instance, the government of New South Wales has committed $45 million over the next five years to aid in development of new solar cell technology, said Walter.

"Many companies are producing leading-edge technology capable of solving serious environmental problems," the federal report stated, but, "a major impediment to successfully promoting and developing Australian environmental technologies is the lack of comprehensive data on many important aspects of the environment management industry." Further, the report said, some industries remain to be convinced of the economic and environmental benefits of cleaner production.

The environmental branch of the Australian Chamber of Manufacturers, with 6,000 member companies across various industries, serves as a liaison between government and industry on energy and environmental matters, said spokesperson Robert Lorenson. The chamber maintains working groups to investigate crucial issues and advises industry on compliance with and impact of government guidelines and other initiatives.

Efforts underway to manage the environment include initiatives to encourage cleaner production in industry and establishment of a National Pollutant Inventory and a national Greenhouse Gases Inventory, according to the Department of the Environment's 1993–94 annual report. The Environment Protection Agency is also undertaking a major revision of its environmental impact assessment legislation.

Although a recession several years ago prompted the belief among many that environmentalism was a "negative" that clashed with job development, attitudes are changing. The federal government reports that "environmental concern is growing rapidly among Australian communities." An estimated 800,000 people are members of environmental groups, according to a report published by the Australian Overseas Information Service. In a survey sponsored by the *Sydney Morning Herald* last December, 57% of respondents rated environmental protection higher than economic growth.

## New Zealand

New Zealand, the size of California, has one of the world's highest standards of living with an economy dependent mainly on farming and foreign trade. Its earliest settlers, the Maori, first landed on the beaches in canoes 1,000 years ago and stayed to pursue a life that was agricultural, rather than nomadic. Visitors arriving in New Zealand for the first time usually remark upon the crystal clear quality of the air. Movie makers "are astounded at the physical beauty of the country," the government cheerfully reports. New Zealand's diversity of natural landscapes is the result of mountainous terrain and proximity to the Pacific volcanic "rim of fire." The climate supports dense rainforest and lush grasslands.

New Zealand's isolation protects it from most of the pollution of the industrialized world. Its relatively small population of 3.4 million and few heavy industries mean life there is more pastoral than in other, more crowded nations, and traditional pollution problems have not yet emerged.

Gary Hook

**Clean and green.** New Zealanders are striving to maintain the purity of their environment and the quality of their health.

"Clean and Green," a slogan used in government and marketing, describes not only New Zealand's international image, but also, it would like you to think, its environmental and closely linked economic outlook. As appealing as it may seem, however, this image of awareness "shouldn't lead you to think that we've taken care of things," says Catherine Wallace, co-chairman of Environmental and Conservation Organizations (ECO) of New Zealand, the umbrella organization for 80 volunteer environmental organizations representing about 400,000 people.

Before European settlement in the 1830s, more than 70% of the country was covered in rainforest occupied mostly by birds, with no mammals or snakes, said Lindsay A. Gow, acting secretary for the environment, in an address in Washington, DC, June 6 of this year. Burning and clearing for agriculture and settlement have left that figure at 24%, according to a Ministry for the Environment information bulletin. In effect, Wallace said, "we've skinned the country more or less alive."

As in Australia, global ozone depletion and near-tropical latitudes mean that New Zealand experiences high rates of skin cancer. The nation also has high asthma rates.

### The Maoris Onward

Though the Maori cleared some of the land by burning, concern for the environment is a part of their tribal philosophy, which considers all things to be connected by their life essence, and sees a duty to ensure that resources remain plentiful for future generations. In 1887, a Maori chief donated a mountain to be reserved as the first national park.

Through the 1960s and 1970s, as development increased and New Zealand began a short-lived effort to encourage the establishment of large, energy-intensive industry, a patchwork of laws sprang up to deal with individual conservation and pollution problems. But times were changing, pressure from environmentalists was mounting, and in 1984, the government created the Ministry for the Environment, an office of about 100 staffers who advise 13 regional councils on how to handle pollution problems, and the Department of Conservation, which advises on biodiversity, preservation of the land, and other such issues.

New Zealand has recently sealed its antinuclear status. Opposed to atmospheric and underground testing of nuclear weapons from the 1960s on, the New Zealand Parliament passed a resolution in 1995 that would keep nuclear power and nuclear weaponry out of the country.

In 1991, the Resource Management Act was passed, making sustainable management a nationally legislated goal. As the law is gradually implemented, builders, fishers, farmers, manufacturers, and others will have to consider the environmental impact of any new project as well as any potential economic benefit. The law requires environmental permits for projects involving land use, subdivision, discharge, coastal areas, and bodies of water. Permit renewals for existing projects also come under the law's jurisdiction. Regional councils are responsible for enforcement and may issue abatement notices, enforcement orders, and penalties for offenses.

Simon Hales, a member of ECO's executive committee and a research fellow in the Department of Health at the University of Otago, considers New Zealand ahead of many nations on its waste management policy. The 1991 Resource Management Act mandates that industry must pay for the pollution it generates. Reuse, recycling, recovery, and reduction of waste production are encouraged. Under development is an Environmental Risk Management Authority, which will establish guidelines on management of hazards from chemicals and newly introduced organisms or species, whether they are imported or biogenetically engineered.

The Maori, in many cases denied stewardship of their lands when the British settled in the 1800s, are also participating in environ-

mental issues. The Centre for Maori Studies and Research within Lincoln University works with indigenous people on issues that include biodiversity, water quality, and population planning. "Formal Maori tribal authorities are active in considering resource issues," Gow said in a speech this year.

### In the Air

New Zealand as yet has no national air or water quality standards. What the Resource Management Act doesn't specifically cover, voluntary guidelines issued by the central government do, says Dave Brash, manager of pollution and risk management at the Ministry for the Environment. As in Australia, regional governments are responsible for implementing and enforcing guidelines issued by the national government. Implementation is voluntary, but usually occurs, Brash said. He notes that so far "the air is pretty clear. The thing you notice is the visibility. It's incredible."

For years, prevailing winds have maintained this visibility. New Zealand's increasing concentration of vehicular traffic, however, means that air problems are emerging. In Christchurch, air pollution is noticeable, particularly in winter, Brash said, when atmospheric inversions trap auto exhaust and smoke from wood fires. Problems have been noticed also in Auckland and other, smaller cities, he said, noting the ministry is gathering information on the problem for a database.

In June, the national government mandated that starting next year all new cars will have to run on lead-free gasoline and reduce lead emitted into the air, said Hales.

Skin cancer is a leading health problem. "In 1992, 1 in 31 New Zealanders born, if exposed to current cancer rates, could expect to be diagnosed as having melanoma before the age of 75," according to a report from the Public Health Commission titled *Melanoma: The Public Health Commission's Advice to the Minister of Health.* "By around 2005, this risk could more than double, to 1 in 14, or over 3,000 cases," the report said, adding that 884 new cases were registered in 1990. This represents an increase from the early 1970s, when only 298 cases were reported per year, the report said. The ministry is conducting a survey of New Zealanders' sun-exposure habits with the goal of issuing sun protection guidelines.

According to the Ministry of Health, another health problem, asthma, has become more common in children and young adults in the last 20–30 years in New Zealand. In its report, *Asthma and the Indoor Environment*, the ministry estimated as many as 34% of children and young adults may suffer from asthma or may have experienced asthmalike symptoms. Potential causes, still not definitively determined, include tobacco exposure, dust mites, tighter house construction, and fumes and humidity from carpeting.

NZ Ministry for the Environment

**Dave Brash**—A clean and green image is a competitive advantage.

### In the Water

River and stream headwaters are "pristine, or nearly pristine," Brash says. But 50% of New Zealand's land is used for agriculture, and as the waters pass through these areas, runoff causes bacterial contamination and supplies nutrients for algae blooms. Specific measures have decreased point-source problems, such as runoff from dairy sheds, Brash says, but nonpoint source pollution remains a problem. The Ministry of Environment is experimenting with various measures, such as teaching farmers new environmental management systems and supplying them with guides to minimizing runoff.

Another inadvertent threat to human health is the Australian possum, an imported mammal. Possums, in addition to gnawing their way through acres of greenery, contribute significantly to the spread of bovine tuberculosis, which at high levels threatens meat and dairy export and may also contribute to the spread of waterborne diseases.

To control the possum population, farmers have used a controversial chemical called Compound 1080 (sodium fluoroacetate), cyanide, and phosphorous. A 1994 report by the Office of the Parliamentary Commissioner for the Environment titled *Possum Management in New Zealand*, states that all of these chemicals are "capable of changing the physical, chemical, or biological condition of land, water, or air and therefore can be considered 'contaminants' under the Resource Management Act of 1991." The report states that New Zealand is the world's largest user of Compound 1080 and possibly the largest user of cyanide and phosphorous.

Compound 1080 has raised public concern for two reasons: it kills non-targeted species as well as possums, and aerial spraying of the chemical may contaminate small watercourses.

Despite this concern, Compound 1080 is biodegradable, and according to the report, although "current evidence on the environmental and health effects of 1080 cannot prove absolute safety, " the risks of significant contamination is low and is outweighed by the benefits of use because a more cost-effective control method is not available.

### The 21st Century

For 2 months in 1994, the government held meetings in 15 locations nationwide to hear comment on the "Environment 2010 Strategy," which the federal government describes as the first comprehensive statement laying down broad directions for environment and development. These are voluntary guidelines, rather than mandatory rules, and the stated goals are to protect, conserve, manage, and enhance resources while reducing, managing, and stabilizing various risks and forms of pollution.

**Possum problems.** Toxic chemicals used to kill possums may be hazardous to human health.

Some environmentalists are not impressed. Wallace calls it "greenwash" and noted that besides lacking clearly stated objectives and seemingly subjugating environmental issues to economic ones, "it's got no statutory force." Said Hales, "It's good the government decided to have a policy, but it certainly doesn't go as far as people would like on some issues." Hales would like to see more emphasis on energy efficiency, guidelines or laws to reduce the production of carbon dioxide, and action on how to manage increasing traffic and its exhaust products.

In response, Brash says, "We've very much seen the linkage here between trade and environment. We have a clean and green image. It's a competitive advantage, and we have to maintain credibility. The tourism people are very aware of this issue. We're certainly not sitting on our hands."

Often referred to as isolated because of the thousands of miles of ocean that separate them from other industrialized countries, Australia and New Zealand are hardly isolated from the world environmental community. Both countries participate regularly in the conferences on global environmental problems such as greenhouse gases. Australia is supplying $1.49 billion in foreign assistance for environmental programs during fiscal year 1995–96, according to a World Environment Report. "I think it's important to look at everything in an ecological framework, planet-wide," said Hales. "We're not isolated at all."

**Jan Ziegler in the U.S.**
**Philip Callan in Australia**

# Northern Exposures:

## Cleaning up Canada

Olivia James

Canada, the second largest nation in the world, extends across almost 4 million square miles from the Atlantic Ocean westward to the rocky shores of the Pacific Ocean and from borders shared with the United States in the south 3,000 miles north to the frigid waters of the Arctic Ocean. The country's coastline, the longest in the world, spans more than 150,000 miles. Between coasts, Canada is rich in lakes, rivers, forests, and barren rocks that not only shape the landscape but also mold the economy. Pulp and paper production, agriculture, and mining are major industries. Unfortunately, emissions and runoff from these industries, from manufacturing processes, from hydroelectric plants, and from fossil fuel combustion have polluted the once pristine air and water. Persistent toxic compounds such as PCBs, dioxins, furans, DDT, and heavy metals have been polluting the Great Lakes, shared between the United States and Canada for more than 60 years. Pulp mill effluent has spread organochlorines into the waterways. Incinerators have spewed heavy metals into the atmosphere. All these contaminants are taking their toll directly or indirectly on the lives of Canada's 28 million residents, two-thirds of whom live close to the northern United States. Many of the indigenous peoples live in the Arctic archipelago.

A self-governing member of the British Commonwealth, Canada is a federation of ten provinces and two territories. Each level of government has clearly delineated powers spelled out in Canada's 1982 constitution. Environment Canada, much like the U.S. EPA, is responsible for environmental concerns of national importance such as international and interprovincial waters and interprovincial transport of substances. The provincial Ministries of the Environment have considerably more authority than U.S. state environmental agencies to legislate regulations, set standards and guidelines, and monitor substance emissions. The Canadian Committee of Resource of Environment Ministers, chaired by the federal Environmental Minister, meet to resolve provincial differences when they occur.

Environment Canada, unlike the U.S. EPA, does not make declarations on human health. That is the mandate of Health Canada. Similar to the U.S. Department of Health and Human Services, Health Canada concentrates only on human health issues. However, under the Canadian Environmental Protection Act (CEPA), enacted in 1988, Health Canada and Environment Canada jointly assess substances to determine if they are toxic. When, for example, fish are collected for study, a department of Environment Canada does the laboratory work, but Health Canada determines the risk to human health and issues the warnings. Pesticide use is governed by federal regulations, each approved for a specific use by the Pest Control Products Act, which regularly reviews pesticides in conjunction with Health Canada and Fisheries & Oceans.

Five years ago, the Canadian government committed $3 billion to a comprehensive five-year environmental cleanup program now extended to six years called the Green Plan. These monies are in addition to the estimated $730 million budgeted annually by Environment Canada for cleaning up the environment. Included in the Green Plan is the Health and Environmental Action Plan, under which human health hazards from acid rain, airborne toxins, and ground-level ozone, and drinking water safety, and waste management practices are assessed.

AP Wide World Photos

**Vancouver view.** Canadians have made concerted efforts to improve the quality of the environment in their cities.

In 1995, the federal government instituted the Toxic Substances Management Policy. The policy has two components: one deals with persistent bioaccumulating toxic substances and explicitly targets them for virtual elimination or eradication to below the detection level, the other mandates that risk assessment be carried out under the aegis of the CEPA. Substances of natural origin such as heavy metals and polycyclic aromatic hydrocarbons are assessed for risk under the policy's second component. If the substance is found to cause harm to either humans or the environment, federal regulations are imposed to control its use.

"The Toxic Substances Management Policy is the first of its type that recognizes virtual elimination," says John Carey, director of the Aquatic Ecosystem Conservation Branch of Environment Canada. "I can't think of any other jurisdiction in the world that has selected compounds for virtual elimination."

"In Canada," says Ralph Daley, executive director of Environment Canada's National Water Research Institute, "environmental advocacy groups are major players in influencing public opinion. And, I guess, public opinion and actual science drives the federal government to set these policies.

"What's important about this policy," continues Daley, "is that

government, as a matter of national policy, has recognized that there are persistent, bioaccumulating substances which by virtue of their unpredictable impact on the environment simply cannot be tolerated by the environment. That issue has not been recognized before as a matter of national policy."

Industry has had specific and substantial roles in formulating this policy. When a compound is to be designated by the government as a persistent, bioaccumulating toxic substance subject to virtual elimination, it will be announced to the public. Industry may then challenge the government's position on that substance. Industry can request a "reverse onus" designation after presenting evidence to the government as to why the substance should be removed from the list.

"The government has provided a built-in opportunity for industry to do what it has requested," continues Daley. "Industry will also be consultants and consensus-makers in the policy's action plan to regulate virtual elimination."

Not all of industry is happy with this policy. "Our fundamental concern is the definitions used by CEPA such as bioaccumulative and persistent," states Charles Ferguson, vice-president for Environment, Health and Safety at the International Nickel Corporation (INCO). "Those are great terms for defining chemicals, but we deal with natural metals, nickel, and copper. Those minerals are persistent, so the process says we're guilty because of that. Our problem with this policy is that it doesn't determine risk."

## Purging Pulp Mill Effluent

Paper-making is a major industry in Canada. The abundance of forests in northern Ontario, Alberta, and British Columbia were ideally suited for launching the pulp and paper industry. Yet, while this industry contributes considerably to Canada's GNP, it has also contributed significantly to pollution of nearby waterways.

For years, pulp and paper mills were accused of discharging effluent with high concentrations of organochlorines such as chlorinated dibenzofurans and dioxins into adjacent rivers. Impaired reproduction in fish suggested that something in the effluent was affecting fish metabolism. PCBs, dioxins, and furans have been shown to alter thyroid function in laboratory animals and to exert estrogenlike activity, and scientists suggest that these changes may apply to humans as well. In 1992, a federal regulatory package for the pulp and paper industry was passed into law.

"As far as I know, Canada is the first country to establish controls on dioxin in pulp mill effluence," says Carey. "The regulatory package states that the level of dioxin in pulp mill effluence must be less than 10 parts per quadrillion. We established the limit for tetrachlorodibenzylfuran as well."

Environment Canada

**Ralph Daley**—In Canada, public opinion and science drive federal policy-making.

To meet the dioxin regulations, no mill can use elemental chlorine for bleaching. Since 1990, use of elemental chlorine has steadily dropped, and today it is only 30% of what it was five years ago. Most of Canada's 122 pulp and paper mills now use chlorine dioxide instead of elemental chlorine.

Preliminary results of a joint federal–provincial project investigating contaminants, drinking water quality, and hydrology show fairly low concentrations of organochlorinated compounds in Canada's water. "One reason for the lower levels may be that pulp mills are changing their chlorine bleaching process to chlorine dioxide," says Brian Brownlee, a research scientist with the National Water Research Institute, Canada Center for Inland Waters.

During the last three years, Ian Smith, coordinator for Lake-wide Management of the Ontario Ministry for the Environment estimates that Canadian pulp and paper mills have reduced their emissions of organochlorines between 60% and 95%.

"Ontario pulp mills have spent in excess of $250 million to virtually eliminate dioxin from their effluent," says Smith. "We won't see the changes in the environment very quickly. It might be 10 years before we see dioxin levels falling because dioxin is persistent and will linger a long time in the environment."

## Freshening up the Great Lakes

Extending 1,160 miles in a unbroken line from Duluth, Minnesota, up to the St. Lawrence Seaway, the Great Lakes cover an area of 95,000 square miles. Except for Lake Michigan, the remaining four freshwater lakes flow along both the Canadian and U.S. borders. These lakes are vital to the economy of both countries, facilitating the flow of raw materials, agricultural goods, and manufactured products. The opening of the St. Lawrence Seaway in 1959 expedited the movement of autos, grain, lumber, minerals, and other products to and from world markets, enhancing the financial health of adjacent communities.

The shores of the Great Lakes are dotted with manufacturing, thermal and nuclear-generating, and chemical processing plants. Farmlands line the rivers emptying into the Great Lakes. While these industries benefit from proximity to shipping, they also contribute significantly to the pollution of these bodies of water. The most severe pollutants are persistent toxic chemicals, particularly PCBs. According to Michael Gilbertson, biologist for the

AP Wide World Photos

**No more environmental logjams?** Cooperation between the paper industries, government, and environmentalists has resulted in cleaner water.

International Joint Commission on the Great Lakes, industrial contamination, agricultural pesticide runoff, and long-range air-transported substances have been polluting the Great Lakes with PCBs, dioxin, DDT, and dieldrin for some 60 years.

Significant amounts of organochlorines were produced during the 1950s and 1960s. According to Gilbertson, large organochlorine manufacturing facilities up and down the Niagara River, some of which produced sources of dioxins such as the insecticide mirex and Agent Orange, discharged these substances directly into the Great Lakes or into tributaries that empty into the lakes.

Cleanup of the Great Lakes began in the early 1970s, precipitated by deformities in fish-eating birds such as osprey, cormorant, gulls, and herons, and by high bird embryo mortality. "Since then," Gilbertson says, "a large number of species have been monitored at a variety of locations. The results have given us a fairly complete view of the long-term effects organochlorines are having on fowl in the Great Lakes as well as a very good idea of how seriously affected they are. We know far more about how these chemicals affect birds than we know about how they affect humans. The research on human health is probably about 10 years behind the research that was done on wildlife in the Great Lakes."

The studies on waterfowl aroused concern about the effects of organochlorines on humans. Epidemiological studies showed that children whose mothers ate at least one pound per month of fish from Lake Michigan for a few years before pregnancy were smaller and had shorter gestational periods than children whose mothers had not eaten fish. At birth, the infants also showed neurological deficits. When tested again at 7 months and at 4 years, there were significant differences in startle reaction and the ability to process information. These findings, correlated with the levels of PCBs in the umbilical cord blood, indicated that the higher the PCB blood levels, the greater the neurological deficits.

In 1972, Canada and the United States formulated the first Great Lakes Water Quality Agreement under the Boundary Waters Treaty. The agreement, says Gilbertson, had more to do with eutrophication and building sewage treatment plants than with toxic substances. The agreement was renegotiated in 1978 to reflect the growing concern over toxic substances polluting the Great Lakes. Discharge of any or all persistent toxic substances was to be drastically curtailed.

Great Lakes United

**Fish and fowl.** Toxic effects on fish and birds in and around the Great Lakes prompted research on the human health effects of PCBs, dioxins, and other toxicants.

The Great Lakes Health Effects Program was created in 1989 as part of the Canadian Great Lakes Action Plan. The program conducted research and monitored toxic chemicals and their effects on human health. The five-year program was renewed in 1994 and renamed Great Lakes 2000. Its stated goal is to protect health and promote a healthy environment.

Gilbertson says, "We know we've done a pretty good job in getting rid of some toxic chemicals. But, I think evidence from the epidemiological studies suggests we still have quite a ways to go. The levels of these chemicals are still high enough to be affecting fetuses. I don't think there is any unequivocal evidence from the Great Lakes Basin that the incidence of cancer is better or worse around here. What we've really been showing is that there is a whole other area of toxicology that is important for the next generation."


International Joint Commission

**Michael Gilbertson**—Epidemiological studies show there is still a ways to go in cleaning up the Great Lakes.

## Sprucing up the St. Lawrence

The St. Lawrence River, though not technically a part of the Great Lakes, is a conduit between these massive bodies of land-locked lakes and the Atlantic Ocean. Over the years, ships and industry emitting pollutants into this serpentine river have contaminated its waters. The Cornwall-Massena area of the river, riddled with PCBs, dioxins, and mercury, was declared an area of concern for cleanup by the International Joint Commission.

In 1988, the St. Lawrence Action Plan was developed between the federal government and Quebec. In 1994, Ottawa and Quebec jointly funded the renamed Lawrence Vision 2000 plan with $191 million. Embodied in the plan are methods to prevent pollution, conserve the river's ecosystem, and reduce industrial discharges into the river. The plan's long-term objective is to eliminate persistent and bioaccumulating toxic substances released by more than 55 plants along the river and its tributaries. Essential ingredients of St. Lawrence Vision 2000 are monitoring contaminants in human tissues and developing strategies to reduce risk to human health.

In addition, according to Alain Bebit, Information Officer for Environment Canada for the Quebec region, enormous cleanup projects are underway in the contaminated harbors of Montreal and Quebec City. "These harbors, over the years, have been greatly contaminated with heavy metals and organic compounds emitted from petroleum refineries, other industries, and ocean-going ships along the river," Bebit says.

## Wiping out Waste

Substances buried in soil contribute to water quality problems. More than half of Canada's annual 8 million tons of hazardous waste goes untreated. Gilbertson describes the potential threat at one hazardous waste landfill. The Hyde Park Dump, located on the Niagara River in the

U.S., contains two tons of 2,3,7,8-tetrachloro-*p*-dibenzodioxin underground. This 16-acre area is the largest single storage site of dioxin in the world. The problem with Hyde Park and other waste sites like it, is its location on relatively permeable rocks that make up the Niagara Gorge. There is potential for these chemicals to seep into surrounding soil and migrate from the site where they were deposited closer to the gorge. "I don't think any dioxin has yet broken through the gorge, but fingers of nonaqueous-phase pollutants have made their way out of the site and into Canada" says Gilbertson.

The Ministry of the Environment in Ottawa faces a $2 billion cleanup bill for abandoned contaminated waste sites that are under federal jurisdiction. By contrast, the U.S. Congress in 1980 legislated $1.6 billion a year for five years to clean up hazardous waste sites.

Waste management is a continuing problem. According to Dwayne Moore, senior evaluator in the Commercial Chemicals Evaluation Branch of Environment Canada, old landfills are reaching their capacity. "We're having a very difficult time finding replacement sites," says Moore. "Whenever a site is selected, local residents, quite understandably, became very vocal and fight it."

The goal of the Canadian government is to cut overall waste production in half by the year 2000. Eliminating half of the nearly 30 million tons of garbage generated each year requires better disposal of waste as well as recycling and finding new markets for reusable materials.

John Jackson, president of Great Lakes United (a coalition of business, labor, and conservation organizations from the United States, Canada, and native Canadian tribes referred to as First Nations), says, "We have to realize that these are valuable resources that we're throwing away. Instead, we should be reusing them. Reuse is much more efficient energy-wise and material-wise."

Recycling programs have gained popularity in Canada. Municipalities are enjoying the economic windfall of recycling programs. The dollar value of used newspapers, for example, has risen considerably in the last few years. Centrally located bins or curbside pickup of newspapers, cans, and bottles significantly reduce the amount of waste that winds up in landfills or incinerators.

"The cost of recycling newspapers and other items is high because environmental costs are not being factored in," Jackson says. "For example, many Canadian pulp and paper companies rely heavily on sales in the U.S.; increasingly, U.S. newspapers are not buying newsprint unless it has a certain percentage of recycled content, putting pressure on our pulp and paper industry to acquire used newspaper. Otherwise, they might lose the U.S. market because of its procurement requirements."

According to Jackson, a lot of industrial waste from Canada is trucked by private haulers into Ohio and Michigan. Advocacy groups believe that instead of dumping, companies need to concentrate on reducing industrial waste. "Our concern is if companies ship the waste somewhere else, there is no incentive to stop producing it because local residents will not have to complain about living next to landfills or incinerators," Jackson says.

Similarly, reducing the need for hazardous waste sites begins by modifying the industrial production of toxic substances. "If we don't use these hazardous chemicals in the first place, then the waste they produce won't be hazardous," suggests Jackson. "[The waste will] still be a problem but it won't contaminate the environment."

## Sweeping in Clean Air

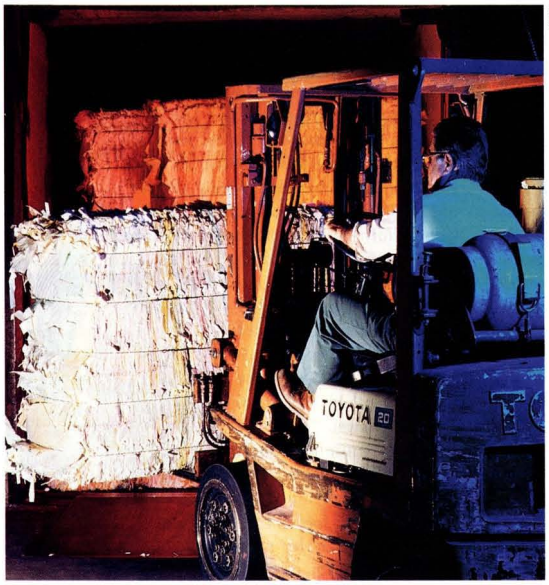
Hazardous substances such as organochlorines and pesticides are found not only in water and soil but are also carried on prevailing winds. These substances are as much an air pollution problem in Canada as the carbon dioxide, nitrogen oxides, and particulate matter emitted by industrial smokestacks, automobile tailpipes, and natural decay. Legislative responsibility for air pollution is shared between the federal and provincial governments. The CEPA oversees national air quality, while the provinces directly manage most environmental and resource issues within their borders. In 1994, a Hazardous Air Pollutants Task Group was established to define hazardous air pollutants and design a long-term strategy to manage them.

Air pollutants in major Canadian cities have been monitored since 1969 by the National Air Pollution Surveillance (NAPS) Network. More than 100 monitoring stations located in industrial, commercial, and residential areas measure levels of sulfur dioxide, carbon monoxide, nitrogen dioxide, ozone, and total suspended particulates.

Sulfur dioxide is generated by ore smelting, coal and oil burning, and gas and oil processing. When sulfur dioxide levels are high, people with asthma and bronchitis suffer respiratory problems. Carbon monoxide is emitted from vehicles, heating homes, and industrial production. When carbon monoxide levels are high, cardiovascular symptoms increase in people with heart disease, blood chemistries change, and respiratory problems rise. Nitrogen dioxide forms when vehicular and industrial fuels are burned at high temperatures. A major contributor to smog, nitrogen oxide can irritate the lungs, reducing their resistance to infection as well as to asthma. The number of times that sulfur dioxide, nitrogen dioxide, and carbon monoxide exceeded their maximum acceptable levels fell from an average of 10 per station in 1979 to well below one per station in 1992, according to an Environment Canada bulletin.

Ground-level ozone is a main component of smog. Ozone is formed by the reaction between volatile organic compounds and nitrous oxide in the presence of sunlight, and it causes pulmonary inflammation even in low doses. Studies have shown a positive association between daily hospital admissions for acute respiratory illnesses such as asthma and bronchitis and the level of ozone and sulfates.

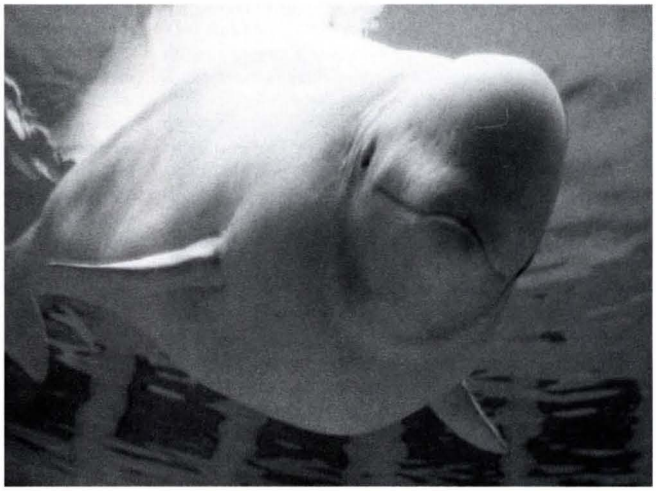
Doug Russell, director of the Air Issues Branch of Environment




RCO

**Bales of our existence.** Recycling programs such as those that divert paper from the waste stream are reducing the need for landfills.

Canada, claims that ground-level ozone is responsible for much of the visible air pollutants in Canada. "We have identified three primary areas in Canada where ground-level ozone is most prevalent," says Russell. "Most of the smog is found in the Windsor to Quebec City corridor where we have major industry. The other two areas are the southwestern areas of Nova Scotia and New Brunswick and the lower Fraser Valley in British Columbia." Environment Canada is now targeting ground-level ozone and expects to reduce it by 40% by the year 2000.



Great Lakes United

**A whale of a problem.** Methylmercury and organochlorines bioaccumulate in fish and aquatic mammals like the beluga whale that make up a large portion of the diet of Canadian aborigines.

NAPS also monitors levels of total suspended particulates. Mining processes, vehicles, thermal power plants, and waste incinerators spew unacceptable levels of particulates into the atmosphere in Canadian cities such as Hamilton, Calgary, and Toronto, says David Bates, professor emeritus at the University of British Columbia School of Medicine. Particles smaller than 10 microns, known as $PM_{10}$, are generated by diesel as well as gasoline-powered vehicles. Health effects can be detected at concentrations as low as 100 $\mu g/m^3$.

"Data collected in the U.S. and Canada indicates a direct relationship between $PM_{10}$ levels and daily mortality," suggests Bates. "A large study in Ontario shows that as aerosol sulfate levels rise, of which $PM_{10}$ is a component, hospital admissions for cardiovascular disease increase as well. There is a direct link between mortality from heart disease and lung disease as the $PM_{10}$ levels rise." According to Bates, this association holds for all of the 25 cities worldwide in which total suspended particulates have been studied. Canada is part of an international coalition working to reduce emissions of sulfur dioxide and the other pollutants that generate ground-level ozone.

## Environmental Injustice

The aboriginal peoples of Canada—American Indians, Inuits, and metis (people of mixed Indian and French origins)—constitute about 1.5% of Canada's population. Yet, in spite of their relatively small numbers, Canada's native peoples are exposed more often to dangerous pollutants than Canadians in general.

"Aboriginal people, as part of their lifestyle, are frequently more exposed to environmental contaminants than the general population," reports Brian Wheatley, director for research and development of environmental contaminants for Health Canada. As an example, Wheatley cites his investigation of methylmercury levels carried out in 514 native communities across Canada. Of the 38,571 aboriginal people tested, 608 individuals had blood levels of methylmercury in excess of 100 parts per billion (ppb). Based on World Health Organization standards, Health Canada defines blood levels of methylmercury as "acceptable" if below 20 ppb and levels higher than 100 ppb as "at risk." The highest mean levels were found in the Inuit living in the Northwest Territories. In a study conducted by Éric Dewailly, director of Environmental Health Services, Quebec Community Health Department, the Inuit in northern Quebec had an average blood concentration of 22 ppb (reported as 104 nanomoles/liter) compared to 78% of Quebec City subjects who had a mercury level lower than 2 ppb (10 nanomoles/liter).

"The common link is almost certainly the traditional native lifestyle and the consumption of fish and sea mammals," Wheatley says. "Methylmercury, like organochlorines, bioaccumulates up the food chain in fish and aquatic mammals. Some cases can be associated with local industrial activity and the general increase in mercury in the global environment," said Wheatley.

Although the levels of mercury detected are too low to directly endanger adults, there is concern about neurotoxic effects on infants. In addition, there is great concern about the indirect effects of methylmercury on native peoples. Henry Lickers, director of the Environmental Division of the Mohawk Council of Akewsasne, says, "The Akewsasne reserve has been called the most polluted in Canada because of the multitude of contaminants such as PCBs, mercury, mirex, and more. People are no longer able to carry out traditional economies of fishing, hunting, trapping, and farming. Unable to share resources among our people by using traditional economies, we're seeing more non-traditional, disruptive economies coming from smuggling and gambling."

Wheatley also cites the impact of a recent survey of PCBs and other contaminants in breast milk from women on the Akewsasne reserve. The results showed PCB levels to be low, but the perception that their food was dangerous to eat caused many Akewsasne Mohawkes to change their lifestyle. Instead of eating a fish diet, high in protein, many have replaced fish with a relatively high carbohydrate diet. Such a change may lead to other problems. "We know there appears to be a predisposition in native people across Canada to develop adult-onset diabetes," Wheatley says. "Fifty years ago there was no diabetes in Akewsasne. The prevalence of diabetes on this reserve now is four times the Canadian average."

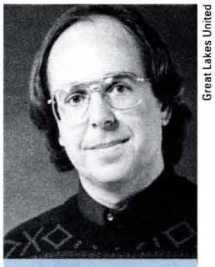
Health Canada

**Brian Wheatley**—Aboriginal people are more at risk from environmental contaminants.

The change from native foods has other health implications as well. "There are many benefits of traditional food," claims Dewailly. "The overall mortality and morbidity trends among the Inuit clearly show that heart disease and cancer are less prevalent among the Inuit. Their diet, high in lipids from sea mammals and fish, protects

them from these diseases. We have to balance the theoretical risk of the contaminants we're studying to the proven benefits of a traditional diet. We're pretty sure that the benefits of the food far outweigh the risks."

Breast milk has been monitored by Health Canada's Health Protection Branch since 1967 to determine infant exposure to environmental pollutants. W. Harvey Newsome of the Food Research Division of Health Canada recently reported the results of 497 human milk samples in a 1995 article in *Chemosphere*. Samples from across Canada were analyzed for 24 organochlorine pesticides and industrial chemicals as well as for 40 PCB congeners. "Compared to earlier surveys in Canada, concentrations of most residues determined declined and were lower than those reported recently in European countries," Newsome said.



Great Lakes United

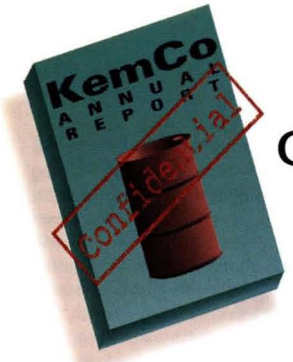
**John Jackson**—If we don't use hazardous products, we won't create hazardous waste.

### Future Trends

By most standards, Canada has done a remarkable job of cleaning up its environment. Pulp and paper mills, for example, have considerably reduced their emissions of organochlorines. The Great Lakes are being purged of pesticides as well as PCBs, dioxin, dieldrin, and heavy metals. Wildlife is beginning to return to the wetlands that had been damaged by toxic compounds. The volume of residential waste, along with industrial hazardous waste, is diminishing as communities and industry alike turn to recycling and reuse. The atmosphere is clearer and cleaner because leaded fuels were abolished in the early 1980s and provincial Environmental Ministries have enacted legislation limiting the number and kinds of pollutants that smokestacks can emit.

And this is only the beginning. Federal and provincial governments are working together on projects to clean up the St. Lawrence Seaway. The recently enacted Toxic Substances Management Plan will ensure that persistent, bioaccumulating toxic materials will be virtually eliminated. However, cautions Ontario's Smith, "In the future, toxic pollutants will come more from multiple, small sources such as transportation, combustion, and incineration. We're moving away from regulating heavy industry and moving into new areas and new threats to the environment."

**Marilyn Citron**




# Can You Keep a Secret?

The Toxic Substances Control Act (TSCA) of 1976 was designed to identify and control chemicals that might pose a public health risk, but chemical manufacturers have consistently taken advantage of the law's confidentiality provisions to withhold data from the public. The liberal exemptions claimed by industry, however, have not gone unnoticed by state environmental regulators, public interest groups, and scientists. Growing criticism of the law's confidential business information (CBI) provision is now leading to reform efforts in which industry is participating.

In the environmental legislation of the 1970s, aimed specifically toward improving land, water, and air quality by focusing on the release of chemicals, TSCA took aim at chemical manufacture itself. In a new book, *Reducing Toxics: A New Approach to Policy and Industrial Decisionmaking,* compiled by the Pollution Prevention Education and Research Center at UCLA, TSCA is called the "jewel in the crown of environmental protection" because of its vast potential for preemptive action. TSCA requires that manufacturers notify the EPA of the production of specific chemicals, including those in premanufacturing stages, and their potential for effects on public health and the environment. It also empowers the EPA to step in and halt the manufacture of those chemicals if the agency believes that the information the manufacturers provided was insufficient.

However, TSCA also recognizes the competitive reality of the marketplace by extending to manufacturers the right to claim some of the information on chemicals as confidential. Although manufacturers are still required to submit information on chemicals being manufactured or planned, they can request that the submitted data be classified as confidential business information for purposes of maintaining trade secrets and competitive advantages that often are the result of costly research investments.

## Too Tolerant?

By all accounts, the EPA has been very tolerant of these requests. During the two decades of TSCA data collection, "EPA has thoroughly adhered to the confidentiality process, maintaining CBI protection as if national security secrets were at stake," according to Janice Mazurek, Robert Gottlieb, and Julie Roque, authors of a chapter in *Reducing Toxics.* "At the same time, however, CBI claims by industry during this period have become so excessive that they, more than any single aspect of the act's implementation, have fundamentally frustrated the intent of TSCA and transformed a treasure trove of information potentially available for pollution prevention into a guarded fortress of inaccessible data."

Foremost among those who seek access to these data are state environmental agencies, many of whom have been performing their own risk assessments over the last 20 years, but who are excluded from access to the confidential information held by the EPA. In large part, the exclusion of state regulators from the notification process may be a reflection of the period during which TSCA was born. "When TSCA was enacted in 1976, the states were only in their infancy in handling a lot of sensitive scientific and commercial-type information," says Paul Wright, a senior attorney with the Dow Chemical Company. "Back in 1976, nobody was handling it, really, except the companies." Today, according to Roger Kanerva, environmental policy advisor to the Illinois EPA, more than half the states have some type of legislated pollution-prevention programs, and a 1993 needs assessment by the Forum on State and Tribal Toxics Action found that 82% of the respondents (48 states responded) reported anywhere from 1 to 6 ongoing toxics regulations programs.

According to Scott Sherlock, a lawyer in the information management division of the EPA's Office of Pollution Prevention and Toxics (OPPT), the realization that states should be parties to CBI became widely shared within a few years after TSCA's passage. In fact, when Congressman David Durenberger (R-Minnesota) sought to amend TSCA in 1984 with a bill that was perceived as being industry friendly, the legislation sought to include states as recipients of the CBI information.

In subsequent years, TSCA has continued to attract attention and criticism, mostly from environmentalists and mostly for failure to live up to initial expectations. Essentially, critics argue that TSCA simply has not resulted in the envisioned chemical control. They also point to the extensive CBI privilege being claimed by industry and the fact that information is not getting to the states. According to Sherlock, in 1990 the EPA sought to "revitalize" TSCA in several ways, including "an effort to reduce CBI claims." The EPA embarked on a specific CBI reform program designed, in Sherlock's words, "to examine CBI, what kinds of claims are made, and also examine how the claims are made and whether they're reasonable under the statute."

The CBI language in TSCA is contained in Section 14(a), which limits confidentiality to "trade secrets or financial information" and Section 14(b), which restricts confidentiality to data that disclose "processes used in the manufacturing or processing of a chemical substance or mixture or, in the case of a mixture . . . any data which discloses the portion of the mixture comprised by any of the chemical substances in the mixture." No matter what the original intent was, it has become routine for industry to claim confidentiality for chemicals which they are required to list in the premanufacturing notices, or PMNs, they are required to submit to the EPA. "There's an assumption that anything claimed as confidential is a valid claim," says Sherlock, "and therefore, in order for us to limit the claims, we have to actually physically go after them. Since we get over 2,000 PMNs a year and 99 percent of those have CBI claims, it's a problem for us."

Part of the EPA's revitalization effort was a contract with a consultant, Hamp-

shire Research Associates, Inc., to specifically address CBI's influence on TSCA implementation. The Hampshire report, published in March 1992, was highly critical of the leniency with which CBI designations had been granted. "EPA practices for safeguarding CBI have effectively prevented damage to submitters from disclosure," the report stated, "but EPA appears to be providing protection to a considerable body of data that is not entitled to such protection; thus resources that could be applied to the protection of legitimate trade secret information are presumably being diverted for the protection of frivolous claims."

## A Plan of Action

The Hampshire report initiated a series of EPA public meetings, a process which included chemical industry representatives—in particular, the Chemical Manufacturers Association (CMA)—as participants in the development of proposals for change. The result was an action plan that was released in June 1994 as a statement of "specific actions the Office will undertake to reduce inappropriate CBI claims." Essentially, the plan is a series of methods for the EPA to reduce inappropriate claims, such as educational workshops and communication of examples of appropriate and inappropriate CBI claims.

Also in 1994, Congressman Harry M. Reid (D-Nevada), chair of the Senate's Subcommittee on Toxic Substances, Research and Development of the Committee on Environment and Public Works, called for a TSCA reauthorization hearing and a report from the General Accounting Office on the effectiveness of the law. Among its findings, the GAO reported that although TSCA has resulted in an inventory of some 72,000 chemicals, its track record for regulating them has been limited to controlling nine chemicals determined to pose unreasonable health risks.

The GAO identified an inherent problem in TSCA's power to restrict chemical manufacture: "the act's legal standards are so high that they have usually discouraged EPA from using these authorities." The report noted that a 1991 ruling by the U.S. Court of Appeals for the Fifth Circuit held that the burden is on the EPA to justify that the products it bans present an unreasonable risk. The GAO report states: "To make an unreasonable risk determination, the act requires EPA to consider more than whether the chemical is toxic or harmful to humans, animals, plants, and other organisms. The agency is to also determine the magnitude of human and environmental exposures to the chemical. Once it determines the extent of the risks presented by the chemical, EPA must determine whether these risks are unreasonable. According to EPA officials, the agency must, in effect, perform a cost-benefit analysis, considering the economic and societal costs of placing controls on the chemical." The report also found that confidentiality claims are "excessive" and that states should have access to CBI because it "would provide the public with another line of defense to protect health and the environment."

After the Republican sweep in the midterm elections last November, it became obvious that TSCA reauthorization was a dead issue for the time being; the EPA's only new direction on TSCA would be provided by its action plan. While the plan didn't specify any definite proposals to extend CBI to state regulatory agencies, it did refer to discussions between OPPT, the Forum on States and Tribal Toxic Action (FOSTTA, an EPA-sponsored group), and the CMA, and the exploration of potential ways for states to receive CBI.

The result of the discussions, completed this spring, is a pilot project that will involve six states. Alabama, California, Georgia, Illinois, New York, and Wisconsin will receive CBI information from the EPA for a 60-day period and then submit reports on how it will affect risk assessments in those states. The CMA is taking credit for the plan, which gets around TSCA's statutory restrictions on dissemination of confidential data by naming the states as federal contractors allowed to access CBI.

Kanerva had been pushing for states to receive CBI ever since the early 1980s, when his agency sought to develop a comprehensive toxics control strategy and contacted the EPA about chemical production in Illinois, only to run into the locked door of CBI. "How could we hope to ultimately ensure chemical safety to citizens in Illinois if we were not even able to find out what chemicals were in production and use?" asked Kanerva when he testified last year at one of the TSCA reauthorization hearings. "From that time forward, CBI became a symbol for us of poor public policy that needed to be changed." Kanerva also argues that the evolution of such environmental "right-to-know" laws as the Emergency Planning and Community Right-to-Know Act (EPCRA) of 1986 have rendered TSCA somewhat of an anachronism. He points out that EPCRA also recognizes the need for confidential information, but does not presume protection for trade secrets like TSCA does. According to Kanerva, EPCRA allows states to obtain "any information" that is submitted to the federal EPA for an EPCRA confidentiality request.

According to Wright, industry's interest in the issue of CBI sharing with states arose about two years ago when TSCA was coming up for reauthorization, and arguments like Kinerva's found sympathetic ears. "We felt it was sort of a nonsensical situation," Wright said. "If the state could get it from us directly [under a revised TSCA], they probably ought to be able to get it from U.S. EPA. EPA, though, took the position that they weren't permitted under TSCA to allow states access to it because the statute reads that federal employees and federal contractors are the only ones who can have access to it."

Essentially then, according to Wright, when the CMA came up with the contractor plan, they came up with a legal theory that means "we don't have to wait for Congress." The chemical industry's central concern is one of efficiency. That is, if a revised TSCA would require chemical manufacturers to submit CBI to any state that asks for it, "then instead of us supplying information to one government entity—the U.S. EPA—we'll be submitting it to 51 government entities—the U.S. EPA plus all 50 states," Wright said. "That kills a lot of trees to print the paper, but it doesn't accomplish anything."

## The Effect of Sharing

Wright says that chemical manufacturers recognize the interest that states have in CBI data, but he contends that confidential information must continue to be liberally designated as such—including chemicals in all premanufacturing notices. "I have to continue to protect that information as confidential," he said, "because if I don't and my competitors get hold of it, then I've invested tens of millions of dollars in research and I won't be able to recoup it because everybody in the world is going to know about it. You have to worry about shareholder value and shareholder derivative value if you start giving away the company property. There's a well-founded legal principle of intellectual property and rights to that property. If we give away that property, it's the same as if we give away a 50-acre tract of land."

The CMA Director of Product Stewardship Charles Walton agrees that states deserve access to CBI, but only so long as the information truly remains confidential. "We want to make sure that this information is fully protected and is not disclosable based on any circumstances that states may come up with," he said.

One of CMA's concerns, he said, involves freedom of information statutes and whether a freedom of information request might provide an avenue for competitors to gain access to confidential information.

Walton and Wright both downplay the effect that sharing CBI with states will have, but they concede that chemical manufacturers have filed too many CBI requests. Wright says that improper CBI requests are more the result of ignorance than intent. "For instance," Wright said, "some companies didn't know that they could claim one sentence or one word in a document as confidential; they felt they had to claim the whole document." He said that the CMA has responded by holding workshops on how to draft CBI requests "as narrowly as you can."

While the EPA and industry may be making strides in reducing improper CBI claims and expanding the confidential data to state regulators, some observers say these are efforts that would be made unnecessary by a stronger law. Janice Mazurek, a research associate at the Washington, DC-based Resources for the Future, a nonpartisan environmental policy group, assesses TSCA as "a missed opportunity for pollution prevention." Said Mazurek, "If you go back and look at what the original framers intended, TSCA was really supposed to be the statute that collected information about development, production, and use of toxic substances." TSCA's framers recognized the need for CBIs, she continued, "but I think that EPA, for lack of will, really loosely interpreted what did and didn't constitute confidential business information over the years since it was implementing TSCA, and it hasn't been until the last four or five years that EPA has begun to sit down with industry and other stakeholders and make them aware of some of these problems and, for example, challenge egregious CBI claims."

Now that the chances for statutory changes to TSCA are dead—for the moment, at least—the current efforts by both the EPA and industry to reduce CBIs and extend that information to a few states is at least a sensible alternative. "Let's face it," Mazurek said. "The train is obviously heading down the tracks to devolution of tough federal environmental responsibility and a greater role for the states. One of the greatest obstacles we're finding in our research here at [Resources for the Future] is that under most of the statutes there simply isn't enough adequate monitoring data. Certainly TSCA, as the Hampshire study showed, caused EPA to collect the most comprehensive health and safety database available anywhere in the country, and states that can demonstrate that they can provide the same kind of protection of confidential information should certainly have access to that."

**Richard Dahl**

# ICOH

25TH INTERNATIONAL CONGRESS ON OCCUPATIONAL HEALTH 1996

STOCKHOLM SEPT 15–20 1996

**Stockholm, Sweden**
**September 15–20, 1996**

The Congress will be a world-wide forum to share the latest scientific advances within all principal fields of occupational safety and health. The application of these advances in occupational health practice will also be presented. Topics of the congress include the influence on health and well-being of chemical and physical factors, at the work site, as well as the impact of ergonomics, psychosocial factors, work organization and new technology. visitors to earlier ICOH congress will recognize the general structure of ICOH'96.

## Courses

Courses on "Continuous Quality Improvement in Occupational Health Services" and "Risk Assessment of Carcinogens" will be held in Stockholm, Sweden, and Helsinki, Finland, in conjunction with the congress. The courses are being organized by the Nordic Institute for Advanced Training in Occupational Health (NIVA).

## Keynote addresses

Topics to be reviewed in the keynotes include:

- Dose concepts in occupational health.
- Electromagnetic fields and cancer.
- Gender and work.
- Occupational health in a global perspective.
- Participatory approaches in occupational health research.
- Prevention of musculoskeletal disorders.
- Promoting safe behavior.
- Working conditions and cardiovascular disease.

For more information contact:

ICOH Congress
National Institute of Occupational Health
S-171 84 Solna
SWEDEN
FAX: (46) 8 82 05 56

# *A Breath of* Fresh Technology

An innovative technique for measuring and visualizing the concentrations of air pollutants couples infrared optical remote sensing with computer-assisted tomography (CAT) to estimate human exposures more accurately. The method, which draws on technology traditionally reserved for medical CAT scanning, promises to outperform current devices for measuring emissions. Once perfected, the detection system, known as Remote Sensing Computed Tomography (RSCT), could be used to enhance workplace health and safety in industries such as petroleum refineries and synthetic organic chemical manufacturing facilities.

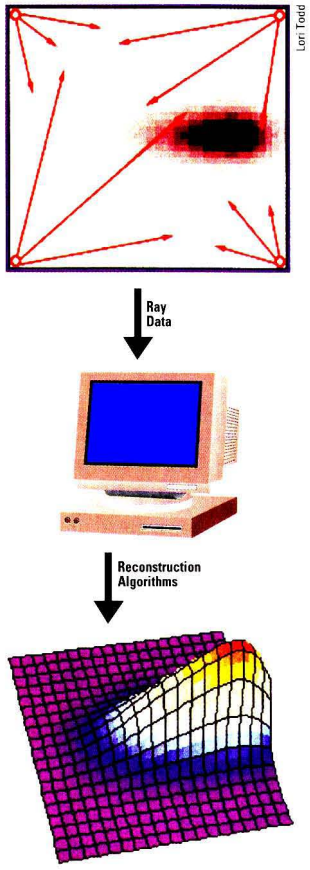
RSCT is the brainchild of Lori Todd, an assistant professor in the Department of Environmental Sciences and Engineering at the University of North Carolina at Chapel Hill. Todd and her research team are refining this technology, which uses multiple beams of infrared light to scan an indoor manufacturing area or outdoor industrial site for airborne pollutants, then creates two-dimensional computerized grids mapping the locations and concentrations of the pollutants.

"I adapted the concept of CAT scanning used in the medical field," says Todd. "Instead of shooting radiation at many angles through a body to view the organs, I am shooting a network of infrared beams through air at many angles to reconstruct [chemical] concentrations." RSCT can simultaneously measure over 100 different chemicals, whereas current detection methods are usually limited to a much smaller number.

## Filling a Gap

As the former deputy chief of environmental toxicology in New York City's Department of Health, Todd was frequently called to inspect sites where workers complained of exposure to toxic chemicals. She found the detection equipment used for such assessments lacking: "I did air sampling and saw a need for something to give more reliable information about what people are exposed to," says Todd, who is a certified industrial hygienist.

The sampling method used in the 1980s is essentially the same in use today: technicians normally use hand-held instruments to pull contaminated air onto sampling media that must be sent to a laboratory where analysis of the pollutants can take more than a week. Time spent analyzing pollutants is one of several drawbacks to this method, according to Todd. In addition, technicians gather data from a limited number of fixed samples over an area (a room in a manufacturing plant where chemicals are processed, for example), which yields poor spatial resolution of the concentrations measured. And, since sample collection is integrated over time, the resulting data give poor temporal resolution of contaminant concentrations as well. "For ambient air, scientists are attempting to infer the nature, motion, and dispersion of pollutants, and validate numerical transport models using only this limited sampling data," Todd says.




**Remote Sensing and Computed Tomography.** Data from infrared scans are fed into a tomographic algorithm to create a two-dimensional map of chemical concentrations.

Another problem is that a limited number of measurements taken on a single day are used to estimate worker exposures. These measurements do not reflect day-to-day variability or account for the flow of contaminants, both of which strongly influence human exposure.

Todd says that she saw a gap to fill: "Nothing else could provide real-time measurements of multiple chemicals over large distances," Todd explains. Undeterred by the skepticism of some of her associates, Todd conducted a feasibility study of her idea while pursuing her doctorate in occupational health and computer science at UNC. Todd's novel ideas have since captured the attention of the petroleum and furniture manufacturing industries, the EPA, and the National Institute for Occupational Safety and Health. In 1994 the National Science Foundation named Todd a Presidential Faculty Fellow, placing her in an elite group of 15 top scientists. The honor, conferred by President Clinton, brought with it a $500,000 grant to UNC-Chapel Hill to further Todd's research.

## Perfecting the Technology

Todd assembled a research team including a biomedical engineer and a full-time programmer to develop the optical remote sensing system. The system scans the air for pollutants, then produces a two-dimensional pollutant concentration map that is "spatially and temporally resolved over large outdoor areas or smaller indoor environments," Todd says. In a significant departure from current sampling technology, the remote sensors gather real-time measurements that provide a path-integrated concentration over an entire beam path, not at a single point.

Most of the team's work to date has been theoretical and experimental—either in computer simulations or in a greenhouselike university laboratory. The greenhouse chamber contains two open-path, broad-band Fourier transform infrared spectrometers (two of only four scanning FTIRs manufactured) on loan to Todd by the U.S. Department of Energy. FTIR pairs a light source and scanning detector with a retroflector placed opposite the area to be sampled. Retroflectors contain mirrors in a cubic design that reflect light directly back to the source. Using retroflectors helps to compensate for atmospheric shifts due to wind or temperature changes that could alter a reading of the pollutants in a given place.

Steve McCaw, Image Associates

**Spotlight on pollutants.** Lori Todd prepares to scan the air in her greenhouse test chamber.

Todd is trying to determine optimal placement of the remote sensing sources and detectors. "You need some kind of symmetry in placing the equipment," she explains. "You don't want to skew the results with equipment placement."

Once measurements have been gathered by the scanner, the data are fed into a tomographic algorithm that, within minutes, reconstructs a gridded, two-dimensional map showing chemical concentrations within the sampled area. Each map provides spatial chemical concentration data, while a series of maps generated from continuously gathered data provide temporal information as well, and could be used to visualize contaminant flow over time. Todd's team is currently trying to determine the minimum number of light beams necessary to construct an accurate map, in an attempt to keep the application cost effective.

When the researchers move their experiments to the field, they will also grapple with the problem of objects such as furnishings or other equipment that may obstruct the paths of light. Another difficulty is air's inherently dynamic qualities: unlike a medical CAT scan that analyzes an unmoving human body, RSCT scans for air toxics present in a constantly moving medium.

## A New Alternative

The hand-held "sniffers" manufacturers and processors currently use to sample air are positioned at every possible seam, joint, valve, pipe flange, and pump to detect fugitive emissions, according to Rima Dishakjian, a chemist in the EPA's Emission Measurement Center in Research Triangle Park, North Carolina. Dishakjian is following Todd's research, which is partially funded by the EPA through a three-year cooperative agreement. "These random leaks in equipment contribute a lot to air pollution," Dishakjian says. A large chemical or petroleum processor may have "hundreds of thousands of potential leaky sources," she says. Thus, a more sophisticated measuring and imaging system may attract a large manufacturer for whom compliance and workplace safety monitoring is an ongoing task, although cost would also be a factor. According to Todd, the smaller RSCT instrument costs about $50,000, while the larger one costs nearly three times that amount.

According to Dishakjian, a couple of instruments set up on the perimeter of a process, for example, would be sufficient to track fugitive emissions in a given area. "You would get a quick response—continuously," she says. "Once the area is mapped, you would be able to pinpoint the source of a leak." Todd's system would also immediately reveal a major system breakdown or catastrophic leak, facilitating more timely worker evacuation.

"Todd's research is interesting; it bears following," says Howard Feldman, research program coordinator for the American Petroleum Institute in Washington, DC. However, he cautions, "the practical applicability remains to be proven."

Feldman agrees that remote sensing coupled with computer tomography would be desirable for refineries if research and testing prove it commercially viable. The API collaborated with Todd's team, the EPA, and several other consultants in a field test conducted earlier this year in Duke Forest, a privately owned reserve in Durham, North Carolina. "We're interested in examining the possibility of using remote sensing for measuring emissions from a volume source, for example, a single stack that [releases] emissions in three dimensions," says Feldman. In particular, he adds, "we were interested in examining how emissions from a volume source dispersed."

While continuing her work in the laboratory, Todd plans to do more field tests—this time in industrial facilities. This is one of the next steps to getting the technology to the marketplace, which, according to the EPA's Dishakjian, may happen in three to four years "assuming success at every stage." Even then, the system wouldn't completely replace current point-source sampling techniques, particularly as a tool for compliance monitoring under Clean Air Act guidelines, because compliance standards are tied to the sampling techniques currently in use. Todd's system would offer an attractive alternative. "First we would have to show equivalency of this technique to the original one," Dishakjian says. "Then, people would have a choice."

"Compliance is a hard field to change," Todd concedes. "It's hard to get new methods approved." Nevertheless, Todd says, an industrial hygienist could use the more sophisticated system to get better information about the type and location of chemicals in the workplace, which would ultimately lead to healthier environments, not to mention healthier workers.

**Jennifer Medlin**

## Suggested Reading


Todd LA, Leith D. Remote sensing and computed tomography in industrial hygeine. Am Ind Hyg Assoc J 51:224–233 (1990).

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Review

# Biokinetics of Nuclear Fuel Compounds and Biological Effects of Nonuniform Radiation

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Environmental releases of insoluble nuclear fuel compounds may occur at nuclear power plants during normal operation, after nuclear power plant accidents, and as a consequence of nuclear weapons testing. For example, the Chernobyl fallout contained extensive amounts of pulverized nuclear fuel composed of uranium and its nonvolatile fission products. The effects of these highly radioactive particles, also called hot particles, on humans are not well known due to lack of reliable data on the extent of the exposure. However, the biokinetics and biological effects of nuclear fuel compounds have been investigated in a number of experimental studies using various cellular systems and laboratory animals. In this article, we review the biokinetic properties and effects of insoluble nuclear fuel compounds, with special reference to $UO_2$, $PuO_2$, and nonvolatile, long-lived ß-emitters Zr, Nb, Ru, and Ce. First, the data on hot particles, including sources, dosimetry, and human exposure are discussed. Second, the biokinetics of insoluble nuclear fuel compounds in the gastrointestinal tract and respiratory tract are reviewed. Finally, short- and long-term biological effects of nonuniform α- and ß-irradiation on the gastrointestinal tract, lungs, and skin are discussed. *Key words*: biokinetics, cancer, hot particles, ionizing radiation, nuclear fission. *Environ Health Perspect* 103:920–934 (1995)

Exposure to radioactive particles made up of radionuclides from fission or activation reactions and/or actinides with activities up to millions of becquerels, also called hot particles, has been identified as an important radiological health problem in the nuclear power industry. For example, the Chernobyl nuclear power plant accident caused extensive global radioactive fallout which exposed millions of people to both volatile and nonvolatile radionuclides. It has been estimated that the fallout exposed the population of Europe to a dose of 0.2 mSv in the first year (*1*); the worldwide, average, annual effective dose from natural sources is estimated to be 2.4 mSv (*2*). However, approximate global dose estimates can never predict health effects of radioactive fallout composed of several radionuclides with different physicochemical properties. The Chernobyl fallout had a special feature, different from the earlier observed nuclear reactor accidents: it contained extensive amounts of pulverized nuclear fuel from the reactor which first exploded and then burned for several days. The nuclear fuel particles were composed of uranium and its nonvolatile fission products, including the ß-emitters zirconium-95 ($^{95}$Zr), niobium-95 ($^{95}$Nb), ruthenium-103 ($^{103}$Ru), $^{106}$Ru, cerium-141 ($^{141}$Ce), and $^{144}$Ce (*3–8*).

Accidental exposure to nuclear fuel particles can occur via inhalation, ingestion, or via direct skin contamination. Due to the poor water-solubility of actinide particles, such as uranium oxide ($UO_2$) and plutonium oxide ($PuO_2$) particles, and consequently a low intake of the particles in the body, the most important organs or tissues from the radiobiological point of view are the lungs, the gastrointestinal tract, and skin. Knowledge about the biokinetic properties of nuclear fuel particles, in addition to the knowledge about the biological effects of these unique radiation sources, is essential in risk assessment of the health effects of the fuel particles.

Exposure to insoluble radioactive particles often leads to an exposure situation where only a minor fraction of the target tissue or organ is irradiated due to a short range of α- and ß-irradiation (from a few microns to a few millimeters). The biological effects of nonuniform radiation exposure compared to uniform radiation exposure have been investigated in a number of theoretical and experimental studies for nearly three decades. The so-called "hot particle hypothesis" is based on the assumption that a nonuniformly distributed radiation dose is more carcinogenic than an equal radiation dose delivered uniformly to the same organ or tissue (*9,10*). The hypothesis was originally based on the effects of α-radiation. This hypothesis has repeatedly been refuted by many theoretical and experimental studies (*11–15*). As a consequence of the Chernobyl power plant accident, several theoretical and experimental studies coordinated by the International Atomic Energy Agency were initiated to study possible health hazards associated with the exposure to ß-emitting hot particles (*16*). Recent experimental studies indicate that conventional dose risk assessment models may underestimate the biological significance of ß-emitting hot particles (*17–21*).

The primary aim of this overview is to describe the biokinetic properties of insoluble nuclear fuel compounds and the effects of nonuniform radiation exposure. First, we summarize data on hot particles, including sources, dosimetry, and human exposure. Second, data on the absorption, distribution and elimination of insoluble actinide particles in the gastrointestinal tract and respiratory tract, with special reference to insoluble $UO_2$ and $PuO_2$ particles, and nonvolatile, long-lived ß-emitters Zr, Nb, Ru, and Ce are presented. Finally, we discuss short- and long-term biological effects of nonuniform α- and ß-irradiation on the gastrointestinal tract, lungs, and skin.

## Hot Particles

### Sources

A hot particle is defined [applied to skin according to the National Council on Radiation Protection (*14*)] as a discrete radioactive fragment with a high specific activity (up to millions of Becquerels), insoluble in water, and not larger than approximately 1 mm in any dimension. Hot particles emit α-radiation, ß-radiation, or ß/γ–radiation. Natural uranium contains 0.7% of the fissionable uranium isotope $^{235}$U (*22*). This natural isotope of uranium is is currently the primary fuel used in nuclear power plants. Other fissionable nuclear fuels are $^{239}$Pu and $^{233}$U.

Both pressurized water reactors and boiling water reactors can cause hot particle contamination in the nuclear power plant environment (*23*). The particles usually contain activation products, such as $^{60}$Co, or fission products of uranium. Activation product particles originate mainly from the high-cobalt stellite used in valve seats. Fission product particles are mainly released after failures in the nuclear fuel processes. The radioactivity of the $^{60}$Co particles ranges from 40 Bq to 20 MBq with size range between a few microns and several millimeters. The radioactivity of fission

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products containing particles ranges from 40 Bq to 400 kBq with the same size range (*24*). In the United States, after examination of 61 nuclear power stations, 44 high-activity hot particles were found (*25*). The particles were both activation (metallic particles) containing mainly $^{60}Co$ with a size range from several microns to several millimeters, activities from 37 Bq to 37 MBq, and fission and fuel activation products with sizes and activities nearly of the same order. Mandjukov et al. (*26*) also found high-activity hot particles (85% activation, 15% fuel fragments) in the Kozloduy nuclear power plant in Bulgaria in 1992.

Hot particles containing α-emitters have been observed in the effluent from nuclear fuel reprocessing plants. For example, the effluent from the Sellafield reprocessing plant in the UK (*27*) has been found to contain small quantities of the α-emitting transuranium elements neptunium (Np), Pu, americium (Am), and curium (Cm) (*28*). Similar particles have been found in environmental samples taken near the plant.

The most important anthropogenic source for environmental radiation is nuclear weapons testing in the atmosphere (*27*). Carbon-14 is the main contributor to the dose, i.e., 2.6 mSv to the population in the north temperate zone, whereas the dose from fission products and plutonium is only 0.6 mSv (*27*). ß-emitting hot particles isolated in the fallout of nuclear weapon tests had a mean geometric diameter of about 4 μm and a total activity up to 100 Bq. The main part of the particles contained the following components: $^{95}Zr/^{95}Nb$, molybdenum-99 ($^{99}Mo$), $^{131}I$, tellurium-132 ($^{132}Te$)/$^{132}I$), barium-140 ($^{140}Ba$)/lanthanum ($^{140}La$), $^{141}Ce$, and neodymium ($^{147}Nd$) (*29*).

Nuclear fallout from nuclear reactor accidents can contain both volatile, such as $^{137}Cs$ and $^{131}I$, and nonvolatile fission products of uranium. The Chernobyl fallout in 1986 contained all nonvolatile fission products, including the ß-emitters $^{95}Zr$, $^{95}Nb$, $^{103}Ru$, $^{106}Ru$, $^{141}Ce$, and $^{144}Ce$ attached to uranium matrix (Table 1) (*3–6*). The total release was estimated to 290 PBq. About 50% of the releases were cesium isotopes, and 43% were nonvolatile fission products, whereas only 1.8% of the fallout was composed of plutonium isotopes (*27*). Hot particles in the Chernobyl fallout had a geometric diameter ranging from a few microns (Finland) to about 100 μm (Kiev). The total activity of the particles varied between about 30 Bq (average activity 130 Bq in Finland) (*5,6*) to over 1000 kBq (Poland) (*8*). Hot particles were also observed in the fallout from a nuclear incident in 1992 at the power plant in Sosnovyi Bor, Russia (*30*). Gases and a small amount of cerium and zirconium isotopes containing nuclear fuel particles were identified in the surface air in Finland.

## Dosimetry

An α-active hot particle emits radiation which only penetrates up to 100 μm in a biological tissue. For example, the absorbed dose rate in a simulated tissue-equivalent object at the distance of 10 μm from a 1 Bq $^{241}Am$ source (average α-energy, 5.4 MeV) is about 70 Gy/hr, whereas at the distance of 40 μm, it decreases to about 2 Gy/hr (*31*). The radiation dose in the immediate vicinity of the α-active particle is very high and would be expected to kill most of the cells exposed to the radiation.

The range of a ß-radiation in a biological tissue can be in the order of a few millimeters depending on the ß-energy. A ß-emitting hot particle creates a dose gradient around it, causing cell death near the particle. The cells around the lethal zone obtain a sublethal dose, which does not kill the cells but is large enough to cause a random, malignant transformation (*17–21*). In Figure 1, a dosimetric model for a hot particle containing 1000 Bq of $^{106}Ru$ or $^{144}Ce$ is shown. For example, at the distance of 1 mm from the particle, the radiation dose rate is 0.01 Gy/hr, and at the distance of 0.2 mm, the dose rate is about 1 Gy/hr (*5*). Pöllänen and Toivonen (*32*) have calculated skin doses from large uranium fuel particles. The nuclide composition of the particles was estimated from the inventory of the Chernobyl reactor. For example, a uranium nuclear fuel particle of size 40 μm, deposited on the skin, can cause a dose of 1.6 Gy/cm$^2$. Hofmann et al. (*33*) calculated radiation doses and lung cancer risk for a hot particle composed entirely of $^{103}Ru$. The authors concluded that in the immediate vicinity of a particle the doses are so high that all cells are killed and no tumors will arise. At intermediate distances, the probability for lung cancer induction exhibits a distinct maximum. According to the ICRP (*34*), inhalation of a 300 Bq (geometric diameter 1 μm) $^{103}Ru$ hot particle results in an average lung dose of $3.2 \times 10^{-2}$ mGy/year. Burkart (*35*) concluded that the limited knowledge on the biological response of affected tissue and the limited experimental and epidemiological database for ß-active hot particles requires additional information to elucidate the main characteristics of lung irradiation by hot particles.

The hot particle hypothesis was proposed by Geesaman (*9*) and Tamplin and Cochran (*10*). It implied that highly nonuniform radiation, for example on the skin or lungs, might be five orders of magnitude more harmful than uniform radiation. Most of the theoretical (*12,14,15*) and experimental studies (see Table 3) have refuted the hot particle hypothesis. However, we have observed that hot particle-induced carcinogenesis is associated with specific biological mechanisms that cannot be explained by mathematical modeling (*17–21*).

**Table 1.** Physical data of nonvolatile fission products in an average hot particle (activity 130 Bq, aerodynamic size 10 μm) found following the Chernobyl fallout in Finland (*6*)

| Radionuclide | Physical $t_{1/2}$ (days) | Activity (Bq) | Average ß-energy (MeV) |
|---|---|---|---|
| Cerium-144 | 285 | 21.5 | 1.208[a] |
| Cerium-141 | 32 | 26.7 | 0.144 |
| Ruthenium-103 | 39 | 23.2 | 0.062 |
| Ruthenium-106 | 368 | 5.42 | 1.415[b] |
| Zirconium-95 | 64 | 27.5 | 0.115 |
| Niobium-95 | 35 | 25.8 | 0.046 |

[a]Daughter nuclide $^{144}Pr$.
[b]Daughter nuclide $^{106}Rh$.

## Human Exposure

***Occupational exposure.*** Within nuclear power plants, hot particles may be attached to the workers due to electrical charges inherent in the plastic protective clothing worn by nuclear power plant workers (*23*). A high-activity hot particle can cause considerable local skin doses to exposed employees (*25*). Hot particles can also deposit on conjunctival tissue of workers, such as in the eye (*36*). Fuel cladding failure events may increase radiation exposure rates by an estimated 540% in some areas of the nuclear power plant during routine operations (*37*).

McInroy et al. (*38*) have reported a whole-body distribution of $^{239}Pu$ in an occupationally exposed worker. The worker was involved in operations with plutonium exposure from 1945 to 1982, approximately 10.5 months before his death. At the time of death the body contained 246 Bq of $^{239}Pu$, of which 52.8% was found in the lungs and associated lymph nodes. The remaining 47.2% was mostly in the skeleton (44%), the liver (42%), with the remainder (14%) in the rest of the body. Studies on the 42-year follow-up of 26 Manhattan Project plutonium workers estimated that the plutonium depositions, including lung burdens, range from 52 to 3180 Bq with a median value of 500 Bq (*39*).

Health effects of α-active hot $^{239}Pu$ particles have been investigated in a few epidemiologic studies (*39,41–44*). The exposed populations have either been workers in nuclear weapon facilities or workers

accidentally exposed. The results have not shown any excess mortality or cancer incidence among the exposed persons.

***Chernobyl fallout.*** Particulate fallout from Chernobyl extended over 1000 km from the accident site (*3–5*), exposing millions of people. In the vicinity of the Chernobyl nuclear power plant, a large number of people were exposed to both external γ-irradiation and highly penetrating ß-irradiation from pulverized nuclear fuel, which was mainly deposited on the skin. The total number of the victims was 237; 140 persons were exposed to between 1 and 2 Gy, and 97 to between 2 and 8 Gy whole-body doses (*45*). All patients with total body doses higher than 4 Gy had skin burns. The ß-emitters were deposited on the skin and clothes of the victims and caused severe burns (*45,46*) that often led to their deaths.

In more distant areas from the accident site exposure to hot particles occurred mainly via ingestion or inhalation. For example, the plutonium body contents of Gomel citizens in Russia, 4–5 years after the Chernobyl accident, were on average 3–4 times higher than the global levels (*40*). In Poland, the estimated annual intake of plutonium in the diet was 774 mBq/year in the first year after the Chernobyl accident and decreased to approximately 90 mBq/year in the sixth year (*47*). Lung doses in farmers living in the 30-km isolated zone around the power plant were estimated to be of the order of several milliSieverts during the first year after the accident (*48*). The number of hot particle-induced lung cancer cases for the Bulgarian population was estimated as four to six, or approximately 10% of the total number of lung cancer cases which are expected from external and internal irradiation after the Chernobyl accident (*49*). Balashazy et al. (*7*) estimated the lung cancer risk due to inhalation of Chernobyl-released hot particles to be less than $10^{-10}$ for individuals living in Budapest. In Finland, the epidemiological studies have focused on the fallout and incidence of childhood leukemia (*50*). However, human exposure to the Chernobyl hot particles has not been verified experimentally. In Finland as a whole, no increase in the incidence of childhood leukemia was observed from 1976 to 1992. However, some indication of an increase in the incidence of the disease was observed in the area with the highest exposure based on one or two extra cases per year.

## Biokinetics of Nuclear Fuel Compounds in the Gastrointestinal Tract

Radionuclides can be ingested either by direct intake of contaminated food or water or by swallowing inhaled material that has been cleared from the respiratory tract. Radionuclide absorption can occur from all segments of the gastrointestinal (GI) tract. The small intestine is generally the primary site of systemic uptake due to the large surface area of villus. Water-insoluble radionuclides that are not absorbed during GI transport or those that are first absorbed then subsequently secreted into the GI lumen and not reabsorbed are primarily excreted in feces. The excretion rate may vary greatly even within the same animal population because of unusual evacuation habits or unexplained physiological differences, for example (*51*).

Behavior of particulate material in the GI tract is affected by many factors. For example, large insoluble particles appear to pass through the tract more slowly than much smaller particles ingested in solution (*51*). Solid particles may be absorbed via phagocytosis through Peyer's patches into lymph (*52*). Peyer's patches are lymphoid, follicular aggregates on the intestinal mucosa. Another mechanism of particle uptake is persorption (*53*), which means that as epithelial cells are sloughed off at the tip of the villus, a gap in the membrane is temporarily created, allowing entry of materials that are not membrane permeable. There is evidence for and against the persorption hypothesis (*54*). A third mechanism for particle transport is epithelial membrane damage. Absorption of particles can be enhanced after exposure to certain chemicals (*52*).

### Actinides

Human data show that the actinide elements thorium (Th), Np, Pu, Am, and Cm

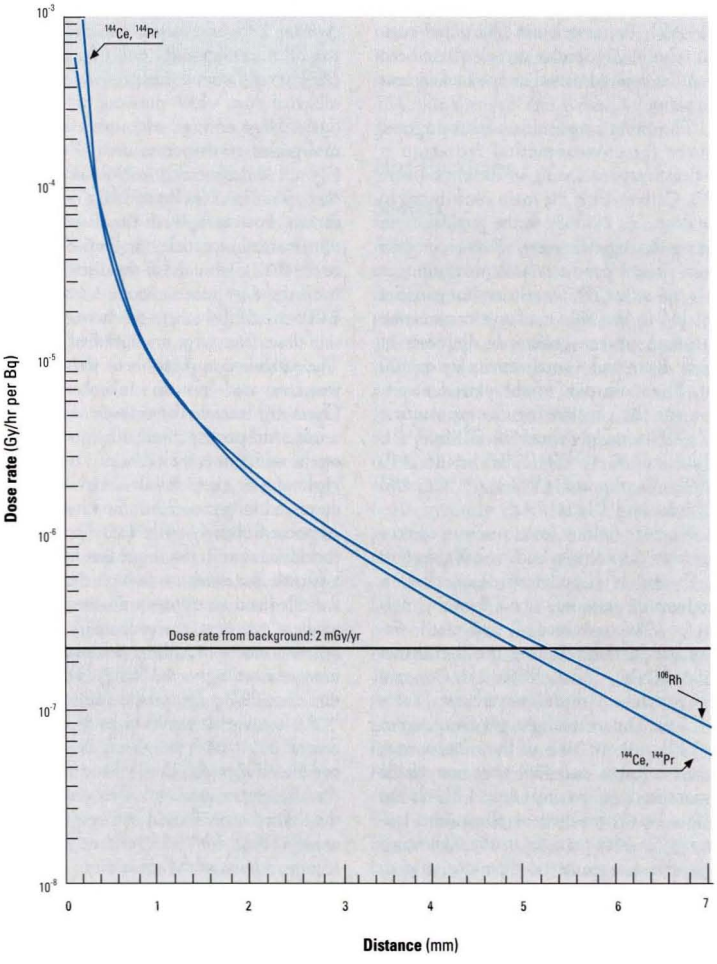


**Figure 1.** Radiation dose rate from a hot particle containing 1000 Bq of ruthenium-106 or cerium-144 and daughter nuclides rhodium-106 and praseodymium-144.

are absorbed poorly from the GI tract (*55*). The values for their fractional absorption are $2 \times 10^{-4}$ ($10^{-4}$ for Am). The gastrointestinal absorption of uranium is greater than that seen with the other actinides. This is consistent with its different solution chemistry. Uranium also behaves differently within the body, with a low intake in tissues other than bone. Uranium is primarily excreted via urine with associated retention in the kidneys (*56*). The average human GI absorption of U is most likely 1–2% and is probably independent of age or the mass of U ingested (*57*). Chemical and biological data for U and Pu are shown in Table 2.

In animal experiments, factors affecting the GI absorption of actinides, and particularly Pu, have been studied extensively. Values of fractional absorption in newborn animals, depending on the chemical form of the compound, vary between $10^{-2}$ and $3 \times 10^{-5}$ (*55*). Values for adult animals are somewhat lower: between $10^{-3}$ and $3 \times 10^{-5}$. Oxides of Pu have very low absorption values, typically $<10^{-5}$. Fritsch et al. (*58*) have shown that absorption in neonatal rats is limited mainly to the first hours after ingestion, whereas in guinea pigs and primates, ingested Pu was retained in macrophages located beneath the intestinal epithelial cells. In neonatal primates, increased absorption appears to be due to uptake in distal epithelial cells. Higher values of absorption of many elements have been observed in fasted animals. Absorption of plutonium can be increased by an order of magnitude in fasted animals (*59*). This is presumably due to decreases in concentration of binding ligands which normally act as direct competitors for absorption.

Fractional absorption of U in hamsters, rabbits, dogs, and baboons is in the range of $3 \times 10^{-3}$ to $2 \times 10^{-2}$, i.e., somewhat lower than for humans (*57*). Absorption values for rats are usually lower; values as low as $4 \times 10^{-4}$ have been reported. The normal U content of a reference man is about 38 μg, of which 66% is found in the skeleton (*57*). The natural U concentration in the human kidney (0.3% of the total body burden) appears to be about twice that in the liver. When U is injected subcutaneously or intravenously, accumulation primarily occurs in the kidneys or bones (*56*). Uranium competes with calcium for sites of deposition. Neutron-induced autoradiographs of retained U in human bones show that under equilibrium conditions it is diffusively distributed throughout the bone volume (*57*). Uranium dioxide, $UO_2$, with a melting point of 2078 ± 20°C, is water-insoluble but soluble in $HNO_3$ and concentrated $H_2SO_4$ (*60*). The rate of dissolution of $UO_2$ in acids depends on the particle size, acid concentration, and temperature (*61*). Hodge and co-workers [see Yuile (*56*)] conducted 1- and 2-year feeding experiments in rats and observed that $UO_2$ was not absorbed by the GI tract. The chemical analyses showed no increase in the U content in the kidneys or bone.

## Fission Products

Zr, Nb, and Ru belong to the transition metals and Ce to the inner transition metals (also called lanthanides or rare earths). These metals are water-insoluble in their dioxide form (*60*). Several experiments have been performed to study GI absorption of these uranium fission products in laboratory animals (*34,62*). The chemical and biological characteristics of these nuclides are shown in Table 2. The generally accepted values of fractional absorption for Zr, Nb, and Ce are less than $10^{-4}$ and $3 \times 10^{-2}$ for Ru. The values are based on the studies with single nuclides in ion salts (e.g., chlorides, oxalates, and nitrates) (*34*). GI absorption of $^{95}$Nb oxalate in the mouse, rat, and monkey is approximately 1% (*63*). In young rats, the whole body retention of $^{141}$Ce nitrate is less than 0.04%, and absorption of various salts of $^{95}$Zr/$^{95}$Nb is approximately 0.8% (*64*). In miniature swine, a whole-body retention of $^{144}$Ce chloride of less than 0.01% has been reported (*65*). GI absorption of $^{95}$Nb citrate in guinea pigs has been estimated as 0.8% (*66*).

A few studies have shown that whole-body retention of $^{95}$Zr, $^{95}$Nb, $^{141}$Ce, and $^{144}$Ce is significantly higher in suckling than in adult rats (*67–69*). The absorption can be 10–100 times higher in neonates. This relative increase in absorption is probably due to retention of the radionuclides in the intestine where pinocytosis is more active in younger animals. For example, insoluble radionuclides such as cerium and niobium are readily taken up by the intestinal cells in suckling rats and are apparently immobilized in these cells and are cleared from the body only when the cells have migrated up to the villi and have been sloughed into the intestinal lumen (*68*). Increased absorption of ingested radionuclides in neonates is a general phenomenon (*66*) that is observed immediately after birth, followed generally by a progressive reduction over the suckling period (*70*). The absorption appears to be nonspecific due to a greater permeability of the immature intestine for different elements and range of molecular species. This phenomenon may be related to the specific uptake of immunoglobulins from milk, which continues to a reduced extent after "closure" of the intestine to the transfer of these molecules (*71*). Intestinal retention of $^{95}$Nb citrate in newborn guinea pigs is low, unlike retention in other species (*66*), but consistent with observations of the retention of actinide elements in this species (*71*). In the guinea pig, as in humans, this "closure" occurs either before or shortly after birth; maternal immunoglobulins are transferred cross-placentally to the fetus, and the mechanism of intestinal uptake from milk does not operate (*72*). Fasting results in a 75% increase in niobium absorption in guinea pigs (fasting 24 hr and 2 hr after the administration) (*66*).

Distribution studies with ion salts of $^{95}$Zr, $^{95}$Nb, $^{103}$Ru, and $^{144}$Ce in different animals species have shown that these radionuclides are primarily deposited in the bone both after oral and intravenous administration (*63,69,73*). Some uptake also occurs in the liver, kidney, spleen, and testis. Ruthenium, however, does not accumulate as strongly in bone as other radionuclides (*73*).

The fission products have entirely different biokinetic properties when administered in particulate form. Lang and Raunemaa (*74*) studied the behavior of simulated nuclear fuel particles in the GI tract of the rat. Whole-body autoradiography and γ-spectrometric tissue analyses showed no absorption of $^{141}$Ce, $^{144}$Ce, $^{103}$Ru, $^{95}$Zr, or $^{95}$Nb from the GI tract. None of the radionuclides could be detected in the liver, kidney, muscle, bone, brain, blood, or urine. Approximately 98% of the total radioactivity was excreted in feces within 3 days post-exposure. Intestinal

**Table 2.** Chemical and biological data of uranium, plutonium, and nonvolatile uranium fission products (*60,63*)

| Element | Solubility in dioxide form | Fractional absorption by ingestion | Fractional absorption by inhalation | Biological $t_{1/2}$ (days), total body |
|---|---|---|---|---|
| Uranium | Insoluble in $H_2O$;soluble in $HNO_3$, conc. $H_2SO_4$ | $<10^{-4}$ | 0.25 | 100 |
| Plutonium | Insoluble in $H_2O$; soluble in conc. $H_2SO_4$, HN03, HF | $3 \times 10^{-5}$ | 0.25 | $6.5 \times 10^4$ |
| Cerium | Insoluble in $H_2O$; soluble in $H_2SO_4$, $HNO_3$ | $<10^{-4}$ | 0.25 | 563 |
| Ruthenium | Insoluble in $H_2O$, alkali; soluble in fused alkali | 0.03 | 0.27 | 7.3 |
| Zirconium | Insoluble in $H_2O$; soluble in $H_2SO_4$, HF | $<10^{-4}$ | 0.25 | 450 |
| Niobium | Insoluble in $H_2O$, alkali; slightly soluble in alkali | $<10^{-4}$ | 0.25 | 760 |

retention for $^{141}Ce$, $^{144}Ce$, $^{103}Ru$, and $^{95}Zr$ at 1 day after administration was between 2 and 3%; for $^{95}Nb$ retention it was about 6%. These results indicated that $UO_2$ fission products must first be released from the matrix of particles prior to absorption of nuclides across the intestinal mucosa. This is also supported by the observations of Mirell and Blahd (*75*) who performed whole-body measurements of an American tour group who were exposed in Kiev to the initial Chernobyl reactor accident plume. They found that ingestion of particulate fission products ($^{141}Ce$, $^{144}Ce$, $^{131}I$, $^{103}Ru$, $^{137}Cs$, $^{95}Zr$/Nb) appeared to result in a relatively short element-independent retention. Consequently, fission products in the fused particulate form renders them virtually inert in metabolic terms and the radionuclides are not metabolized along biological pathways characteristic for the elementary form (*34*). Ingestion of nuclear fuel particles does not lead to significant extraction of fission products from particles and their deposition into tissues where they may be retained for long periods of time.

## Effects of Nuclear Fuel Compounds on the Gastrointestinal Tract

The toxicity of poorly absorbed, ingested radionuclides depends on the energy of the nuclide, the mass of the intestinal contents, and how long the nuclide remains in the gastrointestinal tract. The high rate of cell proliferation in the small intestine makes this tissue especially sensitive to ionizing radiation (*76,77*). The most radiosensitive cells in the intestine are the crypt cells. In humans, the crypt cells of the duodenum may lie 1.5 cm beneath the surface, whereas in the rat, the distance is approximately 0.02 cm. Consequently, the biological effects of poorly absorbed radionuclides depend on the type of radiation (i.e., the differences in ranges) the nuclides emit.

Sullivan et al. (*78*) exposed rats to the β-emitter yttrium-91 ($^{91}Y$) as the $YCl_3$ at 925 MBq/kg (dose to large intestine about 30 Gy) and to a suspension of $^{239}PuO_2$ (α-dose to the surface of the small intestine about 1000 Gy). Exposure to $^{91}Y$ caused severe damage to the cecum and colon, such as disruption of the crypt pattern, cystic dilation of the crypts, irregularity of the surface epithelium, cytoplasmic vacuolization, and edema and hyperemia of the submucosa. Plutonium α-particles induced only mild and superficial lesions confined to the cecum and colon. The authors concluded that ingested α-emitters did not induce acute toxicity, in contrast to ß-emitters, because of the poor penetration of α-irradiation to the radiosensitive crypt cells (*78*). In contrast, in neonatal rats, acute intestinal lesions leading to death were observed after gavage of $^{238}Pu$ (IV)-citrate (122 kBq per animal) (*79*). The neonatal rats might be more sensitive to α-irradiation because they have immature and poorly invaginated crypts compared to other mammalian species (*80*).

Studies with insoluble $^{238}PuO_2$ (*81*) and simulated nuclear fuel particles [depleted uranium in graphite matrix and strontium-89 chloride (*51*)] have shown that prolonged retention of particles in the intestine produces localized pathological lesions. In the pig intestine, $^{238}PuO_2$ particles caused villar-tip necrosis and inflammation in a 1-cm region surrounding the particles (*81*). In the rat, simulated nuclear fuel particles caused partial denudation of the epithelium and/or ulceration, and less severe damage with changes in the architectural structure in the villi and marked cellular atypia in the epithelial cells (*51*). Sikov et al. (*51*) concluded that the same amount of radiation administered uniformly in solution would not have caused serious damage. The potential for damage from an insoluble radioactive particle depends on the length of time the particle remains at a given point in its progress through the GI tract. Sullivan et al. (*76*) exposed suckling, weanling, and adult rats by gavage and adult beagle dogs by ingestion to high-energy (1.4 MeV average) $^{106}Ru$-rhodium-106 ($^{106}Rh$) chloride solution. The $LD_{50}$ values for suckling, weanling, and adult rats were 55,000, 666,000 and 333,000 kBq/kg, respectively. The newborn rats were most sensitive because of the absorption of the radionuclide into the mucosa of the lower small intestine where it can destroy that segment. In the same study the low-energy ß-emitter promethium ($^{147}Pm$) caused death in rats by damaging the large bowel. The $LD_{50}$ for $^{106}Ru$-$^{106}Rh$ in dogs was about 129,500 kBq/kg. The signs of intestinal injury, duration of injury, and the probabilities of tissue repair were much different in the dog than in the rat (*76*). The midcolon and lower colon of dogs were usually denuded at focal sites rather than in widespread areas.

## Biokinetics of Nuclear Fuel Compounds in the Respiratory Tract

Inhalation is considered the most likely route of accidental intake of radionuclides (*82*). Inhalation of particles can lead to deposition in the nasopharyngeal, tracheobronchial, and pulmonary regions of the respiratory tract. The extent of particle deposition is a function of particle size, shape, and density of the aerosol, lung structure, and respiratory characteristics such as breathing rate, tidal volume, and expiratory reserve volume (*62*). It is also well documented that anatomic and physiological differences exist among experimental animals that may influence deposition patterns (*83*). For example, the simulation models for inhaled materials project an eightfold difference among rats, guinea pigs, dogs, and nonhuman primates in the lung concentration of particles per gram of lung after a 2-year chronic inhalation exposure to the same aerosol for 8 hr/day, 5 days/week (*84*). The largest lung accumulation would occur in guinea pigs, the smallest in rats. Deposited particles are taken up by endocytosis, either by phagocytosis or pinocytosis, during the first few hours after deposition. Particles are rapidly phagocytized by pulmonary alveolar macrophages or other phagocytic cells. Some particles may directly enter the alveolar interstitium by pinocytosis (*84*). There are also indications that the particles in the lungs are redistributed or aggregated due to macrophage migration and grouping (*85*), which may change the radiation dose or injury pattern. Water-insoluble radionuclides are absorbed in accordance with their dissolution rate, partition coefficient, and residence time in the respiratory tract (*62*).

The dominant routes for physical translocation of particles from the pulmonary region are the mucociliary escalator or the lung-associated lymph system, presumably phagocytosis. In addition to chemical dissolution of particles, physical forces may also influence the dissolution rate indirectly by altering the form of the particles and the surface area available for dissolution. Very small particles may also be translocated directly into the circulatory system (*84*). In addition, translocation of particles into the interstitium appears to be a function of the number of particles; i.e., the delivered dose and dose rate (*86*). Exhalation is a major elimination pathway for undeposited particles and gases.

### Actinides

Oxides and hydroxides of actinides are water-insoluble compounds that have maximal retention half-times in the lungs of over 100 days (*34*). It has been shown that in the rat, inhaled particulate compounds of actinides become stored in phagolysosomes of alveolar macrophages (*87*). Rat alveolar macrophages possess the ability to phagocytize $UO_2$ particles despite the high toxicity the metal exerts on cell membranes (*88*). Soluble actinides, such as $^{241}Am(OH)_3$, are solubilized within lysosomes, bind to cytoso-

lic ferritin, and released from macrophages. The compounds can cross the alveolar membranes as transferrin or as low molecular weight forms. Insoluble $UO_2$ and $PuO_2$ particles remain within the lysosomes of alveolar macrophages and may damage the lysosomal membranes. The particles can thus also be observed free in the cytoplasm (*87*).

Leach et al. (*89,90*) exposed dogs, monkeys, and rats in a 5-year inhalation study to a natural uranium dioxide ($UO_2$) aerosol of approximately 1 µm mass median diameter at a mean concentration of 5 mg/m$^3$. Biological half-times in the dog lung and tracheobronchial lymph nodes were 20 and 26 months, respectively. The analogous values for monkeys were 18 and 65 months, and for rats, 10 and 22 months respectively. Similar high lung-retention values in these animal species have been reported for mixed $UO_2$ and $PuO_2$ particles (*91,92*). Monkeys and rats clear plutonium and americium (in mixed oxide particles) from their lungs faster than dogs. The authors concluded that errors could result from using data from a single animal species to estimate inhalation risk to humans (*89,90*). LaBauve et al. (*93*) reported that in the rhesus monkey, the inhaled $^{239}PuO_2$ was retained in the body with an average effective half-life of 1000 days with some translocation to the pulmonary lymph nodes. Human data suggest that the biological half-time of $UO_2$ in the lung is between 500 and 1500 days (*94*).

Intratracheal instillation produces a much more nonuniform distribution of particles in the lungs than that seen after inhalation exposure (*95–97*). However, some aggregation of $UO_2$ particles seems to occur in the rat lung even after inhalation due to macrophage phagocytosis, probably as early as within 24 hr after the exposure (*85*). Similar observations have been made for $PuO_2$ particles in different animal species (*98*). Formation of large $^{239}PuO_2$ aggregates (>25 particles) may produce a mean dose rate as high as 120 Gy/day to the focal alveolar regions (*99*). The aggregation of actinide particles such as uranium dioxide is probably due to both the physical characteristics of the particles and active macrophage transport.

Relatively insoluble actinide particles which are retained in the lungs for long periods of time are slowly cleared mechanically by mucociliary action, swallowing, and excretion via the GI tract. Particles in the lower airways can also be transported to the tracheobronchial lymph nodes, or blood and further to various tissues. Studies on dogs have shown that the lung and lymph nodes associated with lymphatic drainage of the respiratory tract are the principal sites of α–irradiation from inhaled $^{239}PuO_2$ (*100*). There is also evidence that various types of particles are not completely cleared from the large airways (*101,102*). For example, up to 0.7% of $UO_2$ particles injected in the rat trachea can be found in the trachea 14 days after exposure (*101*). The mechanism of retention in the tracheal wall is not clear, but probably consists of phagocytosis by epithelial cells.

Current studies reveal that the clearance half-time from the rat lung is 247 days for $UO_2$ particles with an aerodynamic diameter of 2.7–3.2 µm after a single exposure (*103*). Mixed $(U,Pu)O_2$ particles appear to have a shorter biological half-time in the rat lung compared to $UO_2$ particles but similar to $PuO_2$ particles (*91,92,104,105*) ranging from 60 to 100 days (*87,102*). The retention of these mixed oxide aerosols is not significantly influenced by particle shape or exposure method. In the baboon, lung retention of pure $PuO_2$ and mixed $(U,Pu)O_2$ was 56–80% of the initial pulmonary burden 1 year after a single inhalation exposure (*92*).

Dissolution of insoluble $UO_2$ and $PuO_2$ particles, which has been demonstrated both *in vitro* and *in vivo* (*106*), appears to be the dominant mechanical process in lung clearance. Uranium is dissolved more readily than Pu or Am in mixed $UO_2$ and $PuO_2$ particles, generally reflecting the physical nature of the $UO_2$–$PuO_2$ matrix. Fragmentation of $^{238}PuO_2$ particles has a significant influence on the lung clearance of particles (*105*). This phenomenon probably reflects the increased surface area of particles which would increase their solubilization rate and direct transfer of nanometer-sized particles from the lungs to various tissues. Observations with $PuO_2$ (*107*) and $CmO_2$ (*108*) have shown that small particles, approximately 1 nm in diameter, are translocated intact from lungs to blood and urine. Cooper et al. (*109*) have shown that $^{233}UO_2$ particles <4 nm in diameter translocate from lungs to blood at the same rapid rate as $^{233}UO_2(NO_3)_2$. The authors suggested that small particles of $UO_2$ are oxidized during the inhalation procedure to $UO_3$, which reacts with salt solutions in the lungs and forms the uranyl ion (*109*). The uranyl ions bind to the major pulmonary surfactant phophatidylcholine and are translocated to blood. In plasma, approximately 50% of the $^{233}U$ is bound to transferrin, 25% to citrate, and 25% to bicarbonate.

Insoluble $UO_2$ particles retained in the lower airways are translocated poorly to other organs. Morris et al. (*103*) found 82% of the total body burden of enriched $UO_2$ in the rat lung at 720 days after exposure. Ten percent of the activity was in the thoracic lymph nodes, 3.9% in bone, 3.2% in soft tissue, and 0.8% in the kidney. The values for other organs and tissues were below 0.1%. Similar observations have been made with mixed $(U,Pu)O_2$ particles (*87*). Only 1% of the initial alveolar deposit was found in the rat liver, spleen, or kidneys throughout the 200-day observation period. Lataillade et al. (*92*) reported that translocation of mixed $UO_2$ and $PuO_2$ from rat and monkey lung after a single inhalation exposure was less than 3% of the initial pulmonary burden. The translocation of Pu from the mixed oxide to the skeleton and liver was greater than that from the industrial $PuO_2$. In mice, only about 0.5% of the inhaled $^{239}PuO_2$ is translocated to other organs (*110*).

## Fission Products

The behavior of uranium nonvolatile fission products in the lungs has not been studied as extensively as their behavior in the GI tract. Chemical and biological characteristics of the nuclides are shown in Table 2. The mean biological half-time of $ZrO_2$ in the human lung has been estimated to be 224 days, and in the beagle dog the calculated value is 301 days (*111*). Thomas et al. (*112*) exposed mice to $^{95}Zr$ and $^{95}Nb$ in oxalic acid. The particles were generated at four different temperatures ranging from 100°C to 1100°C. Over 90% of the whole-body $^{95}Zr$ and $^{95}Nb$ was retained in the lungs at the higher temperatures (600° and 1100°C) and was cleared with a half-time of about 39 days. About 2–3% of sacrifice body burden was found in the bone. At the lower temperatures (100° and 250°C) the retention half-times were 61–62 days, and approximately 80% of the total body burden was found in the skeleton and 3% in the liver 120 days after inhalation. Different metabolism of the radionuclides was sometimes observed depending on the temperature of formation. Zirconium was translocated more readily to the bone than niobium. Inhaled $^{144}CeO_2$ particles are also translocated poorly from the lungs. Lundgren et al. (*113*) observed that, in the rat, 91% of the whole-body activity was found in the lungs 29 days after inhalation. The content in the skeleton was 1.0% at 270 days after inhalation, and 0.15% in the liver at 170 days after inhalation. Lang et al. (*97*) exposed rats to neutron-activated $UO_2$ particles, including the ß-emitters $^{141}Ce$, $^{144}Ce$, $^{103}Ru$, $^{95}Zr$, and $^{95}Nb$. At 1 day after intratracheal instillation, on average 78.1% of the total injected radioactivity was detected in the lungs. Only 0.7% of the activity was found in the trachea, and

the rest was in the gastrointestinal tract (8.3%) and feces (12.9%). One month after instillation, approximately 94% of the retained total body activity was in the lungs, and this decreased to 83% after 3 months. The activities in the liver, kidney, spleen, and bone were <1% of the retained total body activity. The fractional absorption in the liver and bone was significantly lower ($p$ <0.05) than expected (*65,114*), which was probably due to the administration of uranium fission products in particulate form. Clearance of uranium-matrix-associated fission products from the lungs is preferentially dependent on the physical characteristics of the particulate material, as previously described. Particle size is probably the most important factor in the translocation of water-insoluble particles from the lungs to the blood (*105, 107–109*).

## Effects of Nuclear Fuel Compounds on the Lungs

### Short-term Effects

Deposition of insoluble radioactive particles in the alveoli is associated with an elevated lung cancer risk due to the long retention of particles in the lower respiratory tract. However, the significance of the short-term effects of insoluble radioactive particles, such as inflammatory reactions, in the development of lung neoplasms is not well understood. High lung radiation doses are needed to cause early mortality in experimental animals. For example, studies on $^{239}PuO_2$ have shown that early death occurs in baboons and dogs after accumulation of lung doses between 20 and 100 Gy (*115*).

Alveolar macrophages represent the initial defense mechanism of the lungs for clearing particulate matter. Although the alveolar macrophages are not tumor precursor cells, they secrete various cell growth-related factors, such as interleukin-1, tumor necrosis factor, and leukotrienes (*116*). The high toxicity of $UO_2$ on macrophages depends on the interaction of the metal with phospholipids and proteins, resulting in alterations of membrane permeability in phagosomes. Successive events may lead to the leakage of hydrolytic enzymes which may in turn cause severe damage to the cytoplasmic organelles (*88*). At present, it is not known whether lysosomal membrane damage is due to chemical (by uranium) or radiological toxicity (*87*). Moores et al. (*117*) have shown that there is a significant depression in the number of mouse alveolar macrophages after exposure to $^{239}PuO_2$ at initially acquired doses greater than 20 Bq. Morgan and Talbot (*116*) reviewed diverse effects of inhaled α-emitting actinides on mouse alveolar macrophages. For example, $^{239}PuO_2$ exposure appears to increase macrophage size, inhibit the mobility of macrophages, and enhance the phagocytic capacity. Cytoplasmic and lysosomal enzymes such as lactate dehydrogenase and α-glucuronidase are also activated by $^{239}PuO_2$. Induction of nuclear aberrations, particularly micronuclei, can be detected at doses as low as 1 Bq, corresponding to a cumulative radiation dose to lung of 50 mGy (*118*).

Recent data suggest that there are two distinct mechanisms involved in radiation-induced radiation damage: 1) classical pneumonitis, which ultimately leads to pulmonary fibrosis, is primarily due to radiation-induced local cytokine production confined on the field of irradiation; 2) sporadic radiation pneumonitis, which is an immunologically mediated process resulting in bilateral lympholytic alveolitis (*119*). Both animal experiments and human studies show that classical radiation pneumonitis has a threshold dose and a narrow dose–response curve with increased morbidity and mortality over a small dose range (*119*). For example, in rats, inhalation of high-fired $^{239}PuO_2$ (initial lung burden 3.9 kBq) leads to peribronchial particle aggregation, which increases with time and results in well-defined focal inflammatory lesions after 120 days (*120*). The exact mechanisms of radiation pneumonitis induced by insoluble radioactive particles are not fully understood. One possible mechanism is damage to type II cells, causing alterations in the cell differentiation and disturbances in the metabolism of surfactant phospholipids (*121*), or type II proliferation with failure to develop into type I cells (*122*). Taya et al. (*123*) have shown that significant cellular changes, particularly cell proliferation (mainly type II and Clara cells), occur early after exposure of mouse lung to $^{239}PuO_2$ at initial alveolar deposit of 500 Bq. However, the relevance of the results to the late carcinogenic effects of $^{239}PuO_2$ could not be verified because of the limited duration of the study.

Effects of insoluble, internally deposited radionuclides on the pulmonary clearance of inhaled bacteria have been reported in a few studies (*124–126*). For example, inhalation exposure to insoluble $^{144}CeO_2$ and $^{239}PuO_2$ particles reduced the pulmonary clearance of *Staphylococcus aureus* in mice (*124*). The authors suggested that direct radiation injury to the alveolar macrophage population was the likely cause of the reduced clearance (*124*). It has also been shown that inhaled $^{144}Ce$ in a relatively insoluble form results in detectable changes in the pulmonary surfactant, important in the killing of bacteria by pulmonary macrophages (*127*). These studies indicated that insoluble radionuclides may decrease an animal's resistance to bacterial invasion of the lungs and increase the risk for pneumonitis. Studies with simulated nuclear fuel particles (neutron-activated $UO_2$) show that particles induce local inflammatory changes at the cumulative lung doses of 170, 230, 400, and 550 mSv (*97*). As a short-term effect, the particles also appeared to modulate the cytochrome P450 enzyme activities, which in turn may affect the metabolism and effects of xenobiotics and chemical carcinogens (*128*).

### Pulmonary Fibrosis

Fibrosis-associated changes apparently have their origin in the pneumonitic phase (*35,119,129*). At the light microscope level, fibrosis is defined as an increase of connective tissue fibers as a result of inadequate regeneration of parenchyma. Collagen accumulation may alter the normal ratio of type I (coarse fibered) to type III (meshwork) collagen. Cellular disturbances play an important role in the alterations of the alveolar structure involving cell death on the endothelial and epithelial sides of the basement membrane and damage to the immune-competent cells (*130*). Biochemical and histological evidence of fibrosis may be detected as early as 2 months after radiation exposure (*129*). Gas transfer is impaired by fibrosis as a result of thickening of alveolar–capillary barriers and reduction in the effective surface area. Recent studies have shown that irradiation induces gene transcription and results in the induction and release of proinflammatory cytokines and fibroblast mitogens, which ultimately results in pulmonary fibrosis (*119*). Ionizing radiation may induce synthesis of various inflammatory gene products, such as EGR1 (*131*), platelet-derived growth factor (*132*), and necrosis factors (*133*).

Studies with insoluble, fused aluminosilicate particles carrying $^{90}Y$ have demonstrated a typical pneumonitic phase in beagle dogs and lung function changes typical of pneumonitis and fibrosis (*134*). Inhalation of $^{144}Ce$ fused clay particles have shown an early increase in ultrafiltrable hydroxyproline in the lungs of beagle dogs (*135*), suggesting an increase in collagen degradation, preceding the synthetic phase of more soluble collagen. Beagle dogs may have signs of restrictive lung disease 1–5 years after exposure by inhalation to $^{239}PuO_2$ at initial pulmonary burdens of 330–4100 kB/kg of body mass (*136*).

LaBauve et al. (*93*) observed marked alterations in respiratory function in a Rhesus monkey 30 days before its death from pulmonary fibrosis 990 days after inhalation exposure to $^{239}PuO_2$ (estimated initial lung burden 37000 Bq). Exposure of rats to high-fired $^{239}PuO_2$ (initial lung burden 3.9 kBq) resulted in fibrotic lesions 180 days after inhalation (*120*). Some studies with soluble and insoluble $^{239}Pu$ particles have shown that the metabolism of collagen, glycosaminoglycans, and lipids in laboratory animals are all shifted toward enhanced synthesis (*129*). McAnulty et al. (*137*) observed dramatic increases in both synthesis and degradation rates of collagen in the mouse lung after exposure to $^{239}PuO_2$, suggesting an extensive remodeling of the lung connective tissue matrix during development of fibrosis. Preexisting, bleomycin-induced pulmonary fibrosis has been shown to decrease significantly the clearance of $^{239}PuO_2$ in the rat lung (*138*). However, the risk of lung tumors in rats with or without existing pulmonary fibrosis were similar. Diel et al. (*139*) exposed beagle dogs to $^{239}PuO_2$ once (accumulated doses of 23 ± 8 Gy) or repeatedly (22 ± 5 Gy during 7–10 semiannual exposures). Clearance of plutonium from the lungs of dogs exposed repeatedly was slower than in the dogs exposed once. Pulmonary fibrosis accounted for 72% of the radiation-related deaths in the single-exposure study and 87% in the repeated-exposure study. The remaining dogs died from pulmonary cancer. The dose rate did not appear to be an important factor in predicting death from radiation pneumonitis or pulmonary fibrosis.

## Pulmonary Cancer

Tumors in the lung as a direct consequence of inhalation or instillation of radioactive materials are easily demonstrated in animals. However, in rodents, spontaneous or radiation-induced tumors normally occur in the alveolar region, whereas in man they are located in the bronchiolar region (*129*). Studies on Japanese A-bomb survivors and American uranium miners have shown that radiation-induced lung cancers appeared more likely to be of small-cell subtype and less likely to be adenocarcinomas (*140*). The review of lung cancer cases revealed further that the proportion of squamous cell cancer was positively related to smoking history in both populations. Absolute radiogenic risks of radiation-induced lung cancers are similar for both sexes, although baseline lung cancer risks are much higher than they are for females (*141*). Other factors important in risk assessment of the effects of radioactive particles in the lungs involve the particle size, chemical and physical form of the particles, the type of radioactive emission, the physical half-life of the radionuclide and its biological half-time in the lungs. Animal experiments indicate that prolongation of ß-irradiation of the lung from a period of days to years reduces its tumorigenic effectiveness by a factor of about 3, and that chronic α–irradiation of the lung from inhaled $^{230}PuO_2$ is 10 to 20 times more carcinogenic than chronic ß-irradiation (*141*) Burkart (*35*) has stated that both from the point of view of contracted dose and radiosensitivity, the human lung is the most critical organ for late somatic health effects from exposure to ionizing radiation in our environment.

### Alpha-emitters

Several animal studies have been performed to study long-term effects of insoluble α–emitters. Leach et al. (*89,90*) studied long-term effects of natural $UO_2$ particles in the monkey, dog, and rat. Inhalation of 1 µm mass medium diameter particles at a mean concentration of 5 mg/m$^3$, (6 hr per day, 5 days per week) did not cause serious injury in animals during the 5-year exposure. In the following post-exposure period, malignant tumors developed in 31% of the dogs 2–6 years after the exposure. The effects of inhaled $^{238}Pu/^{239}Pu$ dioxides have been studied in rats (*104,142*) , Syrian hamsters (*143*), and mice (*144*). These studies demonstrated, except for studies in mice, that prolonged inhalation of insoluble α-emitters does not enhance pulmonary carcinogenesis as compared to a single exposure. Both $^{238}PuO_2$ and $^{239}PuO_2$ have been shown to cause malignant lung tumors in dogs (*139,145,170–173*). Diel et al. (*139*) exposed beagle dogs to $^{239}PuO_2$ once and repeatedly (7–10 semiannual doses) to 22–23 Gy lung doses by inhalation. In the single exposure, 28% of the dogs died with pulmonary cancer, whereas in the repeated exposure the death rate was 13%. Gillett et al. (*146*) also observed primary liver tumors in beagle dogs exposed by inhalation to $^{239}PuO_2$. In baboons, $^{239}PuO_2$ particles caused slightly differentiated lung carcinoma and bronchogenic adenoma (*115*). Hahn et al. (*147*) also observed fibrosarcoma in the lung of a rhesus monkey after

**Table 3.** Summary of selected experimental studies supporting or refuting the hot particle theory

| Species | Experimental design | Tumor incidence | Reference |
|---|---|---|---|
| Sprague-Dawley rats, male | Single external ß-irradiation of skin with $^{91}Y$-source; grid, sieve, and uniform exposure | Markedly delayed after grid and sieve nonuniform radiation exposure compared with uniform exposure | Albert et al., 1967 (*180*) |
| CBA/H mice, female | Single external ß-irradiation of skin with $^{204}Tl$-source; irradiation over one or two zones | Proportional to the area of irradiated skin | Hulse, 1967 (*181*); Hulse et al., 1983 (*182*); Papworth and Hulse, 1983 (*183*) |
| SAS/4 mice | Single external ß-irradiation of skin with $^{170}Tm$ source; uniform and arrays of nonuniform 8 or 32 sources | 30% Reduction by the 32-point array at low doses; an order-of-magnitude reduction by the 8-point source at low doses | Williams et al., 1986 (*184*); Charles et al., 1988 (*185*) |
| Sprague-Dawley SPF rats | Inhalation of uniformly distributed, soluble $^{244}Cm(N\text{-}O_3)_3$, and particulate $^{239}PuO_2$ (hot particle model) | Uniformly distributed $^{244}Cm\text{-}(NO_3)_3$ up to 5 times more toxic than $239PuO_2$ | Lafuma et al., 1975 (*152*) |
| Syrian golden hamsters | Repeated intratracheal instillation of α-active $^{210}Po$ absorbed onto $Fe_2O_3$ particles or in 0.9% NaCl solution (more uniform distribution) | Incidence nearly similar in both experiments | Little and O'Toole, 1974 (*11*) |
| C3H 10T1/2 cells | Irradiation of cells with Chernobyl-released and simulated nuclear fuel particles | Malignant transformation in each cell culture; expected incidence 0% | Servomaa and Rytömaa 1989,1990 (*17,18*); Servomaa et al., 1992 (*19*) |
| Hairless mice | Implantation of simulated nuclear fuel particles under the dorsal skin | Incidence about 10% in exposed sites; expected incidence 0% | Lang et al., 1993 (*20*); Leszczynski et al., 1994 (*21*) |

inhalation of $^{239}PuO_2$. Sanders and coworkers (*99,148,149*) exposed Wistar rats to $^{239}PuO_2$ aerosol. Survival was significantly reduced only in rats with lung doses >30 Gy. Ninety-nine primary lung tumors were found out of the 2105 exposed animals, of which 92% were malignant and 80% carcinomas. The authors suggested that all types of malignant lung tumors exhibited a threshold at a lung dose >1 Gy (*149*). No significant difference was observed in nonpulmonary tumor location or type between control and exposed rats (*148*). Studies on plutonium-induced pulmonary neoplasms in the rat suggest that the alveolar epithelial surface may be more at risk for neoplasic transformation than the other histological types of proliferative foci (*150*). The majority of plutonium-induced proliferative epithelial lesions and neoplasms in the rat appear to originate from alveolar type II pneumocytes (*151*).

Little and O'Toole (*11*) studied the effects of nonhomogeneously and uniformly distributed $^{210}Po$ on the hamster lung. The average macroscopic dose to the lung was about 200 Sv. Tumor incidence was higher in the animals exposed to $^{210}Po$ in solution than in animals exposed to $^{210}Po$ bound to $Fe_2O_3$ particles with nonuniform dose distribution (see also Table 3). Nonuniform irradiation also resulted in longer latency periods. Lafuma et al. (*152*) exposed rats to soluble, uniformly distributed $^{244}Cm(NO_3)_3$ and insoluble $^{239}PuO_2$, which served as a model for hot particles. Uniformly distributed $^{244}Cm(NO_3)_3$ was up to five times more toxic than particulate $^{239}PuO_2$ (Table 3). Most inhalation studies with relatively insoluble α-emitters appear to produce lung cancers in rats, mice, and dogs at initially acquired doses above 0.04 Mbq/kg, with peak incidences in the range of 0.6–3.7 MBq/kg (*129*). Recent studies by Lundgren et al. (*153*) have shown that the relative biological effectiveness in rats of the α-particle doses to the lungs from inhaled $^{239}PuO_2$ relative to ß-particle doses to the lungs from inhaled $^{144}CeO_2$ is 21 ± 3.

### Beta-emitters

Most experiments with ß-emitters have involved studies with compounds of $^{144}Ce$ in rats (*113,154,155*), mice (*156,157*), and Syrian hamsters (*158*). In hamsters and rats, the incidences of primary lung tumors were more dependent on the cumulative ß-radiation doses to the lungs than the radiation dose-rate pattern. For example, in rats, a mean life time dose of 250 Gy resulted in 91.9% incidence of malignant lung tumors, whereas a 50 Gy dose caused 27% tumor incidence (*137*). The most frequently occurring malignant tumors were adenocarcinomas and squamous cell carcinomas. Even neutron-activated $UO_2$ particles induce benign or malignant tumors in rat lung at cumulative 24-month lung doses of 0.4–0.66 Gy (*128,159*). Squamous cell carcinoma and adenocarcinoma are the most frequently occurring tumors in the rat lung after exposure to ß-irradiation (*155,160*). The incidence of primary lung tumors in rats is a slow process and appears to be related to the cumulative ß-radiation dose. In mice, protraction of the absorbed dose resulted in a sparing from the life-shortening effects of $^{144}Ce$ pulmonary irradiation (*156*). Seventy-day-old mice were more sensitive to development of late-occurring effects of inhaled $^{144}CeO_2$ than 260- and 450-day-old mice. Experiments on beagle dogs have shown that protracted irradiation of the lungs with $^{144}Ce$ or $^{90}Sr$ result in a relatively high radiation dose and produce more total lung tumors but fewer lung tumors per Gray than less protracted irradiation with $^{90}Y$ and $^{91}Y$ (*161*). The carcinomas included adenocarcinomas, squamous cell carcinomas, or combinations of these types. Hemangiosarcomas were induced in animals that were exposed to $^{144}Ce$ and $^{90}Sr$ but were not found after $^{90}Y$ or $^{91}Y$ exposures; this tumor type was not even found in earlier inhalation exposure of beagle dogs to $^{239}PuO_2$ (*162*). Boecker et al. (*163*) exposed beagle dogs once, briefly, by inhalation, to the ß-emitter $^{91}Y$ or to the α-emitter $^{239}PuO_2$. $^{239}PuO_2$ was more effective in producing lung cancer than was $^{91}Y$; risk coefficients for $^{239}Pu/^{91}Y$ ranged from 10 to 18.

## Molecular Mechanisms of Lung Carcinogenesis Induced by Insoluble Nuclear Fuel Particles

The conversion of lung cells from normal to malignant involves a series of molecular changes, including the inactivation of tumor suppressor genes, the activation of dominant oncogenes, or other disturbances in normal cellular processes (*164*). The p53 tumor-suppressor gene appears to have a central role even in radiation-induced neoplasms of the lung. For example, p53 mutations have been observed in lung tumors of miners exposed to radon (*165,166*) and in lung cancers from radiation-exposed and nonexposed atomic-bomb survivors from Hiroshima (*167*).

Molecular changes in the lungs associated with radiation carcinogenesis after exposure to insoluble nuclear fuel particles are not well understood. However, the p53 tumor suppressor gene appears to have a central role even in radiation lung carcinogenesis induced by insoluble nuclear fuel particles. We observed both overexpression and mutations in the p53 gene in malignant tumors in the rat lung after exposure to neutron-activated $UO_2$ particles (*159*). The base change was identical in each case. Transition of C:G to T:A in the CG dinucleotide as a result of low-LET radiation may be similar to CC to TT double-base change in UV-associated skin cancer (*168,169*) or AGG to ATG transversion in high-LET-radiation related lung cancer (*166*).

Some studies have also been performed to investigate molecular mechanisms in the lungs after exposure to $^{239}PuO_2$. The expression of epidermal growth factor receptor (EGFR) was detected in plutonium-induced lung neoplasms in dogs (*170*). Forty-seven percent of lung tumors expressed EGFR; however, the expression was not correlated with tumor etiology (e.g., spontaneous versus radiation-induced), but did correlate with specific histologic phenotypes. The same group (*171*) observed an increased (59%) expression of transforming growth factor-α (TGF-α) in plutonium-induced lung neoplasms in the dog. Twenty-seven percent (32/117) of radiation-induced proliferative epithelial foci expressed TGF-α, and many of these foci (8/32) expressed both EGFR and TGF-α. The results indicated that the foci exhibiting increased expression of the growth factor or its receptor represented preneoplastic lesions which were at greater risk for progression to neoplasia. Even a significant increase in EGFR binding has been observed in plutonium-induced dog lung tumors (*172*). Davila et al. (*173*) have also observed severe depression of immune response in tumor-bearing beagle dogs which had been exposed to $^{239}PuO_2$. Overexpression of TGF-α and EGFR have also been observed in plutonium-induced malignant tumors in rat (*174*). These data suggested that increased amounts of TGF-α were early alterations in the progression of plutonium-induced squamous cell carcinoma, and the increases may occur in parallel with overexpression of the receptor for this growth factor.

Stegelmeier et al. (*175*) have also investigated molecular and genetic alterations of Ki-*ras* in preneoplastic foci and neoplasms in the lungs of rats that had inhaled $^{239}PuO_2$. Specific Ki-*ras* point mutations were present in 46% of the radiation-induced malignant neoplasms. Similar mutation frequencies were observed in radiation-induced adenomas and foci of alveolar epithelial hyperplasia, but no mutations were identified in normal lung tissue. The findings suggested that Ki-*ras* activation, not alterations in expression, is an early lesion associated with many radia-

tion-induced, proliferative pulmonary lesions and that this molecular alteration may be an important component of both radiation-induced and spontaneous pulmonary carcinogenesis in the rat.

## Biological Effects of Nonuniform Radiation on the Skin

### Acute Effects

The response of the skin to ionizing radiation is highly complex and depends to a large extent on the exposure conditions (*176*). The basic underlying pathogenic mechanisms following hot particle exposure appear to be different from classical radiation damage to skin. The primary lesion resulting from irradiation with hot particles is acute ulceration (*176*). The depth and size of the ulcer depends on the skin surface dose and the energy of the radiation from the particle. Before the development of an ulcer, a small pale, circular area with a slight bluish tinge can be detected, which is frequently surrounded by a halo of erythema. Within a few days of irradiation, pyknosis of nuclei of endothelial cells and fibroblasts can be seen in the papillary dermis, and within 5–7 days the papillary dermis is largely without cell nuclei. These changes result from the direct cell death of these cells in interphase after doses >100 Gy. Acutely produced ulcers of the skin tend to heal rapidly if they do not become infected, and the lesion leaves a small scar with the appearance of a small dimple (*20,176*).

The $ED_{50}$ (effective dose) values for acute ulceration from $^{90}Sr/^{90}Y$ and thullium-170 ($^{170}Tm$) particles <1 mm in diameter is about 250 Gy (*177*). Moist desquamation cannot be seen after hot particle irradiation, but late dermal atrophy may develop at doses below the threshold for ulcer formation (*178*). Lang et al. (*20*) exposed hairless and nude mice to neutron-activated $UO_2$ particles. Within the first two weeks, an ulcer (diameter 1–4 mm) with erythematous and thick edges developed. At about 3 weeks, the hyperplastic epidermis often had a papillomalike appearance. Histologically, a five- to sixfold increase in epidermal thickness was observed. Inflammatory and giant cells of foreign body type were seen in the dermis It is currently believed (*14,15*) that the only type of hot particle-induced lesion of concern is acute ulceration or breakdown with subsequent infection leading to ulceration.

### Carcinogenesis

Ionizing radiation is a complete carcinogen (i.e. initiator and promotor), in rodent skin (*13*). In rats and mice, and in humans, the times between irradiation and appearance of tumors, as fractions of life span of the species, is similar. Protraction of radiation dose produces a reduction in its carcinogenicity in rats, whereas in mice no sparing effect has been observed. The human data support a significantly lower skin cancer incidence, at least two orders of magnitude lower than that in rodents (*179*). Some of this discrepancy may be because the human tumors are mostly basal cell carcinomas, whereas only a few radiation-induced cancers in the experimental animals are of this type.

Most of the long-term studies on skin carcinogenesis after hot particle exposure have refuted the hot particle theory (Table 3). Albert et al. (*180*) showed that the rat skin tumor yield after grid and sieve nonuniform radiation exposure was markedly delayed compared with uniform exposure. Hulse and colleagues (*181–183*) irradiated CBA mice with thallium-204 ($^{204}Tl$) particles using 12 different surface doses (5.4–260 Gy) and 4 different dose rates (1.7–200 cGy/min). The average latent period for tumor formation was 7 months, and more than 70% of the tumors were of dermal origin, 30% epidermal, and more than 60% were malignant. The tumor yield was proportional to the area of skin irradiated (*182*). Williams and his colleagues (*184,185*) exposed 1200 SAS/4 mice to uniform $^{170}Tm$-sources (8.6 $cm^2$) and nonuniform $^{170}Tm$ sources, which were arrays of either 32 or 8 sources, each 2 mm in diameter, distributed over 8 $cm^2$. Average skin doses varied from 2–100 Gy. The nonuniform irradiation showed a 30% reduction in tumor incidence by the 32-point array at the lower mean doses compared with the response from uniform sources. The 8-point array showed an order-of-magnitude reduction in tumor incidence compared to uniform irradiation at low doses. Even national and international radiation protection organizations have stated their objection to the hot particle theory (*14,15*).

Our current observations (*20,21*) do not agree with the previously reported results. We exposed hairless and nude mice to neutron-activated $UO_2$ particles by implanting the particles under the skin, which permitted a continuous long-term exposure of the skin to hard ß-irradiation. The results suggested that there was an excess of skin cancers in mice exposed to hot particles compared with the numbers estimated using a conventional, nonthreshold stochastic model of radiation-induced cancer (see Table 3). The results of the previous studies have undoubtedly been correct, but the conclusions have been too generalized. The results in our studies showed further that any direct mathematical–statistical extrapolation is not always appropriate but requires judgmental evaluation of biological mechanisms.

### Biological Mechanisms of Skin Carcinogenesis Induced by Hot Particles

Development of skin cancer has been shown to be associated with the activation of many genes, particularly oncogenes and tumor-suppressor genes. Current evidence indicates that carcinogenesis is a multistep process (*186,187*). Activation of *ras* (*188*) and c-*myc* oncogenes (*188,189*) has been observed in radiation-induced skin tumors in rats. Overexpression of *ras* oncogenes has been found in many preneoplastic tumors, suggesting that *ras* activation is often an early event in tumor formation (*187*). Biopsy studies have shown that in radiation-induced rat skin tumors, c-*myc* functions as a late-stage progression-related oncogene (*189*). Physical parameters such as LET, dose, and dose rate may also affect oncogene activation patterns (*190*).

Our observations (*20,21*) suggest that the development of hot-particle-induced skin cancer depends on a few essential cellular and molecular mechanisms. The development of a permanent wound is an essential step in the carcinogenesis induced by nonuniform β-irradiation. The wound acts as a promoter by stimulating the proliferation of surrounding mutated cells. The skin exposure to simulated nuclear fuel particles revealed further that expression of p53 tumor-suppressor protein was frequent (28%) at the exposed sites (*21*). In some cases p53 protein was detected not only in the nucleus but also in the cytoplasm of the epithelial cells. The expression of the oncoproteins $p62^{c\text{-}fos}$ and $21^{N\text{-}ras}$ was also markedly elevated in all the p53-expressing skin samples. The results showed that apparent carcinogenesis-related molecular changes occur frequently in the mouse skin well before the development of a distinct tumor, and probably even before premalignant changes can be detected by conventional histopathological analysis.

The key feature in carcinogenesis is that an agent can increase the incidence of cancer in one of two ways: it can specifically damage the DNA in a cell or increase the number of cell divisions, thereby providing a greater opportunity for (spontaneous) genetic errors during DNA replication (*191*). In hot particle exposure, both mechanisms are simultaneously involved and, possibly even more important in view of the multistage model of carcinogenesis, the number of cell divisions is increased in the

same cells in which specific radiation-induced DNA damage is most likely to occur. Our *in vivo* studies (*20,21*) directly support the general multistage model of carcinogenesis according to which the mechanism is based on genotoxic (DNA damage) and nongenotoxic (cell proliferation) effects. These observations are also supported by our *in vitro* experiments where C3H10T1/2 cells were exposed to Chernobyl-released and simulated nuclear fuel particles (*17–19*). Malignant foci developed in all cell cultures usually 2–4 mm from the radiation source. In addition, almost all the tested 11 oncogenes were activated by radiation, though in none of them was this change common.

## Conclusions

Environmental releases of insoluble nuclear fuel particles may occur both in nuclear power plants in normal operation and following nuclear power plant accidents. The effects of hot particles on humans have been assessed in a few epidemiological and theoretical studies based on occupational exposure to $PuO_2$ particles and exposure to Chernobyl-released uranium particles. The results have so far been only speculative due to the lack of detailed and reliable data on the exposure. However, the biokinetics and biological effects of nuclear fuel compounds have been investigated in a number of experimental studies using various cellular systems and laboratory animals.

Ingestion of insoluble nuclear fuel compounds does not pose a serious radiological health problem. $UO_2$ and $PuO_2$ particles are not absorbed to any significant extent from the GI tract of experimental animals. Fission products $^{144}Ce$, $^{141}Ce$, $^{103}Ru$, $^{95}Zr$, and $^{95}Nb$ are also absorbed poorly in their elementary form, whereas they are almost metabolically inert in the fused particulate form in the uranium matrix. However, in neonatal animals the absorption is higher. A slight retention of compounds may occur in the intestinal cells, but only extensive amounts of nuclear fuel material with prolonged retention in the GI tract may cause serious lesions in the radiosensitive cells crypt cells of the intestine.

Inhalation of insoluble nuclear fuel compounds induce both benign and malignant lung tumors in experimental animals. The elevated cancer risk is due to the long retention of particles in the lower respiratory tract. However, both intratracheal instillation and inhalation of insoluble nuclear fuel particles seems to lead to a nonuniform distribution of particles in the lungs and therefore complicates the assessment of the lung cancer risk based purely on the conventional dose calculations. Translocation of $PuO_2$ and $UO_2$ particles and fission products $^{144}Ce$, $^{141}Ce$, $^{103}Ru$, $^{95}Zr$, $^{95}Nb$ in the particulate form from the lungs to other organs or tissues is poor.

The development of hot particle-induced cancer has been investigated in a number of experimental and theoretical studies. Most of the studies have suggested that nonuniform distribution of ionizing radiation is less carcinogenic than the same amount of radiation delivered uniformly to the same organ or tissue. However, current observations indicate that the development of a permanent wound is an essential step in carcinogenesis induced by nonuniform ß-irradiation. This is also the primary lesion in the skin resulting from hot particle irradiation. The wound acts as a promoter by stimulating the proliferation of surrounding mutated cells. The experimental design in most of the studies has not allowed the development of a permanent wound, which in part may explain the obvious discrepancy between the results. In addition, the contradictory results can also be explained by the different effects of nonuniform α- and ß-irradiation on biological material.

Exposure to insoluble nuclear fuel particles may induce various changes at the molecular level, which can be observed long before the development of a tumor. Overexpression and mutations of genes regulating cell proliferation and cell growth have been observed both *in vitro* and *in vivo*. The tumor-suppressor gene p53 may play a central role even in carcinogenesis induced by nonuniform radiation exposure.

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# Health Effects in a Casual Sample of Immigrants to Israel from Areas Contaminated by the Chernobyl Explosion

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We analyzed questionnaire and physician examination data for 1560 new immigrants from the former USSR divided into three groups by potential exposure to Chernobyl radiation. Two groups were chosen according to soil contamination by cesium-137 at former residences, as confirmed by our findings in a $^{137}Cs$ body burden study. The third group consisted of "liquidators," persons who worked at the Chernobyl site after the disaster. Liquidators had greater self-reported incidences of symptoms commonly accepted as acute effects of radiation exposure, increases in prevalence of hypertension, and more health complaints. Excesses of bronchial asthma and health complaints were reported in children from the more exposed communities. Asthma prevalence in children potentially exposed *in utero* appears to be increased eightfold. Older adults from more exposed areas had more hypertension as assessed by history and measurements. These findings suggest the possible association of radiation exposure with several nonmalignant effects. *Key words*: bronchial asthma epidemiology, cesium-137 soil contamination, Chernobyl fallout, environmental radioactivity, hypertension epidemiology, internal radiation exposure, ionizing radiation, noncancer radiation effects, occupational radiation exposures. *Environ Health Perspect* 103:936–941 (1995)

Health effects in connection with the Chernobyl explosion, which occurred 26 April 1986, are of widespread concern in Israel because about 100,000 people immigrated into the country from possibly contaminated zones between 1990 and 1993. Nonmalignant health disorders associated with Chernobyl exposure have been reported in populations from many regions of Byelorus, Ukraine, and Russia. Health problems are especially prominent among people who were deliberately exposed due to their involvement in cleanup work after the disaster (*1–3*). These clean-up workers are called "liquidators" because they participated in liquidating the sequelae of the Chernobyl nuclear power station disaster.

A major problem in the study of such environmental contamination is that valid and reliable measurements of exposure are not generally available (*4*). Most of our subjects had undergone measurements of the body burden of cesium-137, using a portable, whole-body counter, in the autumn of 1991 provided to us by the Canadian Department of Health and Welfare. This same isotope ($^{137}Cs$) was the basis for mapping ground-level contamination by the International Atomic Energy Agency (IAEA) (*5*) and Russian authorities. This map allowed us to classify the probable exposure of individuals according to the places they lived after the disaster, which is usually the place from which they emigrated.

We thus defined two groups with different exposures to long-term Chernobyl radiation to determine if certain health effects differed between these groups. A third group, liquidators or salvage personnel, was presumed to be deliberately exposed to radiation in connection with their work assignments, and thus were subject to higher external and internal exposure, albeit to different isotopes and over different time periods, than the other two groups.

## Methods

The study sample consisted of 1560 immigrants (885 adults, 675 children and adolescents to age 18 years) who arrived in Israel during 1990–1991.

***Sample selection.*** In spring of 1991, a group of faculty members from Ben Gurion University, Faculty of Health Sciences, recognizing the unusual problems facing some of these immigrants, established a clinic for counseling and evaluation. Individuals and families came to the "Chernobyl Clinic," based at Soroka Medical Center, on a voluntary basis. In autumn 1991, a portable, whole-body counter for measuring $^{137}Cs$ was made available by the Canadian Department of Health and Welfare. We then invited (by radio and newspapers) recent immigrants concerned about their health in connection with possible exposure to Chernobyl radiation to come in for a health evaluation. In a 5-week period, 1244 people underwent $^{137}Cs$ body burden measurements, interviews, and physical examinations. In subsequent months, a further 316 immigrants were questioned and examined. In reviewing our initial findings, we were impressed by the frequency of thyroid enlargement, which we had not initially planned to evaluate. So, during the summer of 1992, we standardized our thyroid examination procedures and evaluated thyroid function and autoantibodies. We invited for reexamination all of the children seen in autumn 1991, and 180 of them returned, along with 129 additional children and their parents. These participants, along with the people who attended the Chernobyl Clinic seeking evaluation and counseling, accounted for 316 additional immigrants who were included in the total 1560 people in the study.

***Questionnaires.*** Questionnaires (available on request from E. Kordysh) were administered after pretesting to obtain demographic data, exposure history, medical history, health status, smoking and dietary history, and, for children, history of birth and development.

***Gradients in assumed dose.*** We designated communities in areas with $^{137}Cs$ soil contamination at the level <37 GBq/km$^2$ as "less exposed" and those with >37 GBq/km$^2$ as "more exposed," based on map data obtained by IAEA (*5*). This designation has been confirmed by our findings in the $^{137}Cs$ body burden study (*6*), as shown in Figure 1.

The population sample was stratified into 3 distinct groups by potential exposure: 1) liquidators (47 men and 8 women), persons who worked on ameliorating the consequences of the Chernobyl explosion, 2) 291 males and 397 females who had lived in more exposed areas, and 3) 351 males and

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466 females who had lived in the less exposed areas. The latter two groups are collectively referred to as "residents." The more exposed areas in Figure 1 include Gomel, Chernobyl, Mogilev, Mozyr, Korosten, Kalinkovichi, Narovlja, Klintzy, and Rechitza. All other areas, including Kiev, are classified as less exposed.

The group of liquidators is not homogeneous by kind of job (it includes deactivators, building workers, drivers, medical and sanitary personnel, and service workers), nor by the period and duration of work (work was performed from the first days after the fire and later, and for less or more than 1 month). The maximal accumulated dose reported is 25 rem. For most people, dose information is not reliable; for many it is missing. It is reasonable to assume a greater exposure in the first few weeks after the disaster than in a later period. We recognize the relevance for an exposure index of measured dose, calendar time of first employment, duration of employment, type and location of work, documented change in white or red cell counts, use of protective equipment, and occurrence of symptoms possibly related to radiation exposures. Such information is potentially available in records of these salvage workers; we also used a questionnaire to compile such data and are currently evaluating these data.

***Possible effects of radiation exposure.*** We analyzed data reported for generally recognized symptoms of acute radiation sickness—nose bleeds, nausea, hair loss, diarrhea, bleeding into skin, blood in urine and stool, and drop in white blood cell count—which took place in residents in the first 2 months after the fire and in liquidators subsequent to the time of work. We analyzed physician-diagnosed as well as self-reported chronic health impairments. Diseases and symptoms were tabulated according to the *Classification of Health Problems in Primary Care* (*7*), used by Kupat Holim, the health care organization to which many of the subjects were referred.

***Physical examination.*** Physicians measured pulse rate and arterial blood pressure (seated position) as well as weight and height of each subject. Consistent reporting of these measurements was restricted to the sample studied in the autumn 1991. The staff available during the body burden study allowed the use of the same physicians for examinations, who were blind to the exposure history. This practice could not be replicated for subjects seen later. These measurements were done blind as to former residence of the subjects. We examined thyroid and lymphatic glands of all the subjects by palpation.

***Statistical treatment of the data.*** Statistical analysis was performed using EPINFO (USD, Inc., Stone Mountain, Georgia) and SPSS (McGraw Hill, Chicago, Illinois) programs. The ages were stratified into nine groups: 0–5 , 6–9, 10–14, 15–18, 19–28, 29–38, 39–48, 49–58, and 59+ years.

For symptoms associated with acute exposure, distributions corresponded to age in 1986; for other health indices, distributions corresponded to age in 1991. We evaluated data by chi-square tests with Yates correction and, where small numbers were expected, with the Fisher's exact test. For age-stratified data, the Mantel-Haenszel test was used. The Student's *t*-test was used to evaluate the means of systolic and diastolic blood pressure. Adjustments for smoking, weight, and age were made for blood pressure data in adults, as well as for height and body mass in children. Sample distribution by age, sex, and possible exposure is presented in Table 1.

We consider probabilities less than 0.05 satistically significant. For a hypothesis-generating study such as this, statistical tests are useful for pointing to testable, specific hypotheses. Because this was not a hypothesis-testing study, we disregarded the multiplicity of statistical tests.

## Results

Of the 55 liquidators, 25 (45.5%) reported one or more symptoms associated with acute radiation exposure. The frequency of these symptoms was 22.4% among former adult residents compared with liquidators, ($p < 0.003$ ) and 18.8% among children. All symptoms were reported more often by liquidators, but a statistically significant excess was found only for nausea and decreased white blood cell count. Information on white cell count was available for 81.8% of liquidators and 79.6% of residents. Significant differences in reported frequency of hair loss in males was found for liquidators aged 29–38 years (29.4% versus 5.8% for residents; $p < 0.02$).

In female children from the more exposed zones, nose bleeds were significantly more frequent than among female children from the less exposed area (15.7% compared to 7.6%). Among adults males from the more exposed communities, nausea was reported twice as frequently than among the males from less exposed areas, but the difference did not reach conventional levels of significance (Table 2).

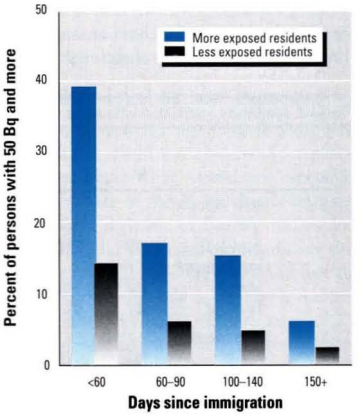


**Figure 1.** Percent of radiocesium tests with 50 Bq and more by days since immigration and former residence. $p < 0.05$ for each time-period difference. Reproduced with permission (*6*).

**Table 1.** Distribution of subjects by age, gender, and possible exposure, examined in 1991–1992

| | | More exposed | | Less exposed | | Liquidators | |
|---|---|---|---|---|---|---|---|
| Age | | Male | Female | Male | Female | Male | Female |
| 0–5 | Total[a] | 42 | 39 | 41 | 38 | | |
| | Initial | 27 | 27 | 31 | 25 | | |
| 6–9 | Total | 41 | 44 | 48 | 48 | | |
| | Initial | 31 | 33 | 30 | 38 | | |
| 10–14 | Total | 69 | 66 | 64 | 37 | | |
| | Initial | 54 | 66 | 44 | 22 | | |
| 15–18 | Total | 22 | 33 | 20 | 23 | | |
| | Initial | 19 | 23 | 15 | 15 | | |
| 19–28 | Total | 18 | 26 | 24 | 46 | 9 | 2 |
| | Initial | 14 | 22 | 18 | 40 | 7 | 2 |
| 29–38 | Total | 31 | 85 | 57 | 104 | 16 | 3 |
| | Initial | 23 | 72 | 45 | 77 | 13 | 2 |
| 39–48 | Total | 28 | 34 | 38 | 69 | 16 | 1 |
| | Initial | 24 | 27 | 30 | 55 | 14 | 1 |
| 49–58 | Total | 15 | 25 | 24 | 38 | 5 | 0 |
| | Initial | 12 | 21 | 23 | 32 | 4 | |
| 59+ | Total | 25 | 45 | 35 | 63 | 1 | 2 |
| | Initial | 19 | 41 | 33 | 56 | 1 | 2 |
| All | Total | 291 | 397 | 351 | 466 | 47 | 8 |
| | Initial | 223 | 332 | 269 | 360 | 39 | 7 |

[a]Total, 1991–1992; initial, 1991.

Among 0- to 14-year-old boys and girls from more exposed areas, rates of asthma were 5.4% and 3.4%, respectively, which is 2.8 and 2.1 times higher than the rates for those from less exposed communities (1.9% for boys and 1.6% for girls; Fig. 2).

In adults, onset of asthma was only reported prior to 1986, and no difference by exposure was found (Fig. 2).

For children who were *in utero* at time of the Chernobyl accident, cumulative asthma incidence reached 20% (5/25) by 1991, which is about eightfold higher than in all the other children (average of 2.4%, $p < 0.001$; Fig. 3). Twelve children from Gomel and six from Kiev were potentially exposed *in utero*. Seven children from other less exposed communities were possibly exposed *in utero*, but none of them developed asthma.

The incidence of asthma among children *in utero* at the time of the explosion is sevenfold and fourfold higher than among children one year older (2.9%, $p < 0.05$ ) or one year younger (5.6%), respectively. Of the five children who were *in utero* at the time of the explosion and who developed asthma in the first five years of life, three were from Gomel, in the more exposed area, and two were from Kiev, in the less exposed area. Recontact with the mothers of these children confirmed that the asthma was persistent and that the children were under clinical treatment for asthma.

In liquidators older than 38 years, the prevalence of physician-diagnosed hypertension was greater than among residents. For liquidators aged 38–48, the rates were 35.3% versus 27.1% in residents; for those aged 49–58, the rates were 60% versus 38.6%; and among those 59 and over, the rates were 66.6% versus 47.8% among residents.

There was a nonsignificant excess of hypertension in the 49- to 58-year-old former residents of more exposed areas (37.2% versus 29.0% in less exposed areas).

A significant relationship between occurrence of symptoms associated with acute radiation exposure and development of hypertension was found among liquidators; 28% who reported such symptoms had hypertension, in contrast to 7.1% who did not report such symptoms.

For the 19 males 59 years old and over from the more exposed areas, the measured blood pressure was significantly greater than for the 33 males from the less exposed areas (153.7/93.4 versus 140.6/86.6). A history of hypertension was paralleled by increased systolic pressure for men aged 49–58: 138.8 for liquidators ($N = 4$, (SD 19.6) versus 132.1 for residents ($N = 34$, SD 11.8).

Rates of goiter prevalence before the Chernobyl disaster were almost equal among residents from more exposed areas (15.1%) and residents from less exposed areas (14.4%). The areas being compared were thought to differ in $^{137}Cs$ pollution, but may not have differed in exposures to external radiation or to radio-iodine.

Thyroid palpation in 309 children (156 from the more exposed area and 153 from the less exposed), performed by experienced physicians, also did not reveal differences in findings of enlargement of the thyroid gland between groups by former residence (Fig. 4).

The prevalences of nodules were 7.7% and 5.5% for children from more exposed and less exposed areas, respectively (Fig. 4). There was a marked contrast between nodules in people from Gomel (6.9%) and Kiev (2.9%). For other communities in the more exposed category, this figure was 9.1%, versus 6.2% in other less exposed communities.

Differences between groups in frequency of other health conditions, assessed both

**Table 2.** Symptoms associated with acute exposure, 1991–1992[a]

| Group | Area | N | Frequency of symptom (%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Nose bleed | Hair loss | Nausea | Diarrhea | Bleeding into skin[b] |
| Adults | More exposed | | | | | | |
| | Males | 107 | 5 (4.7) | 7 (6.5) | 16 (15.0) | 4 (3.7) | 2 (1.9) |
| | Females | 199 | 8 (4.0) | 19 (9.5) | 21 (10.6) | 5 (2.5) | 3 (1.5) |
| | Less exposed | | | | | | |
| | Males | 163 | 5 (3.1) | 10 (6.1) | 14 (8.6) | 5 (3.1) | 2 (1.2) |
| | Females | 307 | 8 (2.6) | 28 (9.1) | 32 (10.4) | 11 (3.6) | 5 (1.6) |
| Children | More exposed | | | | | | |
| | Males | 141 | 14 (9.9) | 3 (2.0) | 16 (11.3) | 6 (4.2) | 2 (1.4) |
| | Females | 172 | 27* (15.7) | 7 (4.1) | 19 (11.0) | 8 (4.6) | 4 (2.3) |
| | Less exposed | | | | | | |
| | Males | 132 | 13 (9.2) | 1 (0.8) | 18 (13.6) | 9 (6.8) | 2 (1.5) |
| | Females | 145 | 11* (7.6) | 7 (4.8) | 18 (12.4) | 6 (4.1) | 2 (1.4) |

[a] Ages are as of 1986. Adults are persons ≥ 19 years old; children are ≤ 18 years old.
[b] Bleeding into skin includes bleeding into urine and feces.
*$p < 0.05$.

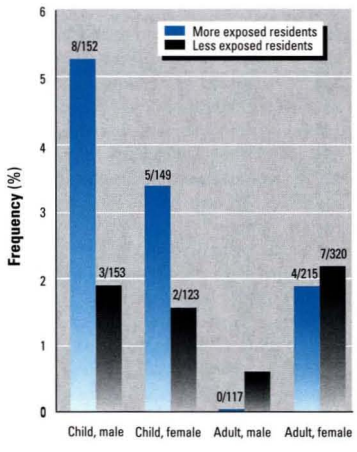


**Figure 2.** Bronchial asthma prevalence by sex and former residence. Number of cases/sample size above bars.

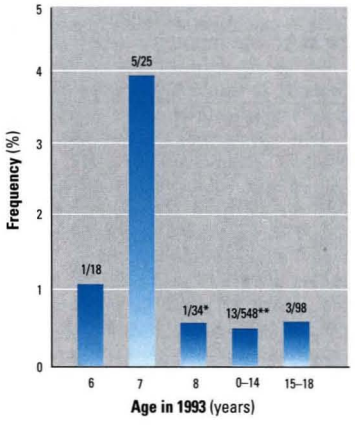


**Figure 3.** Bronchial asthma prevalence in children and adolescents. Children 7 years old were *in utero* in April 1986, when the explosion occurred; children 0-14 include all others. Number of cases/sample size above bars; *$p < 0.05$; **$p < 0.001$ compared to children *in utero* in April 1986.

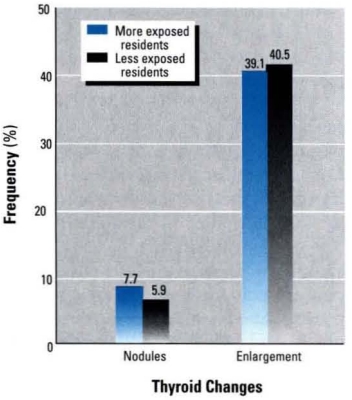


**Figure 4.** Thyroid changes in children by former residence.

by complaints and by physician diagnosis, are shown in Table 3. Liquidators demonstrated significantly higher rates for central nervous system (neurosis, sleep disorders, headache, dizziness), respiratory tract, and cardiovascular system disorders, as well as an increased tendency toward all other symptoms compared to former residents. A similar trend of increased health complaints was found for boys and girls who were born subsequent to the Chernobyl explosion and then lived in more exposed towns as compared to those from less exposed areas. Adolescents from more exposed areas also had more health complaints compared to those from other areas, with significant differences for cardiovascular system complaints.

## Discussion

### Sampling and Bias

The subjects of our study were not drawn from random population samples selected from an immigrant pool, but rather from Jewish immigrant volunteers concerned about their health due to their exposure to Chernobyl radiation; therefore, the possibility of selection bias is real. The subjects we examined came from many parts of Israel for evaluation, so we were able to make the observations we report here. We were able to offer reassurance as to lack of long-term effects from the body burden data and refer subjects for health care when necessary, whether or not their Chernobyl exposures were relevant.

Virtually every family believed that they had been exposed, and in our discussion of possible exposures we confirmed this. Our construction and validation of the two groups of more exposed and less exposed could not have led to a biased participation or response, however, because the classification was not established until after the subjects had been questioned and examined.

For these reasons we do not believe that there could have been an important contribution of selection, response, or observer bias to the differences between the more exposed and less exposed groups. On the other hand, our data cannot be extrapolated to other populations. While we cannot prove that the two different communities do not differ in some other ways, this problem is common in epidemiological analysis. Initial differences associated with presumed exposure differences are conventionally attributed to exposure until a better model or explanation is found. Nevertheless, associations with exposures in this population do increase the *a priori* probability of similar associations in other exposed populations.

Our data on hypertension from the less exposed community do not show significant differences from the general population of the former USSR (*8*). Elderly males from the more exposed areas had higher blood pressure than males of the same ages in Moscow and St. Petersburg (*4*).

Liquidators reported significantly more nausea and decreases in white blood cell counts than in residents; men aged 29–38 from more exposed areas had more hair loss and girls had more nose bleeds. We cannot exclude reporting bias for these findings. Although the interviewers and examiners were usually "blind" as to the exposure class of residents, they were not blind to the status of liquidators.

We could validate the decreases in white or red cell counts if we had access to complete records. Some of the liquidators brought complete health records and those who had a history of decreases in cell counts usually could tell us the actual counts and dates.

Other symptoms of radiation exposure must be considered with caution because of their nonspecificity, latency, and the consequent risk of recall bias. A group who volunteered for study because of health concerns might be more likely to recall symptoms than would a less health-conscious group.

The frequency of symptoms associated with acute radiation sickness was higher than expected. Stather and coauthors (*9*) reviewed dose–response information for acute radiation effects based on data from Hiroshima and Nagasaki and cancer therapy. For brief exposures to low-energy radiation (low LET) for <1 day, the $ED_{50}$ exposures for anorexia, nausea, fatigue, vomiting, and diarrhea are 0.97, 1.4, 1.5, 1.8, and 2.3 Gy, respectively. A threshold for vomiting of 0.5 Gy, is given. These relationships are for exposures to thorax, abdomen, and head and are thought to involve the autonomic nervous system. Skin exposures would be relevant to hair loss and bone marrow exposures to changes in white blood count and possibly to nose bleeds.

### Childhood Asthma and Exposure

Asthma is reported significantly more frequently in children who lived in more exposed areas. What we did not expect to find was that of the 25 children who were *in utero* at the time of the fire and explosion, 5 (20%) developed asthma during the first 5 years of life. This was not due to possible exposure only in the first trimester, when sensitivity of the fetus to external X-ray exposure seems to be greatest, nor was it associated with other problems of growth and development. Children 1 year older or 1 year younger had no such increase. Response or observer bias in these observations does not seem probable.

We do not know of any specific mechanism to explain this observation, nor of comparable reports. We hypothesize that the manifestation of childhood asthma could be associated with an immunological effect of radiation on the developing fetus. Impaired cellular and humoral mechanisms of immunity, as well as autoimmune processes, have been found among children and adults residing in radioactively contaminated areas near Chernobyl (*4,10,11*)) and among liquidators (*12,13*). Kulakov et al. (*14*) reported alterations in immunoglobulins and increases in levels of C-reactive protein in serum of pregnant women living in areas contaminated by the Chernobyl fallout.

Exposure to radiation *in utero* can cause childhood leukemia and other tumors (*15–17*), mental retardation (*18*), and seizures (*19*). A detailed review of these effects is presented by Upton et al. (*20*) and, Nussbaum and Köhnlein (*21*). Under the auspices of the World Health Organization, Prilipko et al. (*22*) are following several thousand children exposed to radiation *in utero* to evaluate evidence of

**Table 3.** Health disorders in immigrants, self-reported and physician-diagnosed, 1991–1992[a]

| | Frequency of disorders (%) | | | | | |
|---|---|---|---|---|---|---|
| | Adults | | Adolescents | | Children | |
| | L ($N$ = 55) | R ($N$ = 830) | ME ($N$ = 55) | LE = ($N$ = 43) | ME ($N$ = 53) | LE ($N$ = 51) |
| Respiratory | 87.3† | 54.5 | 39.6 | 39.2 | 78.8* | 60.9 |
| Central nervous | 65.4** | 52.1 | 32.6 | 37.1 | 51.5† | 15.5 |
| Cardiovascular | 89.1** | 76.1 | 49.1* | 27.5 | 4.5 | 1.5 |
| Gastrointestinal | 74.5 | 69.3 | 37.7 | 25.5 | 40.5 | 30.4 |
| Genitourinary | 70.9 | 62.7 | 32.1 | 19.6 | 36.4 | 26.1 |
| Musculoskeletal | 69.1 | 62.3 | 28.3 | 25.5 | 37.9 | 30.4 |
| General symptoms | 65.4 | 61.1 | 35.9 | 29.4 | 36.4 | 27.5 |

Abbreviations: L, liquidators; R, total residents; ME, residents of more exposed areas; LE, residents of less exposed areas.

[a]Data are for ages as of 1991. Adults are persons ≥ 19 years old; adolescents are 15–18 years old; and children were born after the explosion on 26 April 1986.

*$p$<0.05; **$p$<0.01; †$p$<0.001.

mental retardation. This cohort could also provide data on asthma in radiation-exposed children, although bronchial asthma has not yet been noted in this cohort.

### Changes in Blood Pressure

The concordance between history of hypertension and measurements of blood pressure in elderly residents of more exposed areas compared to those from less exposed is evidence against recall or reporting bias. Hypertension has multiple causes, and we considered only whether, among older subjects, a valid association with Chernobyl could be inferred from our observations. Our results concerning arterial blood pressure are in agreement with data from other studies: incidence of hypertension in people living near Chernobyl has increased (*23*), and a high percentage (27.2%) of borderline arterial hypertension was noted among 107 liquidators, aged to 39 (*24*). People living near Three Mile Island nuclear power station exhibited higher levels of blood pressure than those from control areas (*25*); however, it has been asserted that there was almost no radiation exposure at Three Mile Island. Tsyb reported a dose–response relationship between incidence of cardiovascular disease and exposure in liquidators (*3*). Such effects have been considered a response to a strong psychoemotional tension in liquidators resulting from both their work and radiation phobia (*24*) and a response to chronic stress in residents (*25*).

No doubt, stress occurred among our population, but we do not believe that stress is entirely responsible: adults from more exposed and less exposed areas did not know local contamination ratings and therefore could not have been differentially stressed. Furthermore, stress is believed to cause changes primarily in systolic pressure, but we observed differences in in both components of arterial blood pressure.

Chernogus and Kupchinskaia (*26*) examined hypertensive patients from Kiev and from an area within a 30-km radius of the Chernobyl nuclear power plant and compared T-lymphocyte counts and T-suppressors with those from a sample of normal blood donors. Hypertensive patients had reduced levels of T-lymphocytes, T-suppressors, and T-helpers, an effect which was greater in the residents of the more exposed areas. The authors hypothesized that these decreases reflect mechanisms affecting the progression of vascular disease. Tests of blood serum in 340 liquidators revealed increased levels of autoantibodies to neurospecific brain proteins (*27*). This finding is considered possibly relevant to chronic postradiational encephalopathy, with which hypertension may be associated.

In a series of investigations in liquidators, Voloshin et al. (*28*) using Doppler sonography and magnetic resonance imaging (MRI), detected increased vascular tension and reduced cerebral vessel blood flow. Using MRI, they also noted structural changes in the brain.

Kundiev et al. (*29*) found lead pollution near the damaged reactor and symptoms consistent with lead exposure, suggesting that the observed hypertension may be lead related. Kodama (*30*), reviewing the data on cardiovascular disease following the atomic bomb explosions in Hiroshima and Nagasaki, failed to find evidence of an effect on blood pressure in the majority of studies (*29*).

### Thyroid Changes Associated with Former Residence

We found no increase in the prevalence of enlargement of the thyroid gland (goiter) in more exposed compared to less exposed communities. The exposure estimate was based on the ground level and body burden of $^{137}Cs$, whereas any thyroid effects are more likely to be related to exposure to isotopes of iodine, which would have occurred in the early post-explosion period. We have no reason to believe that $^{137}Cs$ is related to thyroid nodularity. However, Likhtarev et al. (*31*), in deriving thyroid dose assessment in the Ukraine, used $^{137}Cs$ deposition, among other variables, and were able to show a good relationship between derived doses and thyroid cancer.

Our results of blood tests show no deviations from expected levels of antithyroid autoimmune processes or other thyroid function tests in children, which might be related to exposures to $^{131}I$ (Wynberg et al., unpublished data). We found no differences that could be related to $^{137}Cs$ exposure. We also could not define gradients for $^{131}I$.

The data on occurrence of thyroid cancer in radiation-exposed children (*32*) lend importance to the increase in nodularity observed in children from the more exposed communities, even though the results are not significant. Three thyroid cancers, two in adolescents, have been observed in our population.

### Other Chronic Conditions

The excess of chronic health disorders (self-reported and physician-diagnosed) in liquidators compared to residents and in residents from more exposed communities compared to those from less exposed is not explicable as a mere consequence of stress.

Kulakov et al. (*14*), after studying 688 pregnant women and their babies and 7000 delivery records during the 3 years before the Chernobyl accident and 5 years afterward, concluded that health of mothers, fetuses, and children in contaminated areas were significantly affected by radiation. In a district where 53% of women lived with ground level pollution greater than 20 kCi/km$^2$, there was an increase in perinatal mortality for the 3 years after the accident from 15.1% to 17.8%, whereas in another district with only 20% of the women exposed to this level of contamination, the perinatal mortality rate dropped from 11.5% to 7.3%. Guskova (*33*) considers that increasing rates of cardiovascular pathology, endocrine dysfunctions, and worsening of respiratory and digestive system diseases among residents of exposed regions are the result of the chronic stress.

Nussbaum and Kohnlein (*21*) point out some inconsistencies and unresolved questions concerning low-dose health effects of ionizing radiation. They deplore the use of A-bomb survivor studies as a "universal standard." They note that the hypothesis of reduced biological effectiveness of fractionated low-dose exposure compared to that of the same acute dose is not supported by data on human populations. They call attention to observations of other diseases than radiation-induced malignancy, suspected to be associated with relatively low levels of internal exposure. Our observations also indicate that effects other than cancer should be investigated.

## Conclusions

It may be reasonably assumed that the health effects we observed are associated with exposure to radiation from the Chernobyl disaster. However, only some of the effect may be due to radiation itself. The occurrence of stress disorders in persons exposed to radiation from the Chernobyl accident is not a valid argument against the etiological role of radiation alone or in combination with other pollutants. The exposures of relevance could be brief or long term; for example, food contamination by $^{137}Cs$.

As a result of our findings, we are convinced that a preoccupation with carcinogenesis as the principal consequence of Chernobyl exposures has led to a distorted view of health effects of low-level radiation. While such attention has stimulated studies of possible mutagenic mechanisms, mutagenesis may also result in non-neoplastic abnormalities. Elsewhere we report our study of oxygen free radical mechanisms and possible consequences (*34*).

We believe that our data point to opportunities to both relieve the anxieties and improve the health of those who were exposed, as well as contribute to the knowledge of low-level radiation effects.

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# Identifying Chemical Carcinogens and Assessing Potential Risk in Short-term Bioassays Using Transgenic Mouse Models

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Cancer is a worldwide public health concern. Identifying carcinogens and limiting their exposure is one approach to the problem of reducing risk. Currently, epidemiology and rodent bioassays are the means by which putative human carcinogens are identified. Both methods have intrinsic limitations: they are slow and expensive processes with many uncertainties. The development of methods to modify specific genes in the mammalian genome has provided promising new tools for identifying carcinogens and characterizing risk. Transgenic mice may provide advantages in shortening the time required for bioassays and improving the accuracy of carcinogen identification; transgenic mice might now be included in the testing armamentarium without abandoning the two-year bioassay, the current standard. We show that mutagenic carcinogens can be identified with increased sensitivity and specificity using hemizygous p53 mice in which one allele of the p53 gene has been inactivated. Furthermore, the TG.AC transgenic model, carrying a v-Ha-*ras* construct, has developed papillomas and malignant tumors in response to a number of mutagenic and nonmutagenic carcinogens and tumor promoters, but not to noncarcinogens. We present a decision-tree approach that permits, at modest extra cost, the testing of more chemicals with improved ability to extrapolate from rodents to humans. *Key words*: bioassays, carcinogens, risk, transgenic mouse models. *Environ Health Perspect* 103:942–950 (1995)

There has been a continuing, worldwide effort to control the public health and social burden of cancer. One aspect of this effort is the identification of agents that cause cancer in humans and the reduction of exposure to acceptable levels of risk by regulatory action. Human carcinogens have been identified with greatest certainty by epidemiological studies. However, the rodent bioassay has come into prominence because using epidemiology to identify causal agents is feasible only to a limited extent. Identification of human carcinogens by rodent bioassay, however, has a number of important limitations: the most perplexing problem is the uncertainty of whether all the agents that cause tumors in rodents do so in humans. A corollary to this problem is the uncertainty of whether the potency and dose–response relationships are the same in rodents and humans. There is also the cost and duration of the National Toxicology Program (NTP) bioassays, the gold standard for animal bioassays; the expense is so high that only a few chemicals per year can be evaluated. There are many chemicals in commercial use or in the environment that have not been tested, and thousands of new chemicals are synthesized each year. There is a clear need to improve the process of carcinogen identification not just so that more chemicals can be evaluated, but also to achieve a better understanding of human risk. Short-term testing of chemicals in bacteria and cultured cells contributes valuable information, but such assays are regarded as ancillary to epidemiology and animal testing because of their limited biological complexity. For the foreseeable future, improvements in identifying potential human carcinogens will come from improvements in the rodent bioassay.

A useful new tool which has emerged from developments in molecular biology is the transgenic rodent. Such animals have modified genes that cause them to respond to carcinogens in advantageous ways. This paper describes the response characteristics of two of the most promising of these transgenics, p53+/ and TG.AC, and discusses ways in which they may be used to improve carcinogen bioassay.

The p53+/- line responds rapidly to genotoxic carcinogens, and the TG.AC line responds rapidly to nongenotoxic carcinogens. Positive results in either could lead to an NTP bioassay modified to focus on low-level dose response; verification of carcinogenicity could be substantially accomplished by the transgenic assay, and the transgenic assay could provide guidance for dose selection. Thus, the use of these two transgenic models could accomplish several important objectives: 1) accelerated testing of environmental chemicals; 2) prioritization of chemicals for NTP bioassay; and 3) focusing the NTP bioassay on one primary objective, low-level dose response, instead of the dual objective of determining whether an agent is carcinogenic, which requires administration of high doses, and at the same time trying to determine dose–response characteristics at low doses. Conversely, as experience is accrued in the use of these transgenics, it may become evident that with agents that are clearly positive with structural alert evaluations, *in vitro* mutagenicity, and transgenic bioassays, the full NTP bioassay is not needed.

## Transgenic Mouse Models

Appropriate transgenic mouse lines offer the opportunity to develop relatively short-term *in vivo* models to identify potential carcinogens and other toxic agents. Such models include transgenic mice carrying reporter genes that may serve as targets for mutagenic events (*1,2*) or mice carrying specific oncogenes or inactivated tumor-suppressor genes that are important factors contributing to the multistage process of carcinogenesis (*3–5*). Mouse lines with defined genetic alterations that result in overexpression or inactivation of a gene intrinsic to carcinogenesis, but that are insufficient alone for neoplastic conversion, are promising models for chemical carcinogen identification and evaluation. Likely gene targets for alteration that might result in this phenotype are 1) the activation (by mutation) of the c-Ha-*ras* protooncogene, which alters signal transduction and growth control (*6*), and 2) inactivation of the p53 tumor-suppressor gene, which is critical to cell cycle control and DNA repair (*7–9*). These tumor genes are often mutated and/or amplified (c-Ha-*ras*) or mutated and/or lost (p53) in human and rodent tumors (*10–13*).

***p53 +/- mice.*** Mice with only a single wild-type p53 allele provide a distinct target for mutagens and are analogous to humans at risk due to heritable forms of

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cancer, such as the Li-Fraumeni syndrome (*14*). The reduction in p53 gene dosage by this "germline first hit" increases the probability that a second mutagenic event will cause either loss of p53 tumor-suppressor function or gain of transforming activity by requiring (at minimum) only a single mutation (*10–13*). Mice with inactive p53 genes are viable and, in the hemizygous state, have a low background tumor incidence for up to 12 months of age, whereas nullizygous mice have a higher rate of spontaneous tumors at sites apparently determined by the strain's genetic background (*15,16*).

We conducted concurrent 6-month carcinogenesis studies using male and female C57BL/6 mice hemizygous for the wild-type p53 gene together with their homozygous wild-type p53 siblings. We used two carcinogens that exhibited trans-species carcinogenicity (*17*) (i.e., induced tumors in both mice and rats), in long-term rodent bioassays and that were positive in the *Salmonella* mutagenesis assay (*18–20*): *p*-cresidine (*21*) and 4-vinyl-1-cyclohexene diepoxide (VCD) (*22*). For comparison, we used two nonmutagenic carcinogens that were carcinogenic in only one of two test species: *N*-methylolacrylamide (NMOA) (*23*) and reserpine (*24*). As a negative control, we used *p*-anisidine (*25*), an analogue of *p*-cresidine, which was positive in the *Salmonella* assay and negative in rats and mice in two-year carcinogenesis studies. These chemicals represent the opposite ends of the spectrum of biological activity observed in rodent bioassays (*18–20*). To facilitate this comparison, we used the same exposure conditions and chemical doses as used in the 2-year bioassays.

***TG.AC mice.*** TG.AC mice carry a v-Ha-*ras* oncogene fused to the promoter of the ζ-globin gene (*26*). The v-Ha-*ras* transgene has point mutations at codons 12 and 59, and the site of integration of the transgene confers on these mice the characteristic of genetically initiated skin as a target for tumorigenesis in the context of the intensively studied mouse-skin tumorigenesis model (*27*). An important consideration of the TG.AC mouse model is that the transgene is not constitutively expressed in the skin, and the untreated skin appears normal when compared to the skin of the wild-type FVB/N parent strain (*26,28*). In addition, the spontaneous incidence of skin papillomas in the dorsal skin of untreated mice is very low to zero. Heterozygous or homozygous TG.AC mice receiving repetitive topical treatment with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) or other well-described mouse-skin promoters (benzoyl peroxide, mezerein, or methyl ethyl ketone peroxide) readily develop, as early as 4–6 weeks after treatment, benign squamous papillomas which may progress to malignancy (*26,29*).

Our approach to evaluating and using the TG.AC mice is different from that for the p53+/- mice. Many of the chemicals tested initially in TG.AC were used to characterize the phenotype of the line rather than to directly assess its use in identifying carcinogens. As the studies progressed, the potential use of this line to identify putative nongenotoxic carcinogens and to permit extended dose–response analyses became apparent.

The activities of 20 chemicals have been evaluated in homozygous female TG.AC mice using a skin-painting protocol. These chemicals represent well-known mouse skin initiators and promoters (*26,29*), specific intermediate metabolites known to occur in the signal transduction pathways induced by TPA binding to its receptor, protein kinase C, and carcinogens and noncarcinogens identified in the NTP 2-year bioassay. These chemicals include genotoxic and nongenotoxic carcinogens and noncarcinogens that represent a spectrum of chemicals that have exhibited a wide range of biological activities in the 2-year NTP bioassays.

We have used the TG.AC mouse model in both retrospective and prospective studies to examine its potential to identify the activity of possible carcinogens and to complement the standard 13-week prechronic toxicity studies used by the National Toxicology Program (NTP) to identify target organs and dose–response relationships leading to a 2-year rodent bioassay. While the TG.AC mouse might seem most appropriate for evaluating the activity of those chemicals scheduled for a 2-year skin-paint study protocol, our retrospective studies with carcinogens identified in the 2-year NTP bioassays indicated that chemicals administered in the feed or via gavage can also exhibit activity in the skin of topically treated transgenic mice when using a dose range representative of that used in the 2-year bioassay protocol.

## Methods

Mice were treated and maintained in accordance with the NIH Guidelines for Humane Care and Use and under an institutional, peer-reviewed animal study protocol.

***p53 +/- mice.*** Male and female mice hemizygous for wild-type p53 (TSG-p53) and homozygous wild-type siblings were obtained from GenPharm International (Mountain View, California). Treatments began in a staggered fashion at 15–18 weeks of age after a 3-week acclimation period. Male mice were housed singly, and female mice were housed in groups; both were fed a pelleted or powdered defined diet (#D10010, Research Diets, New Brunswick, New Jersey) and water *ad libitum*. Wild-type sibling and hemizygous p53 mouse control groups contained 10 mice each and hemizygous p53 treatment groups contained 15 (low-dose) or 20 (high-dose) mice. Assignment of uneven numbers was determined by the number of mice available and unknown effects of toxicants in these mice. Test chemicals were administered in the diet daily, by topical administration (2 times/week; 100 µl acetone vehicle), or by gavage (5 times/week; corn oil vehicle) for 24 weeks (Table 1). The mice were held an additional 4–6 weeks to allow tumor development after interim sacrifices indicated chemical-specific differences in the rate of appearance of gross lesions. Clinical observations and body weights were recorded weekly. All mice received a gross necropsy and microscopic examination of gross lesions and target tissues. Mice were held 4–6 weeks after cessation of treatment to allow for a staggered termination of the experiments, following determination of tumor progress in those mice with gross lesions.

***TG.AC mice.*** Groups of 10–15 female homozygous TG.AC mice, housed 5 per cage, were first treated when 10 –12 weeks old. Mice were fed Purina Pico Chow, no. 5058, and water *ad libitum*. The dorsal skin at the application site was clipped 1– 2 days before administration of the first dose and as needed throughout the dosing period. Chemicals were administered topically to the clipped area with 200 µl of acetone or 70–95% ethanol 2 to 5 times per week for 20 weeks. Mice treated concurrently with the vehicle solvent or 1.25 µg TPA served as the negative and positive controls, respectively. Mice were examined weekly for the development of skin papillomas at the application site.

## Results

***p53 +/- mice.*** Topically administered VCD induced squamous cell and basal cell carcinomas in male and female F344 rats and male and female B6C3$F_1$ mice in 2-year bioassays (*22,30*) that first appeared after 54 weeks of treatment (Table 1). In the studies described here, both hemizygous p53 and wild-type male and female mice showed an apparent dose-related decrease in body weight gain (not less than 10% of the concurrent control) throughout the VCD skin paint study that was reversed after the treatment period, especially in the females (data not shown). Microscopic analysis of gross lesions revealed that there

**Table 1.** Target organ tumor incidence in the 24-week study in C57BL/6 p53-deficient mice and the NTP 103-week study in B6C3F$_1$ hybrid mice

| Chemical (route: target tissue) | Dose | Time[a] (weeks) | Mouse strain (C57BL/6) Hemizygous p53 M | Hemizygous p53 F | Wild type M | Wild type F | Dose | Time[a] (weeks) | Mouse strain (B6C3F$_1$) Wild type M | Wild type F |
|---|---|---|---|---|---|---|---|---|---|---|
| 4-Vinyl-1-cyclohexene diepoxide[b] | 0 mg | 24 + 4 | 0/7 | 0/8 | 0/5 | 0/5 | 0 mg | 103 | 0/50 | 0/50 |
| (topical: skin) | 12.5 mg | 24 + 4 | 2/7 | 0/8 | — | — | 5 mg | 103 | 40/50 | 37/50 |
| | 25.0 mg | 24 + 4 | 3/10 | 3/8 | 0/5 | 0/5 | 10 mg | 103 | 43/50 | 43/50 |
| *p*-Cresidine | 0% | 24 + 2 | 0/5 | 0/5 | 0/5 | 0/5 | 0% | 103 | 0/50 | 0/50 |
| (diet: urinary bladder) | 0.25% | 24 + 2 | 4/7 | 0/8 | — | — | 0.25% | 103 | 31/31 | 44/46 |
| | 0.5% | 24 + 2 | 9/10 | 4/10 | 0/5 | 0/5 | 0.5% | 103 | 40/42 | 41/46 |
| *p*-Anisidine | 0% | 24 + 6 | 0/5 | 0/5 | 0/5 | 0.5 | 0% | 103 | Noncarcinogenic | |
| (diet: none) | 0.225% | 24 + 6 | 0/8 | 0/7 | — | — | 0.25% | 103 | | |
| | 0.45% | 24 + 6 | 0/10 | 0/10 | 0/5 | 0/5 | 0.5% | 103 | | |
| *N*-Methylolacrylamide | 0 mg/kg | 24 + 6 | 0/7 | 0/7 | 0/5 | 0/5 | 0 mg/kg | 103 | 12/50 | 3/50 |
| (gavage: liver) | 25 mg/kg | 24 + 6 | 0/7 | 0/7 | — | — | 25 mg/kg | 103 | 17/50 | 4/50 |
| | 50 mg/kg | 24 + 6 | 0/10 | 0/10 | 0/5 | 0/5 | 50 mg/kg | 103 | 26/50 | 17/49 |
| Reserpine[c] | 0% | 24 + 6 | 0/7 | 0/8 | 0/5 | 0/5 | 0% | 103 | 0/50 | 0/50 |
| (diet: M, seminal vesicle; | 0.0005% | 24 + 6 | 0/8 | 0/7 | — | — | 0.0005% | 103 | 0/50 | 7/49 |
| F, mammary gland) | 0.001% | 24 + 6 | 0/10 | 0/10 | 0/5 | 0/5 | 0.001% | 103 | 0/50 | 7/48 |

[a]Length of exposure plus holding (weeks) before euthanasia and necropsy.
[b]VCD dosage was administered (mg/mouse) in 200 ml acetone 2 ×/week or 5 ×/week in the 24-week or 105-week studies, respectively; resulting in an equivalent cumulative weekly dose.
[c]Due to excessive weight loss and mortality in the high-dose group, survivors were converted to the low-dose feed concentration after 4 weeks of treatment and maintained in the same cages for the remainder of the study without further loss. Data were also maintained separately because of sustained body weight depression and clinical observations.

was an apparent dose-related increase in skin tumors (squamous cell or basal cell carcinoma or fibrosarcoma) in VCD skin-painted mice hemizygous for the p53 gene, but not in the respective p53 +/- vehicle controls or in the control or high-dose, homozygous wild-type sibling male or female mice (Table 1). The incidence of nodular epidermal hyperplasia appeared to be a continuum with the development of squamous cell carcinomas, which may be significant since carcinomas appeared virtually in the absence of papillomas. Repetitive exposure of the skin to carcinogens has been shown to result in complete loss of p53 and malignant skin tumors without progression through a benign tumor phase (*31,32*).

*p*-Cresidine was mutagenic in the *Salmonella* assay and caused urinary bladder tumors in rats and mice when administered in the diet (*18,21*). In the 2-year studies, the first bladder tumors were observed in B6C3F$_1$ mice at 40 and 44 weeks of treatment for high-dose male and female mice, respectively (*21*) (Table 1). In the current dose-feed studies, p53 +/- and wild type mice treated with *p*-cresidine experienced a similar short-term transient and dose-related decrease in body weight gain, which was greater in males than in females of either mouse line and was similar to that observed in the 2-year studies. It is important that both p53 +/- and wild-type mice exhibited similar systemic toxicity, but the latter did not develop tumors. After 6 months of exposure and a 2-week holding period, microscopic analysis of gross lesions revealed an apparent dose-related increase in urinary bladder transitional cell carcinomas in male and female p53 hemizygous mice, but not in the p53 +/- controls or in the high-dose, homozygous wild-type siblings (Table 1). Tumors were grossly observed as thickened bladder walls or large masses, which frequently were associated with hydronephrosis due to bladder occlusion. These characteristics were similar to the tumors induced in the B6C3F$_1$ mice used in the 2-year bioassay. Microscopically, the carcinomas were invasive masses composed of markedly anaplastic cells and/or cells exhibiting squamous differentiation. In one high-dose p53 +/- female, a squamous papilloma of the bladder mucosa was observed. Deaths related to tumor induction that occurred during the study (Table 2) were especially frequent in male mice hemizygous for wild-type p53.

*p*-Anisidine, an analogue of *p*-cresidine, was mutagenic in the *Salmonella* assay and was not carcinogenic to male or female F344 rats or B6C3F$_1$ mice in 2-year dosed-feed studies (*25*). In those studies, the dose levels used induced body weight depression without an increase in mortality in the treated groups relative to the controls. In the present dosed-feed study, *p*-anisidine (administered equimolar to *p*-cresidine) did not induce any significant clinical observations or depressed body weight gain relative to the control groups (Tables 1 and 2). However, 5 of 15 low-dose p53 +/- mice died of unexplained causes during the study. None of the high-dose p53 +/- mice were affected. No gross lesions or microscopic lesions in the urinary bladder were observed after 24 weeks of dietary administration and a 6-week holding period.

NMOA was nonmutagenic in the *Salmonella* assay, although it contains a chemical structural alert indicating electrophilic potential and was carcinogenic to mice in 2-year bioassays when administered by oral intubation (*18,23*) (Table 1). In the 2-year bioassays, NMOA-treated B6C3F$_1$ male and female mice exhibited an increase in body weight gain relative to controls and increased incidences of tumors in the Harderian gland, liver, lung, and ovary. In the studies with transgenic mice, NMOA depressed body weight gain in male mice (greatest in high-dose, wild-type siblings) and slightly increased body weight gain in female mice (data not shown). Mortality was slightly increased in high-dose male and female mice hemizygous for p53, but no induced tumors were observed (Table 2).

Reserpine was not mutagenic in the *Salmonella* assay, but in the 2-year dosed feed studies, it induced mammary gland tumors in B6C3F$_1$ female mice (*18,24*) (Table 1). In these transgenic mouse studies, reserpine administered at 0.001% of the diet to p53 +/- mice resulted in lethargy, tremors, piloerection, and severe weight loss and death (Table 2), requiring immediate reduction of the high dose after 4 weeks of treatment to the lower dose level for the duration of the study in the hemizygous p53 mice to prevent further deaths. No tumors or gross toxic lesions were

observed in high-dose mice converted to the low dose, or in low-dose mice that survived to the end of the study.

***TG.AC mice.*** In the retrospective studies, seven of eight chemicals were correctly identified as carcinogens or noncarcinogens when administered via the skin paint protocol in TG.AC female mice (Table 3). Benzene (*18,33–35*), a proven human carcinogen and a multisite, trans-species rodent carcinogen, readily induced papillomas in 70% of the transgenic mice by 10 weeks of exposure. Mirex (*36*), a nongenotoxic liver carcinogen when administered in the feed to rats, induced papillomas, which were observed as early as 7 weeks. In the NTP studies, *o*-benzyl-*p*-chlorophenol (*37*), a nonmutagenic carcinogen, caused kidney tumors in male B6C3F$_1$ mice when administered by oral intubation (gavage) (Table 3). When administered topically as a promoter (3 mg, 3 times/week) for 50 weeks following an initiating dose of dimethylbenz[*a*]anthracene, *o*-benzyl-*p*-chlorophenol induced a low incidence of papillomas (2 papillomas/mouse) in 36% of the treated female mice (*38*). Topically applied *o*-benzyl-*p*-chlorophenol (3 mg, 3 times/week for 20 weeks) induced papillomas in 80% of the treated TG.AC mice, with an overall

**Table 2.** Incidence of mortality during the in-life portion of the bioassay before termination

| Chemical | Dose | Time[a] (weeks) | Mouse strain: Hemizygous p53 M | Hemizygous p53 F | Wild type M | Wild type F |
|---|---|---|---|---|---|---|
| 4-Vinyl-1-cyclohexene diepoxide[b] | 0 mg | 24 + 4 | 0/7 | 0/8 | 0/5 | 0/5 |
| | 12.5 mg | 24 + 4 | 0/7 | 2/8 | — | — |
| | 25.0 mg | 24 + 4 | 2/10 | 0/10 | 0/5 | 0/5 |
| *p*-Cresidine | 0% | 24 + 2 | 0/5 | 1/5 | 0/5 | 0/5 |
| | 0.25% | 24 + 2 | 2/8 | 0/7 | — | — |
| | 0.5% | 24 + 2 | 7/10 | 2/10 | 0/5 | 0.5 |
| *p*-Anisidine | 0% | 24 + 6 | 0/5 | 0/5 | 0/5 | 0.5 |
| | 0.225% | 24 + 6 | 2/7 | 3/8 | — | — |
| | 0.45% | 24 + 6 | 0/10 | 0/10 | 1/5 | 0/5 |
| *N*-methylolacrylamide | 0 mg/kg | 24 + 6 | 0/7 | 0/7 | 0/5 | 0/5 |
| | 25 mg/kg | 24 + 6 | 1/7 | 1/7 | — | — |
| | 50 mg/kg | 24 + 6 | 2/10 | 2/10 | 0/5 | 1/5 |
| Reserpine[c] | 0 % | 24 + 6 | 0/7 | 0/8 | 0/5 | 0/5 |
| | 0.0005 % | 24 + 6 | 1/8 | 1/7 | — | — |
| | 0.001 % | 24 + 6 | 7/10 | 6/10 | 1/5 | 1/5 |

[a]Length of exposure plus holding (weeks) before euthanasia and necropsy.
[b]VCD dosage was administered (mg/mouse) in 200 ml acetone 2 ×/week or 5 ×/week in the 24 week or 105 week studies, respectively; resulting in an equivalent cumulative weekly dose.
[c]Due to excessive weight loss and mortality in the high dose group, survivors were converted to the low dose feed concentration after 4 weeks of treatment and maintained in the same cages for the remainder of the study without further loss. Data were also maintained separately because of sustained body weight depression and clinical observations.

**Table 3.** Retrospective evaluation of NTP chemicals for activity in TG.AC transgenic mice

| Chemical | SAL[a] | NTP bioassay: Route[b] | Maximum dose: Rat | Maximum dose: Mouse | Rat[c]: M | Rat[c]: F | Mouse[c]: M | Mouse[c]: F | TG.AC skin paint: LED[d] | TTFP[e] | Avg. papillomas/mouse[f] | % Mice with papillomas[f] | Activity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Benzene | – | G | 200 | 100 | + | + | + | + | 200 µl, 2×/week | 5 | 7.4 | 77 | + |
| Benzethomium chloride | – | SP | 1.5, 5×/week | 1.5, 5×/week | – | – | – | – | 60 µg, 5×/week | 8 | 0.55 | 22 | – |
| *o*-Benzyl-*p*-chlorophenol | – | G | 240 | 480 | – | E (K) | + (K) | – | | | | | |
| | | SP | ND | 3 mg/mouse, 3×/week (50weeks) | ND | ND | +[g] | +[g] | 3 mg, 3 ×/week | 7 | 3.0 | 80 | + |
| 2-Chloroethanol | + | SP | 100, 5 ×/week | 15, 5 ×/week | – | – | –[g] | –[g] | 20 mg, 5 ×/week | 10 | 0.10 | 11 | – |
| *p*-Cresidine | + | F | 1.0 | 0.46 | + (UB) | + (UB) | + (UB) | + (UB) | 80 µg, 2×/week | 6 | 5.0 | 58 | + |
| Ethyl acrylate | – | G | 200 | 200 | + (S) | + (S) | + (S) | + (S) | 30 mg, 3×/week | 15 | 0.6 | 50 | – |
| Mirex | – | F | 0.005 | ND | + (L, AG) | + (L, HS) | ND | ND | 54.5 µg, 3×/week[h] | 7 | 12 | 70 | + |
| Phenol | – | W | 0.5 | 0.5 | – | – | – | – | 3.0 mg, 2×/week | 7 | 0.20 | 0.16 | – |

ND, not done.
[a]SAL: *Salmonella* mutagenicity results provided by E. Zeiger, National Toxicology Program.
[b]Route of administration of test chemical. G, oral gavage, dose in mg/kg, given 5×/week; SP, skin paint, dose is in mg/kg except where otherwise indicated; F, feed, dose in %; W, drinking water, dose in %.
[c]F344 rats and B6C3F$_1$ mice except as noted. Tumor sites in parentheses: K, kidney; UB, urinary bladder; S, stomach; L, liver; AG, adrenal gland; HS, hematopoietic system. E, equivocal results.
[d]LED, lowest effective dose per mouse that induced papillomas at the site of application or highest "no effect" dose administered.
[e]TTFP, time (weeks) to first observation of a skin papilloma in any mouse of that dose group.
[f]At 20 weeks.
[g]CD-1 mice.
[h]Data from a collaborative study with R. Smart, North Carolina State University.

incidence of 3 papillomas/mouse (Table 3). *p*-Cresidine, the genotoxic urinary bladder carcinogen (*18,21*) described above in p53 +/- mouse studies, not only induced papillomas in TG.AC mice, but showed systemic effects by causing the same preneoplastic lesions in the bladder epithelium that were observed in $B6C3F_1$ mice. Benzethonium chloride (*39*) and phenol (*18,40*), both nongenotoxic noncarcinogens, and 2-chloroethanol (*18,41*), a genotoxic noncarcinogen, were inactive in the TG.AC mice. Ethyl acrylate, a nongenotoxic carcinogen (*19,42*) induced only forestomach tumors in the 2-year bioassay, a consequence of gavage exposure and cell proliferation (*43–45*). Ethyl acrylate was inactive in TG.AC mice, and no gross systemic effects were observed at the end of the 20-week skin-paint study that had induced chronic epidermal hyperplasia at the application site (Table 3). It is noteworthy that another trans-species forestomach carcinogen, diglycidyl resorcinol ether (*19,46*), was also inactive in a long-term skin paint study in female Swiss mice (*47*).

Four chemicals undergoing skin-painting studies in NTP bioassays were evaluated prospectively for activity in TG.AC mice using dose ranges representative of those used in the 2-year studies (Table 4). Lauric acid diethanolamine (NTP studies in progress) and methyl ethyl ketone peroxide (*48*) readily induced papillomas as early as 4 and 5 weeks, respectively (Table 4). No papillomas were induced by 20 weeks of treatment with diethanolamine (NTP studies in progress) or triethanolamine (*49*). The accuracy of these predictive studies await the outcome of the NTP bioassays.

## Discussion

Hemizygous p53 mice, but not their homozygous wild-type sibling controls, developed tumors after treatment with two model mutagenic carcinogens, but not with two model nonmutagenic carcinogens or with a mutagenic noncarcinogen. Tumors appeared much sooner, but at the same organ sites, in the hemizygous p53 mice as they did in $B6C3F_1$ mice in the 2-year bioassays with the mutagenic carcinogens. These limited data suggest that the hemizygous p53 mice may exhibit a specificity of response for mutagenic carcinogens as well as increased sensitivity when compared to their normal, wild-type littermates. However, neither of the nonmutagenic carcinogens induced tumors in their expected target tissues in either hemizygous p53 or homozygous wild-type sibling mice when administered for 6 months by the same route used in 2-year bioassays. These short-term bioassay results are consistent with the 2-year NTP bioassays for the mutagenic carcinogens, indicating that the C57BL/6 strain responded similarly to the $B6C3F_1$ hybrid. Although the number of mice used in these studies was small compared to long-term toxicology studies, these data indicate that these mice may have increased susceptibility to mutagenic carcinogens (*15,16,32*) and that mutagenic chemicals are required to initiate the carcinogenic responses observed.

The skin-paint studies in the TG.AC transgenic mice indicate that the model can complement the standard NTP 13-week subchronic bioassays that are used to identify potential target organ sites for toxic effects or neoplasia in the 2-year bioassay. Furthermore, the model is sensitive to chemicals that do not necessarily target the skin when administered by other routes of exposure. Some major advantages of this model are that the skin-tumor response can be visibly observed and induced within a 20-week dosing regimen; furthermore, the dosing protocol (e.g., dose frequency per week) can be varied according to the toxicity of the chemical. The target organ site, the dorsal skin, is normal until stimulated by a series of chemically induced events that lead to the induction of skin papillomas (*26,29*). The latency period is short, with the first tumors appearing as early as 5 weeks and usually by 10 weeks when using a 20-week dosing protocol. Further, in every instance where the treated mice were observed an additional 10–20 weeks after cessation of dosing, a subpopulation of the papillomas progressed to malignancy. Other important considerations are that the life span of homozygous TG.AC mice is well over a year, and the sporadic papilloma incidence at the target site, dorsal skin, is very low to zero in untreated or vehicle-control mice. Untreated TG.AC mouse skin has normal morphological and physiological characteristics that are maintained throughout the mouse's life span. The transgene is not constitutively expressed at the target sites, but is expressed in association with the proliferative cell component observed in early papillomas (*28*). From these limited data, the TG.AC mouse model appears to identify genotoxic and nongenotoxic carcinogens and to discriminate a high proportion of carcinogens and noncarcinogens.

### Dose and Dose-Rate Studies

Another potential advantage of the transgenic models is their capability of providing dosimetric data. A major controversy in toxicology is the nature of the dose response derived from rodent bioassays. Due to the variable potency and toxicity of chemicals and the variable and often high rate of site-specific sporadic tumors (*50*), few bioassays produce data that can be appropriately evaluated at relatively low doses. For example, benzene induced a broad spectrum of tumors in a relatively high proportion of mice in the rodent bioassay (*33,35*). When all significant sites of tumorigenesis were combined, even the low dose (20 mg/kg body weight) induced tumors in approximately 60% of male and female $B6C3F_1$ mice. As shown in Figure 1, these results suggest that the response at all doses could represent the upper portion of a dose–response curve, and the pattern was similar for papillomas induced by benzene in TG.AC mice. However, the prospects that lower doses in $B6C3F_1$ mice could produce data sufficient to allow the true shape of the dose response to be deter-

**Table 4.** Prospective evaluation of NTP chemicals for activity in TG.AC transgenic mice

| | | NTP skin paint bioassay (maximum dose)[a] | | TG.AC skin paint | | | | |
|---|---|---|---|---|---|---|---|---|
| Chemical | SAL[b] | F344 rat | $B6C3F_1$ mouse | LED (mg)[c] | TTFP[d] | Avg. papillomas/mouse[e] | % Mice with papillomas[e] | Activity |
| Diethanolamine | – | 64 | 160 | 20, 5×/week | – | 0.0 | 0.0 | – |
| Lauric acid diethanolamine | – | 100 | 200 | 10, 5×/week | 4 | 6.1 | 92 | + |
| Triethanolamine | – | 250 | 2000 | 30, 5×/week | 7 | 0.17 | 8.3 | – |
| Methyl ethyl ketone peroxide | –,+ | 3.6 mg/rat | 3.6 mg/mouse | 5.0, 2×/week | 5 | 50 | 100 | + |

[a]Results pending completion of 2-year bioassay study and peer review. Doses in mg/kg, except for methyl ethyl ketone peroxide, which was administered five times/week.
[b]SAL, *Salmonella* mutagenicity results provided by E. Zeiger, National Toxicology Program.
[c]LED, lowest effective dose that induced papillomas at the site of application or highest "no effect" dose administered.
[d]TTFP, time (weeks) to first observation of a skin papilloma in any mouse of that dose group.
[e]At 20 weeks.

mined, even by extrapolation, is quite unlikely because of the high proportion of control mice that developed tumors at many of the same sites as the benzene-exposed mice by the end of the 104 week study. Because the frequency of spontaneous tumors increases with time, the distinction between induced and spontaneous tumors becomes more difficult to discern. The principal attribute of the two transgenic mouse lines described here is that induced tumors develop within a time frame in which the spontaneous tumor incidence is effectively zero, thus reducing the confounding effects observed in an aging population.

The benzene study was repeated in TG.AC mice using doses as low as 75 µl/week/mouse. The high dose portion of the dose–response pattern, similar to that for the bioassay, was observed again, but it is noteworthy that the response curve also extended into the low-dose range (Fig. 2). These results suggest that it will be possible to compare the dose response of various chemicals, including known human carcinogens, and to study dose-rate effects of chemicals, including time-to-tumor analysis, which are critical (*51*). Further, in the case of the p53 +/- mouse line, it is possible to design molecular dosimetry studies because mutational spectra of the single functional wild-type p53 alleles may be determined (*12*).

## Identification of Presumptive Carcinogens

Although large numbers of new chemicals are continually being synthesized for potential use, they need not all be subjected to a complete rodent bioassay. Over the past three decades, information has been developed in the characterization of the biological effects of most major structural classes of chemicals. It is possible to use available data to predict the relative toxicity or carcinogenicity of many untested chemicals.

The most predictive mechanistic determinant for chemical carcinogenicity is mutagenic potential (*52*). It has been recognized for many years that chemicals that demonstrate mutagenicity *in vitro* are also carcinogenic to rodents (*34,53*). An extensive evaluation of the association between mutagenicity and carcinogenicity of 114 chemicals that were subjected to rodent carcinogenicity bioassays showed that approximately 70% of the chemicals that were mutagenic in the Ames *Salmonella* mutagenicity assay were rodent carcinogens (*53,54*). Based on this relationship, mutagenicity, as well as other information relating to chemical structure and toxicity, were used to predict carcinogenic potential in two rodent species (*55*). Forty-four chemicals that were undergoing bioassays, for which the results were not yet available, were the focus of an effort to demonstrate that chemical structure, mutagenicity, and data from subchronic toxicity studies were sufficient to prospectively identify many potential carcinogens with a high degree of certainty. The principal results of the effort showed that a chemical of unknown carcinogenicity could be predicted to be in one of three possible categories: probably carcinogenic, probably noncarcinogenic, or of uncertain activity (*56*). All of the chemicals that induced trans-species carcinogenic effects were correctly predicted. Trans-species rodent carcinogens demonstrate similar properties (mutagenicity and toxicity) of proven human carcinogens and represent a more proximate risk to human health than species-restricted carcinogens (*17*). The overall specific predictions were 75% concordant with the results of the rodent bioassays; the least success occurred with the chemicals that produced uncertain effects in the bioassay (i.e., equivocal evidence of carcinogenicity or single-site, sex-specific, and species-specific effects). Integration of data on chemical structure, mutagenicity, and systemic toxicity tended to overpredict carcinogenicity (i.e., 7 of 10 missed predictions were for carcinogenicity) (*56*), but any predictive strategy should be risk-adverse, that is, involve principally false-positive errors (*57–60*).

It also became clear from this exercise that while mutagenicity is a major mechanistic determinant, this property is neither sufficient nor necessary for carcinogenicity. Approximately one-third of the nonmutagenic chemicals tested in bioassays have shown some evidence of carcinogenicity. Conversely, approximately one-third of the chemicals that were mutagenic *in vitro* were not carcinogenic in the bioassay (*52,54*). Thus, mechanism-based predictions were the most accurate for potent carcinogens and for chemicals that were clearly noncarcinogens. Use of this information alone could reduce significantly the dependence on rodent bioassays. However, if further improvements in the prediction process can be achieved, it should be possi-

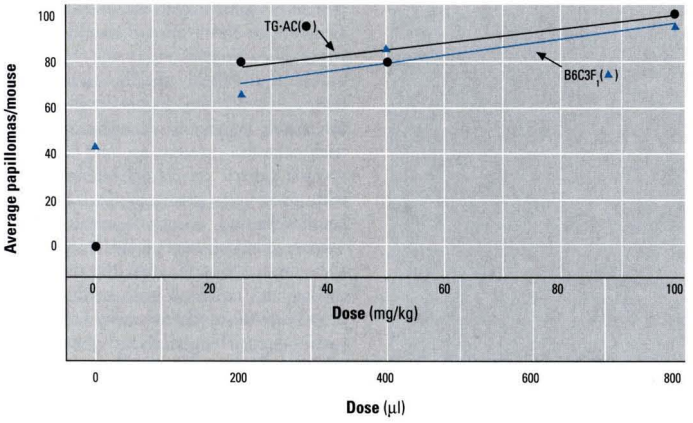


**Figure 1.** Comparison of 2-year bioassay and 20-week dose–reponse studies for cumulative tumor burden in benzene-exposed mice. The graph shows tumors at all sites identified as resulting from chemical exposure in $B6C3F_1$ mice in the 2-year bioassay and papilloma incidence in TG.AC mice exposed to benzene. The doses represent gavage exposure (mg/kg) in $B6C3F_1$ mice or skin paint exposure (µl) in TG.AC mice for 20 weeks.

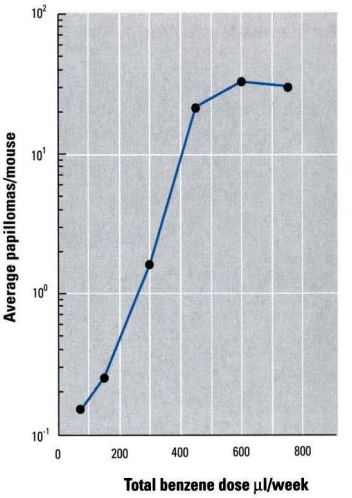


**Figure 2.** Benzene dose response. Groups of 10 TG.AC female mice exposed for 20 weeks to benzene via skin paint. Papillomas were counted weekly and the maximum countable limit was 30 papillomas/mouse.

ble to reduce the uncertainty about the carcinogenic potential of nonmutagenic chemicals and reduce the number of false positives, i.e., *in vitro* mutagens that are incorrectly predicted to be carcinogens. The major impediments to successful predictions are the relatively weak chemicals that induce single-site, species-specific, or equivocal carcinogenic effects, which are generally the consequence of the use of inbred rodent strains (*17*).

## Strategy for the Use of Transgenic Mice

Based on the responses of the p53 +/- and TG.AC mouse lines to specific chemical treatment regimens discussed here, we propose that these lines can be used selectively in conjunction with the 2-year bioassay to confirm predictions based on the aggregate of structure–activity relationships, *in vitro* genotoxicity, and systemic toxicity, ultimately reducing the number of chemicals (and mice) that must be subjected to 2-year rodent bioassays. The strategy is depicted in the decision-tree scheme outlined in Figure 3; a key element is the use of p53 +/- mice for the verification of predicted mutagenic carcinogens.

Few studies have specifically investigated the effects of environmental chemicals on the p53 tumor-suppressor gene in wild-type or genetically altered mice (*16,29,61,62*), and p53 mutations in experimentally induced rodent tumors have not been observed as frequently as in human tumors. This difference may be due to the methods of observation, tumor type, the degree of progression of the neoplasia, etc. Hemizygous p53 mice that lose the function of the remaining wild-type allele may allow a decreased latency through an increased rate of progression to malignancy without altering tumor multiplicity (*32*) or the tissue specificity of a chemical carcinogen. Trans-species carcinogens may involve chemical interaction with highly conserved genes involved in regulation of cell proliferation or differentiation (*7,17,63,64*). Interaction of mutagenic carcinogens with highly conserved genes in the genome may explain, at least partly, why hemizygous p53 mice exhibit a shortened latency while retaining tissue specificity. In addition, genomic stability may decrease with loss of both wild-type p53 alleles (*7,63,64*), possibly because of the loss of the ability to arrest the cell cycle to allow sufficient time for DNA repair and/or to induce apoptosis (*8,65,66*).

Although the number of chemicals evaluated in the p53 +/- line are limited, the results to date support further evaluation of the proposal of using this line to identify or verify mutagenic carcinogens. Because of the relatively rapid induction of tumors and the high proportion of the mice responding, we propose that chemicals with properties that predict mutagenicity and carcinogenicity be tested in this model before conducting a 2-year bioassay. Data relevant to potential target tissues and dose–response patterns can be obtained from a transgenic mouse study that can be conducted within 6-month exposure periods. The uniform sensitivity of the animals allows fewer mice to be used per dose group, and the absence of spontaneous tumors allows for a more categorical determination of carcinogenic potential at lower doses. If the toxicity of the chemical is not known from other studies, preliminary estimates can be made efficiently using wild-type nontransgenic siblings with the same genetic background. Application of the maximum tolerated dose (*67*) can be obviated because an extended dose–response characterization is possible. With a reduction in duration and overall cost, the transgenic mouse experiment may be repeated for verification or to determine additional doses required for dose–response characterization, which is rarely done with the 2-year bioassays.

The second component of the strategy is the use of the TG.AC mouse line. Although mutagenicity is the mechanistic determinant most clearly defined for a presumptive carcinogen, it is possible that some chemicals may not induce tumors through mutations but through epigenetic pathways. Therefore, chemicals that are not carcinogenic in the p53 +/- mouse line might still possess potential for nonmutagenic carcinogenicity. Since the TG.AC mouse line has shown sensitivity to nonmutagenic carcinogens and tumor promoters, a chemical acting via a nonmutagenic mechanism may be detected in these transgenic mice. Further, chemicals that were not tumorigenic in either line may be considered to be presumptive noncarcinogens or in the conventional category of "generally recognized as safe." A crucial question is whether 2-year or lifetime rodent bioassays will still be required to declare a chemical noncarcinogenic. Chemicals that are negative in both of these transgenic mouse lines may still require a complete rodent bioassay because the preliminary data indicate that neither transgenic model will detect all carcinogenic activity in rodents. In particular, nonmutagens that induce single-site carcinogenic effects would not be expected to be effective in the transgenic models. Such highly specific carcinogenic effects are unlikely to be predictable by any foreseeable strategy because the actions of such chemicals are the product of highly specific genetic effects or the interaction with specific gene products (*17*). It is plausible that the study of such chemicals in other transgenic models may provide insights into their modes of action, but the transgenic mouse lines proposed in this strategy will most likely fail to identify many site-specific carcinogens. The preliminary results with ethyl acrylate support this interpretation. Rodents are used as surrogates for humans, and it is essential to note that the aim is to identify carcinogens that may be predicted to be carcinogenic for humans. Several single-site responses in rodents now appear to be very unlikely to predict similar risk for humans (*61,68*).

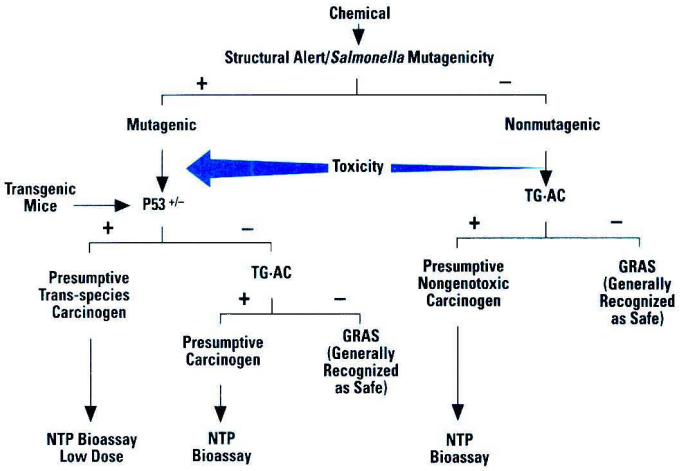


**Figure 3.** Decision tree of strategy for use of transgenic mouse lines to complement the rodent bioassay. Toxicity, in general, as indicated by the empirically determined maximum tolerated dose, increases for mutagenic carcinogens compared to nonmutagenic carcinogens (*17*).

Validation of this strategy can be accomplished by two approaches: one is retrospective, the other prospective. The retrospective approach, taken in the validation of *in vitro* or short-term test methods, has typically involved testing well-defined carcinogens. This approach led to overconfidence in the value of many short-term assays which, when they were more fully characterized for response to noncarcinogens, were found to lack sufficient specificity to be useful (*52,53*). While the selective testing of known carcinogens and noncarcinogens in the TG.AC and p53 +/- mouse lines is necessary, there is also the opportunity for a more direct and objective validation. This prospective approach involves testing chemicals that are currently undergoing long-term bioassays. By providing results, both qualitative and quantitative, before the outcome of the 2-year bioassay is known, even implicit investigator bias (such as in the selection of chemicals) is eliminated. We have implemented this approach with the TG.AC mouse line by prospectively testing four chemicals undergoing 2-year rodent bioassays (Table 4). It is important to emphasize that the hypothesis being tested by this type of validation is that these transgenic models will preferentially identify trans-species carcinogens and noncarcinogens. The TG.AC mouse line is responsive to a number of tumor promoters, but this is not a useful distinction in the context of the rodent bioassay. The principle of trans-species effects (*17*) should apply, since chemicals that can promote only in a single-species or strain are subject to the action of gene(s) or chemical specificity that limit their carcinogenic (or promotion) potential (*67*). Thus, while the TG.AC mouse line may identify chemicals with the capacity to promote tumor development in a mouse two-stage assay, its use is aimed principally at the detection of nonmutagenic carcinogens, for the verification of mutagenic carcinogens positive in the p53 +/- mouse line and for dose–response data.

This strategy can also be applied to problems associated with the development of new products for commercial or medicinal uses. Widespread application of the *Salmonella* mutagen assay has resulted in a marked decrease in the development of new products or chemicals that are positive in that assay. However, approximately 30% of such chemicals have not been carcinogenic when tested in 2-year bioassays. Because most mutagens are carcinogenic and because of the economic and regulatory risks (i.e., what information is needed to override a positive result in a *Salmonella* mutagenesis assay), the development of such products may be abandoned early. The use of the proposed predictive strategy outlined here can result in the identification of such "mutagenic noncarcinogens" and provide a scientific and economic justification for further development or for a more informed decision to conduct a costly bioassay to verify the lack of carcinogenic potential.

This strategy is not presented as a panacea to all of the public health issues associated with environmental carcinogens and risk assessment. It represents the use of new experimental tools to search for answers to such problems, and it can be objectively challenged and validated using ongoing and future bioassays. The strategy provides a mechanistic basis for judging the carcinogenicity of chemicals and for better defining the specific chemical-gene interactions that induce and promote the development of cancers.

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*Organizers: Richard E. Mains, James F. Battey and RobertA.Steiner*
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*Organizers: Michael O'Donnell and Stephen C. West*
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*Organizers: Martin J. Murphy, Alan M. Gewirtz and Si Lok*
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*Organizers: Armand Keating and Joel Greenberger*
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# A Longitudinal Study of Chronic Lead Exposure and Physical Growth in Boston Children

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We investigated the cross-sectional and longitudinal relationships between chronic exposure to lead and physical growth among a cohort of children reassessed 13 years after initial examination. We measured weight, height, and dentin lead levels of 270 children in 1975–78. In 1989–1990 we reexamined 79 of these children for measurement of weight, height, and bone lead levels by means of *in vivo* K X-ray fluorescence. To avoid potential confounding by race and chelation history, analysis was restricted to white subjects without a history of lead chelation therapy. A total of 236 subjects provided complete information for the study of cross-sectional relationship between dentin lead levels and physical growth: 58 subjects for the study of longitudinal relationship between dentin lead levels and changes in physical growth and 54 subjects for the study of longitudinal relationship between bone lead levels and changes in physical growth. Dentin lead levels averaged 14.9 μg/g; tibia and patella lead levels averaged 1.2 and 5.0 μg/g, respectively. With control for potential confounders including age, sex, baseline body size, and mother's socioeconomic status, $\log_{10}$ dentin lead level was positively associated with body mass index as of 1975–1978 (ß = 1.02, $p$ = 0.03) and increase in body mass index between 1975–78 and 1989–90 (ß = 2.65, $p$ = 0.03). Bone lead levels were not significantly associated with physical growth. This is the first study relating chronic lead exposure to body mass index. The results suggest that chronic lead exposure in childhood may result in obesity that persists into adulthood. *Key words*: body mass index, bone lead, growth, lead toxicity, tooth lead. *Environ Health Perspect* 103:952–957 (1995)

Although the adverse effect of overt plumbism on physical growth has long been recognized (*1,2*), the effect of low-level lead exposure on physical growth was first explored by Schwartz et al. using data from the National Health and Nutrition Examination Survey (NHANES) II of 1976–1980 (*3*). The NHANES II data for 2695 children 7 years old indicated that blood lead level (range = 4–35 μg/dl) was a statistically significant predictor of children's height, weight, and chest circumference, with control for age, race, sex, and nutritional covariates. However, the cross-sectional nature of the NHANES II survey limited causal inference regarding the relationship.

The results of subsequent studies have been inconsistent. A retrospective study of the growth of 54 children from birth to 48 months of age suggested a negative correlation between weight gain and higher blood lead between 15 and 24 months of age (*4*). Two longitudinal studies did not find any significant association between blood lead and physical growth (*5,6*). In another longitudinal study, covariate-adjusted heights at 15 and 33 months of age were negatively associated with postnatal blood lead concentrations (*7,8*).

Considering that physical growth is a time-integrated outcome, use of biomarkers of chronic exposure such as tooth lead and bone lead might be more advantageous than blood lead in investigating the association. Blood lead reflects relatively recent exposure over a few months, and the biological half-life of lead in children was several times shorter than that for adults (*9,10*). Indeed, with control for other variables, including the child's medical history, dietary history, behavior, tobacco smoking of parents, and sociodemographic factors, a study of Danish children showed an inverse association between tooth lead and height (*11*).

Lead concentrates in mineralized tissue such as bone and teeth. The concentration of lead in the circumpulpal dentin of deciduous teeth has been a useful biomarker of cumulative lead exposure in studies of the chronic toxicity of lead (*12*). It provides more valid information than does tooth enamel on systemic absorption of environmental lead into the body over several years prior to shedding (*13–16*).

Bone accumulates lead throughout life, eventually serving as the repository for 95% of an individual's lead burden (*17*). Because of the long half-life of lead in bone, bone lead provides an index of cumulative exposure over decades. Direct measurement of lead in bone by means of an *in vivo* X-ray fluorescence (XRF) technique has been developed as a promising biomarker during the last decade (*18,19*). On the basis of the electron orbit where it provokes the emission of fluorescent photons from lead atoms, the XRF technique is classified into L-line XRF (L-XRF) and K-line XRF (K-XRF).

The K-XRF technique is relatively stable and reliable. Its accuracy is not affected by overlying skin thickness or movement, and it permits measurement of lead levels in bone cortex and marrow tissues deeper than L-XRF (*20,21*). It is noninvasive, involves low-level radiation dose (less than 2.5% of the effective dose of a chest X-ray examination in adults), and takes 15–30 min to measure one bone site (longer time renders better precision) (*20,22*).

To our knowledge, no studies examining the effect of chronic lead exposure on body mass index (weight in kilograms divided by the square of the height in meters; BMI) have been previously reported, although BMI, as an index of obesity, has long been shown to be an important risk factor for hypertension, diabetes, and coronary heart disease (*23,24*). We investigated the effect of chronic lead exposure on BMI as well as on weight and height in a cohort that was examined at a 13-year interval. We explored the cross-sectional relationship between dentin lead level and physical growth in each examination of the cohort, then assessed the longitudinal relationship between dentin lead level in child-

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hood and physical growth from childhood through early adulthood. The study protocol was approved by the institutional review boards of Children's Hospital (Boston) and Brigham and Women's Hospital, and informed consent was obtained from all participants in the study.

## Methods

***Subjects.*** The initial sample was chosen from a population of 3329 first and second graders in Chelsea and Somerville, Massachusetts, between 1975 and 1978. From this group, 270 English-speaking children with initial dentin lead levels >24 ppm (upper 10th percentile) or <8.7 ppm (lower 10th percentile) were recruited for a study of neurobehavioral effects of lead. Methods used to define eligible subjects are described in detail elsewhere (*25*).

In 1988, 132 members (mean age, 18.4 years) of this cohort were recruited to participate in a follow-up evaluation of their neuropsychological performance. Of these, 79 subjects (60% of the base population) participated in the additional follow-up in 1989 and 1990. Among those who did not, 5 (3.8%) refused, 34 (25.8%) could not be located, 8 (6.0%) agreed to participate contingent on a return visit to Boston during the data collection period, 5 (3.8%) agreed to participate but repeatedly failed to keep appointments, and 1 withdrew consent during the study.

To control for confounding by race, we restricted the study to white subjects ($N$ = 251 as of 1975–1978). Growth velocity has been known to significantly increase after calcium disodium ethylenediamine tetraacetic acid (EDTA) chelation therapy in lead-poisoned children aged 2–5 years (*26*). Subjects with high dentin lead levels were more likely to have undergone chelation therapy in childhood than those with low levels. Following chelation, subjects might have lower internal doses of lead than expected on the basis of their dentin lead levels. Six subjects had a history of chelation. To eliminate potential confounding by chelation therapy, the principal analyses were restricted to nonchelated subjects. However, results from analyses including the chelated subjects are also presented.

***Physical growth.*** Subjects' weight and height were measured (in light clothes and barefoot) to the nearest quarter pound and quarter inch, respectively, with use of a Health O Meter (Continental Scale Corp., Bridgeview, Illinois) at the initial interview in 1975–1978 and during the examination in 1989–1990.

***Tooth lead measurement.*** Shed deciduous teeth donated by subjects were cleansed ultrasonically, and those with fillings were discarded from consideration. The specimens were then mounted in lead-free wax on the cutting stage of a Buehler low-speed saw (Buehler Ltd., Lake Bluff, Illinois). A 1-mm slice was taken from the central sagittal plane of each tooth at a single pass. The central slice was then placed on an anvil and split with a small chisel along a line from the pulp canal to the dentin–enamel junction. The larger portions of the slices, along with the residual adjacent segments, were filed in numbered pill boxes for later confirmatory analysis. The smaller portion, composed primarily of dentin, was then analyzed for lead by means of anodic stripping voltammetry, as described elsewhere (*27*). If three dentin lead values were available, two concordant values were required; if four values were available, three concordant values were required. We used the mean of all available dentin lead values for a child as the exposure index.

***Bone lead measurement.*** We measured bone lead in the tibia and patella of each patient using our prototype K-XRF instrument (ABIOMED, Inc., Danvers, MA). The physical principles, technical specifications, and validity of K-XRF instruments are described in detail elsewhere (*28*–30). In short, this instrument uses a $^{109}Cd$ $\gamma$-ray source to provoke the emission of fluorescent photons from target tissue that are detected, counted, and arrayed on a spectrum. The net lead signal is determined after subtraction of background counts by means of a linear least-squares algorithm. The lead fluorescence signal is then normalized to the elastic or coherently scattered X-ray signal, which arises predominantly from the calcium and phosphorus present in bone mineral. The unit of measurement is microgram of lead per gram of bone mineral.

Because the instrument provides a continuous, unbiased point estimate that oscillates around the true bone lead value, negative point estimates are sometimes produced when the true bone lead value is close to zero. The instrument also provides an estimate of uncertainty associated with each measurement, which is derived from a goodness of fit calculation of the spectrum curves and is equivalent to a single standard deviation. Although a minimum detectable limit calculation of twice this value has been proposed for interpreting a bone lead estimate for an individual (*31*), use of all point estimates makes better use of the data for a population in epidemiologic studies (*32,33*). Recently, we have specifically addressed the methodological issues of using negative values and values below the conceptual limit of detection in epidemiologic studies (*33*). Our experiment indicated that retaining all values of bone lead concentration provides less bias and greater efficiency in comparing the mean or median levels of bone lead of different populations.

Measurements were taken at the midshaft of the left tibia and at the left patella after each region had been washed with a 50% solution of isopropyl alcohol. The K-XRF beam collimator was seated perpendicular to the bone surface for the tibia and at 30° in the lateral direction for the patella.

***Covariates.*** Information on potential confounding variables and effect modifiers was obtained from data collected by questionnaire and examination in 1975–1978. Age, sex, race, birth weight, mother's socioeconomic status (dichotomized by Hollingshead class I–III versus IV–V), medical history of chelation therapy for lead poisoning as of 1975–1978, and age at the time of K-XRF measurement in 1989–1990 were selected *a priori* as potential covariates.

***Statistical analysis.*** The main purpose of the analysis was to relate physical growth (dependent variables) to lead biomarkers (independent variables) with control for potential covariates. Before the main analysis, extreme outliers in values of height, weight, and BMI were detected by the generalized extreme studentized deviate (ESD) many-outlier procedure, with a Bonferroni-corrected level (*34*). Lowess smoothing plots between covariate-adjusted dependent variables and covariate-adjusted independent variables were used to evaluate the form of the dose–response relationship and to suggest appropriate transformations of variables (*35*).

Three sets of multiple linear regression models were fitted (Fig. 1). First, to determine whether dentin lead level was cross-sectionally associated with physical growth in childhood (1975–1978), regressions of dentin lead on each physical outcome were carried out, with control for age, sex, birth weight, and mother's socioeconomic status (model 1). Second, to determine whether the dentin lead level was prospectively associated with changes in physical growth between 7 and 20 years of age, regressions of dentin lead on each physical outcome were carried out, with control for age increase, age as of 1975–1978, sex, mother's socioeconomic status, and physical growth as of 1975–1978 (model 2). Finally, to determine whether bone lead levels around the age of 20 years were retrospectively associated with changes in physical growth between 7 and 20 years of age, regressions of bone lead levels (i.e., tibia lead levels, patella lead levels, or mean bone lead levels) on each physical outcome were carried out, with control for age increase,

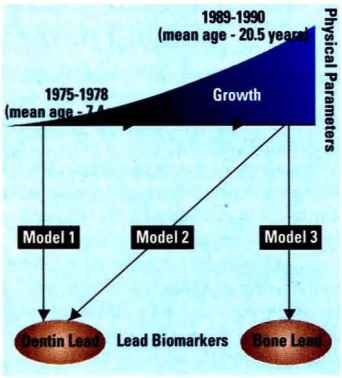


**Figure 1.** Longitudinal structure of the data and the statistical models in the study of the effect of lead exposure on physical growth. The dependent variable of model 1 is physical parameter [weight, height, or body mass index (BMI)] as of 1975–78. Independent variables of model 1 are $\log_{10}$ dentin lead level, age, sex, birth weight, and mother's socioeconomic status as of 1975–78. For models 2 and 3, the dependent variable is physical parameter (weight, height, or BMI) change between 1975–78 and 1989–90. The independent variable of interest is $\log_{10}$ dentin lead level as of 1975–78 for model 2 and bone lead (tibia, patella, or mean bone lead) level for model 3. Other independent variables adjusted in models 2 and 3 are age as of 1975–78, age increase between 1975–78 and 1989–90, sex, mother's socioeconomic status as of 1975–78, and physical parameter (weight, height, or BMI, respectively) age as of 1975–1978, sex, mother's socioeconomic status, and physical growth as of 1975–1978 (model 3-1 for tibia lead levels, model 3-2 for patella lead levels, and model 3-3 for mean bone lead levels).

## Results

A total of 237 white subjects without a history of chelation had complete information for fitting model 1. An extreme outlier at level <0.0002 (0.05/237) for BMI as of 1975–1978 was detected by the ESD procedure. This extremely obese child (weight 49.2 kg, height 1.27 m, and BMI 30.5 kg/m$^2$ at the age of 7.1 years) was excluded from subsequent analyses. The remaining 236 subjects used in fitting model 1 did not significantly differ from the remaining nonchelated subjects in dentin lead, weight, height, BMI, sex, birth weight, or mother's socioeconomic status (Table 1).

Of the 79 participants in the last examination, 60 nonchelated white subjects had complete information for fitting model 2, which requires physical parameter variables measured at both the 1975–1978 and the 1989–1990 examinations. The ESD procedure detected two extreme outliers at level <0.0008 (0.05/61) for BMI change between 1975–1978 and 1989–1990. One outlier, with a BMI increase of 23.2 kg/m$^2$, had a weight of 102.2 kg, a height of 1.60 m, and a BMI of 39.9 kg/m$^2$ at the age of 19.8 years. The other outlier, with a BMI increase of 20.7 kg/m$^2$, had a weight of 112.1 kg, a height of 1.68 m, and a BMI of 39.9 kg/m$^2$ at the age of 21.2 years.

The 58 study subjects used in fitting model 2 did not significantly differ from the remaining nonchelated subjects in dentin lead, physical growth as of 1975–1978, sex, and birth weight. However, mother's socioeconomic status as of 1975–1978 for these subjects was higher ($p$<0.01 by Fisher's exact test). Out of these 58, 4 subjects did not undergo bone lead measurements, resulting in 54 subjects for model 3. They did not significantly differ from the remaining nonchelated subjects in dentin lead, physical growth as of 1975–1978, sex, or birth weight, but mother's socioeconomic status did differ.

At the time of the 1975–1978 examination, weight, height, and BMI averaged 26.1 kg (SD = 5.1, range = 17.5–45.4), 1.26 m (SD = 0.06, range = 1.09–1.49), and 16.3 kg/m$^2$ (SD = 2.3, range = 10.1–25.7), respectively. Dentin lead levels averaged 14.9 (SD = 11.5, range = 2.8–66.3). At the time of the 1989–1990 examination, weight, height, and BMI averaged 69.7 kg (SD = 15.5, range = (49.9–116.7), 1.71 m (SD = 0.10, range = 1.55–1.91), and 23.65 kg/m$^2$ (SD = 3.54, range = 18.09–33.03),

**Table 1.** Characteristics of subjects included in and excluded from analyses of lead and physical growth[a]

| | Model 1: subjects in 1975–78 study ($N$ = 270) | | | Model 2: nonchelated subjects, 1989–90 study ($N$ = 76) | |
|---|---|---|---|---|---|
| Characteristic | Analyzed ($N$ = 236) | Nonchelated, excluded ($N$ = 28)[b] | Chelated, excluded ($N$ = 6) | Analyzed ($N$ = 58) | Excluded ($N$ = 18)[b] |
| Age, 1975–78 (years) | 7.4±0.6 (5.9–9.0) | 7.4±0.6 (6.5–9) | 7.3±0.9 (6.1–8.9) | 7.4±0.5 (6.1–8.8) | 7.4±0.7 (6.5–8.42) |
| Sex (% female) | 47 | 54 | 100 | 53 | 50 |
| Birth weight (kg) | 3.3±0.5 (1.0–4.7) | 3.3±0.6 (2.0–4.6) | 3.2±0.5 (2.7–3.8) | 3.4±0.5 (2.2–4.4) | 3.2±0.7 (1.0–4.3) |
| Mother's socioeconomic status (% high) | 11 | 8 | 0 | 10 | 24 |
| Weight, 1975–78 (kg) | 26.1±5.1 (17.5–45.4) | 27.0±6.0 (19.3–49.1) | 20.5±2.1 (18.5–23.0) | 26.3±5.3 (17.5–43.1) | 26.4±3.7 (22.5–34.2) |
| Height, 1975–78 (m) | 1.26±0.06 (1.09–1.49) | 1.26±0.08 (1.02–1.44) | 1.19±0.04 (1.14–1.25) | 1.27±0.06 (1.13–1.42) | 1.28±0.07 (1.19–1.44) |
| BMI, 1975–78 (kg/m$^2$) | 16.3±2.3 (10.1–25.7) | 16.9±3.7 (13.1–30.5) | 14.4±1.0 (13.0–15.3) | 16.22.1 (13.3–21.8) | 16.2±1.4 (14.1–19.2) |
| Dentin lead as of 1975–78 (ppm) | 14.9±11.5 (2.8–66.3) | 17.0±15.9 (2.6–51.4) | 35.9±15.8 (18.9–61.2) | 12.9±9.8 (2.9–51.8) | 13.6±11.5 (4.6–50.4) |
| $\log_{10}$ dentin lead, 1975–78 (ppm) | 1.06±0.32 (0.44–1.82) | 1.06±0.38 (0.41–1.71) | 1.52±0.19 (1.28–1.79) | 1.00±0.31 (0.46–1.71) | 1.03±0.29 (0.66–1.70) |
| Age, 1989–90 (years) | NA | NA | NA | 20.5±0.7 (18.7–21.8) | 20.3±1.0 (18.8–21.7) |
| Weight, 1989–90 (kg) | NA | NA | NA | 69.7±15.5 (49.9–116.7) | 75.7±16.3 (59.0–112.1) |
| Height, 1989–90 (m) | NA | NA | NA | 1.71±0.10 (1.55–1.91) | 1.28±0.07 (1.19–1.44) |
| BMI, 1989–90 (kg/m$^2$) | NA | NA | NA | 23.6±3.54 (18.09–33.03) | 26.2±6.1 (20.4–39.9) |
| Weight change from 1975–78 to 1989–90 (kg) | NA | NA | NA | 43.5±13.1 (23.8–80.5) | 49.7±15.7 (28.8–79.2) |
| Height change from 1975–78 to 1989–90 (m) | NA | NA | NA | 0.44±0.09 (0.18–0.58) | 0.42±0.08 (0.30–0.60) |
| BMI change from 1975–78 to 1989–90 (kg/m$^2$) | NA | NA | NA | 7.49±2.67 (2.83–15.87) | 10.44±5.55 (4.90–23.15) |
| Tibia lead, 1989–90 (μg/g)[b] | NA | NA | NA | 1.2±4.4 (-7–13) | 2.1±6.3 (-9–19) |
| Patella lead, 1989–90 (μg/g) | NA | NA | NA | 5.08.1 (-10–23) | 5.4±9.9 (-13–25) |
| Mean bone lead, 1989–90 (μg/g) | NA | NA | NA | 3.14.7 (-5.5–14) | 3.9±4.8 (-4–12.5) |

Abbreviations: NA, not applicable; BMI, body mass index.
[a]Means ± SD and ranges (in parentheses) presented where appropriate.
[b]Subjects excluded from analysis are those with incomplete information, and those of nonwhite race.

respectively. Tibia and patella lead estimates averaged 1.2 μg/g (SD = 4.4, range = -7–13), and 5.0 μg/g (SD = 8.1, range = -10–23), respectively.

In exploratory data analyses, the lowess smoothing curves of covariate-adjusted outcome variables and covariate-adjusted predictor variables were curvilinear and slightly upwardly convex. Lowess smoothing after logarithmic transformation of dentin lead levels provided a more linear fit. For the sake of interpretability of the scale, we used $\log_{10}$ dentin lead level in linear regression analyses.

With control for age as of 1975–1978, sex, birth weight, and mother's socioeconomic status, $\log_{10}$ dentin lead in nonchelated, nonoutlying subjects was significantly associated with BMI ($N$ = 236, ß = 1.02, 95% CI, 0.12–1.93, $p$ = 0.03; Table 2), but not with weight or with height. The inclusion of chelated subjects slightly reduced the regression coefficient for BMI of $\log_{10}$ dentin lead ($N$ = 240, ß = 0.88, 95% CI, -0.01–1.76, $p$ = 0.05). Additional inclusion of an extreme outlier obscured the association between BMI and $\log_{10}$ dentin lead ($N$ = 241, ß = 0.65, 95% CI, 0.31–1.60, $p$ = 0.19). When the same regression models were fitted to a subgroup who participated in the 1989–1990 examination and had complete information for model 2, the confidence bounds were slightly widened, which might result from reduced sample size ($N$ = 58, ß = 1.82, 95% CI, -0.06–3.69, $p$ = 0.06).

With control for BMI as of 1975–1978, age increase, age as of 1975–1978, sex, and mother's socioeconomic status, $\log_{10}$ dentin lead was significantly associated with BMI change between 1975–1978 and 1989–1990 among nonchelated, nonoutlying subjects (ß = 2.65, 95% CI, 0.33–4.97, $p$ = 0.03; Table 2). The inclusion of a chelated subject did not appreciably change the regression coefficient for BMI change of $\log_{10}$ dentin lead (ß = 2.50, 95% CI, 0.27–4.74, $p$ = 0.03). However, inclusion of the two outliers obscured the association (ß = 2.48, 95% CI, 0.79–5.75, $p$ = 0.13). There were no significant associations between $\log_{10}$ dentin lead and weight, or between $\log_{10}$ dentin lead and height.

No significant association was observed between bone lead as of 1989–1990 and any of the changes in physical growth between 1975–1978 and 1989–1990, with control for physical growth as of 1975–1978, age increase, age as of 1975–1978, sex, and mother's socioeconomic status. The results were similar when chelated subjects and/or outliers were included.

## Discussion

We observed a weak but significantly positive association between childhood dentin lead level and BMI in both cross-sectional and longitudinal analyses. A 10-fold increase in dentin lead level was associated with an increase of 1.02 kg/m$^2$ in BMI at the age of 7 years. A 10-fold increase in dentin lead level was also associated with an increase in BMI change of 2.65 kg/m$^2$ from age 7 to age 20.

The point estimates of the regression coefficient for weight and for change in weight were positive; those for height and for change in height were negative, but their interval estimates all included zero. Because increased BMI results from increased weight and/or decreased height, it may have served as a more sensitive indicator for the association between physical growth and chronic lead exposure in our study. Bone lead levels at the age of 20 years were not significantly associated with any physical growth changes between 7 and 20 years of age.

Because dentin lead level reflects chronic exposure to lead during the several years prior to the shedding of teeth, our findings suggest that children exposed to lead in early childhood experience greater BMI gain during the period between 7 and 20 years of age than those less exposed. It is possible that obese children ingested more environmental lead from tap water, canned foods, dusts, and paint chips during early childhood. This reverse causality can explain the cross-sectional association, but hardly the longitudinal association. In our longitudinal model, the exposure (the accumulation of lead in the tooth) obviously preceded the effect (the increase in BMI), thereby supporting the attribution of a causal role to lead.

Blood lead level was inversely associated with both height and weight in NHANES II data (*3*). An association of blood lead with BMI was not examined, and the index of exposure was blood lead, not dentin lead, in the NHANES II. Thus, any comparison between this study and the NHANES II study should be made carefully. Nevertheless, it may be interesting to examine whether there is also a significant association between blood lead and BMI in the NHANES II data.

On the basis of coefficients of variation for weight (approximately 20%) and for height (approximately 5%) in our data, we estimated that the cross-sectional analysis of the 1975–1978 data ($N$ = 236) had 80% power to detect the 7% difference in mean of weight and the 1.8% difference in mean of height. Smaller effects would not be detectable with 80% power.

Reduced pituitary responsiveness to hypothalamic stimuli in terms of growth hormone releasing factor or thyrotropin-releasing hormone has been postulated as a pathophysiologic mechanism for lead's effect on physical growth. A recent neuroendocrine study showed that peak human growth hormone and insulinlike growth factor I responses to the L-dopa insulin test were low in lead-poisoned children (*26*). The authors concluded that lead-induced reduction in stature may be due to diminished human growth hormone secretion, which in turn results in reduced insulinlike growth factor I secretion, or that lead may directly inhibit insulinlike growth factor I formation. Blunted response of thyroid-stimulating hormone and growth hormone to stimulatory challenge have been observed in lead-poisoned children (*36*) as well as in rats exposed to low-level lead (*37*). It is possible that our findings reflect similar endocrine effects of low-level lead exposure.

**Table 2.** Partial regression coefficient of $\log_{10}$ dentin lead (ppm) in multiple linear regression of physical growth in study of the relationship between lead exposure and physical growth[a]

| Model ($N$) | Partial regression coefficient dependent variables | | |
|---|---|---|---|
| | Weight (kg) | Height (m) | BMI (kg/m$^2$) |
| 1 (236) | 1.340 (0.991)[b] | -0.009 (0.011) | 1.023* (0.458) |
| 2 (58) | 7.118 (4.176) | -0.028 (0.025) | 2.650* (1.156) |
| 3-1 (54) | -0.038 (0.327) | -0.003 (0.002) | 0.077 (0.088) |
| 3-2 (54) | -0.187 (0.159) | -0.001 (0.001) | -0.042 (0.043) |
| 3-3 (54) | -0.314 (0.286) | -0.003 (0.002) | -0.037 (0.078) |

BMI, body mass index.

[a]Dependent variable of model 1 is weight, height, or BMI as of 1975–78. Independent variables of model 1 are $\log_{10}$ dentin lead level, age, sex, birth weight, and mother's socioeconomic status as of 1975–78. For models 2, 3–1, 3–2 and 3–3, dependent variable is change in weight, in height, or in BMI between 1975–78 and 1989–90. Independent variable of interest is $\log_{10}$ dentin lead level as of 1975–78 for model 2; tibia lead level for model 3–1, patella lead level for model 3–2, and mean bone lead level as of 1989–90 for model 3–3. Other independent variables adjusted in models 2 and 3 are age as of 1975–78, age increase between 1975–78 and 1989–90, sex, mother's socioeconomic status as of 1975–78, and physical parameter (weight, height, or BMI, respectively) as of 1975–78.

[b]Standard errors in parentheses.

*$p$<0.05.

The positive findings of this study may have resulted by chance. As we conducted multiple statistical tests using the same data set, the probability of a type I error may be larger than the nominal level of 0.05. Thus, further study with a larger sample size is encouraged to corroborate our findings.

A possible source of bias is our limited success in locating and enrolling members of the cohort for follow-up because of the increased migration from home that occurs around age 20. However, the characteristics of the population, including dentin lead levels, did not differ significantly between those who participated in this follow-up and those who did not. As physical growth and covariates were measured blindly with respect to the status of dentin and bone lead levels, observation bias is not likely.

We eliminated potential confounding effects of race and chelation by restricting the main analysis to white, nonchelated subjects. However, such potential confounding variables as food intake and parental body size were not controlled for in our study because this information was not available. We intended to reduce confounding effects of those variables by adjusting for mother's socioeconomic status. Still, it is possible that genetic (*38,39*) and nongenetic risk factors (*23*) for increased BMI might result in increased dentin lead levels through unknown mechanism. Further studies more thoroughly controlling for those potential confounders are awaited.

BMI is a major risk factor for elevated blood pressure (*23,24*). It has been noted that blood pressure was positively associated with blood lead levels before and after control for age, BMI, and other related factors (*40–45*). However, the association between blood lead and blood pressure is not conclusive yet (*45*), because it is very weak (1 to 2 mm Hg for every doubling in blood lead levels in middle-aged men) and influenced by the inclusion of covariates in the statistical model such as alcohol consumption and cigarette smoking (*41*).

Our finding that childhood lead exposure may predict increased BMI that persists into adulthood is suggestive of an indirect path to elevated blood pressure related to chronic lead exposure. If our finding is replicable and robust, the ramifications are significant. The previously described association between low-level lead exposure and elevated blood pressure may be stronger than currently assumed, if the effect of lead on obesity is also taken into account.

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# New Books

**Cancer Wars**
*Robert N. Proctor*
New York: Basic Books, 1995, 356 pp. ISBN: 0465027563, $25.

**Casarett and Doull's Toxicology; The Basic Science of Poisons, 5th ed.**
*Curtis D. Klaassen, ed.*
New York: McGraw-Hill, 1995. ISBN: 0071054766, $70.

**Cleaner Production Technologies for Sustainable Development**
*Organization for Economic Cooperation and Development*
Washington, DC: OECD Publications and Information Center, 1995, 100 pp. ISBN: 9264144730, $30.

**Control and Game-Theoretic Models of the Environment**
*Jerzy A. Filar, Carlo Carraro, eds.*
Boston, MA: Birkhauser Boston, 1995, 384 pp. ISBN: 0817638156 (alk. paper), $94.50.

**Ecology Control and Economic Development in East African History**
*Helge Kjekshus*
Athens, OH: Ohio University Press, 1995, 252 pp. ISBN: 0821411322, $17.95.

**The Ecozones of the World; The Ecological Divisions of the Geosphere**
*J. Schultz*
New York: Springer-Verlag, 1995, 449 pp. ISBN: 3540582932 (acid-free paper), $89.

**Environment, Development, Agriculture; Integrated Policy through Human Ecology**
*Bernhard Glaeser*
Armonk, NY: M. E. Sharpe, 1995, 192 pp. ISBN: 1563246929 (cloth, alk. paper), $59.95. 1563246937 (paper, alk. paper), $21.95.

**Environment, Incentives, and the Common Market**
*Frank J. Dietz, Herman R.J. Vollebergh, Jan L. de Vries*
Boston: Kluwer Academic Publishers, 1995. ISBN: 079233602X (alk. paper), $110.50.

**Environmental Medicine; Integrating a Missing Element into Medical Education**
*Andrew M. Pope, David P. Rall, eds.*
Washington, DC: Academy Press, 1995, 400 pp. ISBN: 0309051401, $29.

**Environmental Policy; A Global Perspective for the Twenty-First Century**
*Donald T. Wells*
Upper Saddle River, NJ: Prentice Hall, 1995. ISBN: 0134002199, $24.

**Environmental Science Activities**
*Dorothy B. Rosenthal*
New York: Wiley, 1995, 236 pp. ISBN: 0471076260, $13.95.

**Extraction and the Environment; The Economic Battle to Control our Natural Landscapes**
*Thomas Michael Power*
Washington, DC: Island Press, 1995. ISBN: 1559633689 (acid-free paper), $29.95.

**Handbook of Clinical Toxicology of Animal Venoms**
*Jürg Meier, Julian White, eds.*
Boca Raton, FL: CRC Press, Inc., 1995, 500 pp. ISBN: 0849344891, $120.

**International Management of Hazardous Wastes; The Basel Convention and Related Legal Rules**
*Katharina Kummer*
New York: Clarendon Press, 1995. ISBN: 0198259948, no price available.

**Interpreting Environments**
*Robert Mugerauer*
Austin, TX: University of Texas Press, 1995, 244 pp. ISBN: 0292751788 (cloth), $35. 0292751893 (paper), $14.95.

**Investigating Australian Ecosystems**
*Victorian Association of Environmental Educators*
New York: Cambridge University Press, 1995. ISBN: 0521455936, no price available.

**Opportunities in Environmental Careers**
*Odum Fanning*
Lincolnwood, IL: VGM Career Horizons, 1995. ISBN: 0844245836 (cloth), $13.95. 0844245844 (paper), $10.95.

**Patty's Industrial Hygiene and Toxicology (6 vol set)**
*George D. Clayton, Florence E. Clayton, eds.*
New York: Wiley, 1995. ISBN: 0471547271, $995.

**Photodamage**
*Barbara A. Gilchrest*
Cambridge, MA: Blackwell Science, 1995, 496 pp. ISBN: 0865423431, $95.

**Recycling and Reuse of Industrial Wastes**
*Lawrence Smith, Jeffrey Means, Edwin Barth*
Columbus, OH: Battelle Press, 1995, 116 pp. ISBN: 0935470891, $34.95.

**A Road to Love Canal: Managing Industrial Waste before EPA**
*Craig E. Colten, Peter N. Skinner*
Austin, TX: University of Texas Press, 1995, 240 pp. ISBN: 0292711824 (cloth), $35. 0292711832 (paper), $14.95.

# Calendar

## November

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

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

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

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

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

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

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

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

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

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

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

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

## December

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

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

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

# 1996

## January

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

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

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

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

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

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

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

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

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

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

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

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

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

## February

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

**4–10 February, Sun–Sat. Ion Channels as Therapeutic Targets,** Tamarron, Colorado. Information: Keystone

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Symposia, Drawer 1630, Silverthorne, CO 80498, (303) 262-1230, FAX (303) 262-1525

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

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

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

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

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

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

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

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

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

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

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

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

## March

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

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

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

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

**10–14, March, Sun–Thu. Society of Toxicology,** Anaheim Convention Center, Anaheim California. Information: Trish Strong Society of Toxicology, 1767 Business Center Drive, Suite 302, Reston, VA (703) 438-3115, FAX: (703) 438-3113

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

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

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

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

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

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

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

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

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

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

## April

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

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

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

## May

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

## June

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

## September

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

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

## October

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

## December

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

# 1997

## August

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

# Fellowships, Grants & Awards

**Postdoctoral Fellowships in Toxicology/Epidemiology**

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

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

**European Cancer Centre Two-Year Fellowships for Oncologists**

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

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

Eligibility Criteria: Candidates must meet the following conditions:

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

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

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

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

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

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

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

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

**Earthwatch Field Grants**

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

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

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

Earthwatch field grants cover the costs of maintaining volunteers and principal researchers in the field. They also help with other project expenses, except principal investigator salaries, capital equipment, overhead, and preparation of results for publications.

Applying for grants is a two-stage process. Preliminary proposals are submitted to The Center for Field Research at least 13 months in advance of anticipated field dates. Full proposals are invited upon review of preliminary materials. Proposals are accepted and reviewed year round.

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

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

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

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

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

**Predoctoral Fellowships for Minorities/Disabled Persons**

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

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

**American Honda Foundation**

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

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

Grants generally range from $40,000–$80,000 per

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

**National Cancer Institute**

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

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

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

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

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

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

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

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

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

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

**Senior Scientist Awards**

Applications for Senior Scientist Awards, which provide five years of salary support to outstanding scientists who have demonstrated a high level of productivity, should be submitted to the National Institutes of Drug Abuse, Mental Health, or Alcoholism and Alcohol Abuse by February 1.

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

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

**Great Lakes Protection Fund Call for Preproposals**

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

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

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

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

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

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

The fund also supports efforts to promote collaboration, coordination and regional action through planning and discretionary travel grants. For more information on these grants, please contact the fund: Preproposal Application, Great Lakes Protection Fund, 35 East Wacker Drive, Suite 1880, Chicago, IL 60601.

**Magnetic Fields and Breast Cancer**

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

# Position Announcements

**Clinical Director**

NIEHS's Intramural Program is seeking a tenured Clinical Director to conduct independent clinical research and direct NIEHS's clinical research activities. The successful candidate will be responsible for developing clinical reseach programs to study the environmental component of diseases and dysfunctions; facilitating and evaluating collaborative clinical research between intramural investigators; and ensuring that optimal medical care is provided to all patients participating in NIEHS's protocols through the institute's clinical contracts with Duke and the University of North Carolina. Salary range to $148,400 depends upon qualifications, with relocation expenses available. The position must be filled by a physician. Applicants with certification in internal medicine, gynecology, oncology or other specialties, and demonstrated research and clinical excellence are encouraged to call 919-541-3317 or write Norma Daye, Human Resource Management Branch (HNV115), NIH/NIEHS, MD 1-01, P.O. Box 12233, Research Triangle Park, NC 27709 to request an application package. Complete application packages must be returned to Norma Daye at the above address postmarked by November 7, 1995, to receive consideration.

NIH is an Equal Opportunity Employerapplications from women, minorities and persons with disabilities are strongly encouraged.

**Public Health Scientist**

The Natural Resources Defense Council, a national nonprofit public interest organization, seeks a Senior Scientist to bring scientific analysis and knowledge to advocacy in various forums for the prevention of adverse health and ecological effects of toxic chemical pollution. A PhD or MD/MDH is required, with several years of experience in environmental or public health, or a related field. Candidates should be knowledgeable about cutting-edge toxics issues such as disproportionately impacted subpopulations, endocrine disruption, and other noncancer endpoints, and emerging issues regarding carcinogenesis. The position requires the established ability to keep abreast of scientific advances and work with the public health and academic communities. The ability to conduct outreach activities to build bridges with persons affected by toxics problems is also very important. The salary is $30,000 to $50,000, commensurate with experience. Send resume to: Public Health Program, NRDC, 1350 New York Avenue, NW, Suite 300, Washington, DC 20005. Equal Opportunity Employer.

**Open Rank Faculty Position Announcement—Occupational and Environmental Exposure Assessment**

University of Michigan invites applications for an open rank, tenure-track faculty position in Occupational and Environmental Exposure Assessment. The primary appointment will be in the School of Public Health, Department of Environmental and Industrial Health and will be at a rank and salary commensurate with experience.

Desired candidates will hold wither a PhD in industrial hygiene, epidemiology, environmental health, molecular genetics or other relevant disciplines or an MD with experience in such disciplines. Candidates should have an active interest in innovative and interdisciplinary solutions to theoretical and applied problems in exposure assessment in environmental and occupational settings. Examples of areas of interest include the application of environmental and occupational exposure assessment to exposure-response modeling and risk estimation, and the integration of measures of target organ dose in exposure modeling. Successful candidates will have a demonstrated ability to attract competitive external funding, to publish original research in the peer reviewed literature, and to teach at the graduate level including doctoral level students or medical students. The University of Michigan actively encourages interest from women and minorities and is an Equal Opportunity/Affirmative Action Employer.

Letters of application, accompanied by a curriculum vitae, statement of research and teaching interest, and the names and addresses of three references should be sent to: Thomas Robins, MD, MPH, Associate Professor, The University of Michigan School of Public Health, Department of Environmental and Industrial Health, 1420 Washington Heights, Ann Arbor, Michigan 48109-2029, e-mail: trobins@umich.edu, FAX (313) 763-8095.

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

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

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

Candidates for this position should posses:

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

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

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

**Public Health Scientist**

The San Francisco office of the Natural Resources Defense Council, a national nonprofit public interest organization, seeks a senior scientist with a Ph.D. or M.D. and relevant work experience to promote the prevention of adverse health effects from exposure to toxic chemicals. We will also consider an individual with a Masters Degree and highly relevant work experience.

The position involves bringing scientific analyses and knowledge to advocacy in various forums. Candidates should have expertise in cutting-edgetoxics issues, such as the special vulnerability of children or other disproportionately exposed subpopulations to some toxins, endocrine disruption, or other non-cancer endpoints. The ability to keep abreast of scientific advances, to translate technical issues into simple lay language, and to conduct outreach to persons affected by toxics as well as the scientific and medical communities is required. Salary is commensurate with experience.

Send resume with salary requirements to:
Public Health Program
DR, NRDC, 71
Stevenson, #1825
San Francisco, CA 94105.
Equal Opportunity Employer.
People of color are encouraged to apply.

**Postdoctoral Research Opportunities at the National Institute of Environmental Health Sciences**

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

To apply, please send a cover letter, curriculum vitae, bibliography, and names of three references to the hiring scientist at the maildrop and laboratory listed using the following address: NIEHS, PO Box 12233, Research Triangle Park, North Carolina 27709. In your cover letter, list the position title and the HNV number.

Minorities, women and handicapped individuals are encouraged to apply. All applicants receive consideration without regard to race, religion, color, national origin, sex, physical or mental handicap, political affiliation, age (with statutory exceptions) or any other nonmerit factor. Positions are open until filled.

**Molecular Mechanisms of DNA Repair (HNV88)**

Mechanisms of DNA repair in *Drosophila* are being investigated with focus on the *in vivo* and *in vitro* functions of Rrp1 (recombination repair protein 1). This protein is potentially important in DNA repair and homologous recombination. Future studies will include enzymatic, physical, and genetic characterization of Rrp1.

Contact: Miriam Sander, (919) 541-2799, Laboratory of Molecular Genetics, Maildrop D3-04.

**Molecular Neurobiology (HNV94)**

The signal transduction pathways regulating the expression of neuropeptide and cytokine genes in neural and glial systems are being investigated. Studies on the effects of neuropeptides on the biosynthesis and release of cytokines in microglial cells and potential roles of cytokines in neurodegeneration will be conducted. Applicants should have experience in neuropharmacology, neurochemistry or molecular biology.

Contact: J.S. Hong, (919) 541-2358, Laboratory of Environmental Neurosciences, Maildrop E1-01.

**Ion Homeostasis and Cell Injury (HNV95)**
Changes in ion transport and homeostasis appear to be involved in apoptotic cell death. Studies focus on measuring changes in intracellular calcium, pH, sodium and magnesium in isolated cells using fluorescent indicators in cells stimulated to undergo apoptosis. Alterations in signal transduction pathways which are responsible for the ionic alternations are also under study. Applicants must have experience in ion measurements using fluorescent indicators or experience with cell culture or molecular biology.
Contact: Elizabeth Murphy, (919) 541-3873, Laboratory of Molecular Biophysics, Maildrop 17-05.

**Molecular Dosimetry and Epidemiology (HNV96)**
Knowledge and techniques in molecular biology are applied to investigations designed to determine effects of low-dose exposures to environmental agents. Animal models, cell systems and human samples are used. Studies encompass mutation analysis and signal transduction elements.
Contact: George W. Lucier, (919) 541-3802, Laboratory of Biochemical Risk Analysis, Maildrop A3-02.

**Molecular and Cellular Biology (HNV97)**
The action and function of several nuclear (orphan) receptors in the regulation of gene expression and differentiation are being investigated. Studies involve characterization of response elements, interaction with other transcriptional factors and gene knockouts. Applicants must have training in molecular biology techniques.
Contact: Anton Jetten, (919) 541-2768, Laboratory of Pulmonary Pathobiology, Maildrop D2-01.

**Mechanisms by Which Organisms Produce Mutations (HNV99)**
Studies are aimed at understanding the mechanisms by which organisms produce mutations. Specific projects involve the isolation and molecular characterization of *antimutator* mutants in the bacterium *E. coli*; the genetic and biochemical analysis of DNA replication fidelity in this organism; and a structure-function analysis of the *dnaE* and *dnaQ* genes (encoding, respectively, the DNA polymerase and exonucleolytic proofreading activity).
Contact: Roel M. Schaaper, (919) 541-4250, Laboratory of Molecular Genetics, Maildrop E3-01.

**Mechanisms of DNA Replication (HNV100)**
The regulation and mechanism of human DNA polymerases involved in the replication of nuclear and mitochondrial DNA is being investigated. Attention is on the mutation rate of the mitochondrial and nuclear genome by understanding the enzymology of the mitochondrial and nuclear DNA polymerases. Future studies will include the regulation of these essential enzymes in the cell.
Contact: William Copeland, (919) 541-4792, Laboratory of Molecular Genetics, Maildrop E3-01.

**Reproductive Biology and Toxicology (HNV104)**
The molecular events underlying the abnormal development of the reproductive system associated with exposure to xenobiotic estrogens such as diethylstilbestrol (DES) are being investigated. Particular interest is the biochemical and molecular analysis of transient and permanent alterations in the estrogen-responsive (e.g., lactoferrin) and metabolizing (e.g., sulfotransferase) genes and the implications for human health disease.
Contact: Masahiko Negishi, (919) 541-2404, Laboratory of Reproductive and Developmental Toxicology, Maildrop E4-07.

**Cell Adhesion in Metastasis (HNV105)**
The molecular mechanisms by which cancer cells metastasize are being studied, focusing on the roles of cell surface receptors and cell adhesion. Special interests include the effects of swainsonine, an inhibitor of protein glycosylation, on tumor cells and the hematopoietic system. Candidates should have expertise in cancer biology, molecular biology and biochemistry.
Contact: John Roberts, (919) 541-5023, Laboratory of Molecular Carcinogenesis, Maildrop C2-14.

**Molecular Mechanisms of Respiratory Diseases (HNV110)**
This is a tenure track position to develop an independent research program in cellular and molecular mechanisms of respiratory biology and diseases. Extensive postdoctoral experience in molecular biology, developmental biology, signal transduction or biochemical mechanisms of inflammation is required.
Contact: Paul Nettesheim, (919) 541-3540, Laboratory of Pulmonary Pathobiology, Maildrop D2-01.

**Epitope Mapping (HNV111)**
Mass spectrometry combined with proteolytic foot printing is being used to determine conformational epitopes of recombinant HIV proteins towards monoclonal antibodies. Candidates should have primary experience in protein chemistry, including affinity techniques and proteolytic techniques.
Contact: Kenneth Tomer, (919) 541-1966, Laboratory or Molecular Biophysics, Maildrop 6-01.

**Molecular Biology and Fatty Acid Biochemistry (HNV112)**
Novel human cytochrome P450 enzymes that metabolize fatty acids are cloned and expressed, and the catalytic properties of the recombinant, purified proteins are evaluated by HPLC and GC/MS. The P450 enzymes are localized to specific cell types by immunohistochemistry and *in situ* hybridization and the regulation of P450 gene expression is studies using Northern blot analysis, RT-PCR and protein immunoblotting. Applicants should have a strong background in cell and molecular biology.
Contact: Darryl Zeldin, (919) 541-1169, Laboratory of Pulmonary Pathobiology, Maildrop D2-01.

**Laboratory of Reproductive and Developmental Toxicology (HNV114)**
An independent program of basic research in the field of developmental biology relative to studies in reproductive biology, developmental toxicology, hormone mechanisms, signal transduction, cell growth and differentiation, apoptosis, gene regulation and cancer biology will be initiated. Applicants with the potential for creative research in developmental biology who are studying cellular and molecular mechanisms of mammalian development desired.
Contact: Kenneth Korach, (919) 541-3512, Laboratory of Reproductive and Developmental Toxicology, Maildrop B3-02.

**Gametogenesis (HNV116)**
Genes with stage-specific expression during spermatogenesis are studied to define intrinsic and extrinsic mechanisms regulating development and function of male gametes. We use transgenic mice to dissect promoter regions and gene knockout mice to define the roles of gene products in meiotic and post-meiotic processes. Strong background in cell and molecular biology required.
Contact: E.M. Eddy, (919) 541-3015, Laboratory of Reproductive and Developmental Toxicology, Maildrop C4-01.

**Signal Transduction (HNV117)**
Studies include receptor mechanisms, G-proteins, inositol phosphates, calcium signaling, ion channels, cell growth and differentiation, apoptosis, gene regulation and cancer biology. Priority will be given to applicants utilizing cellular and molecular approaches to study intermediate steps in signal transduction pathways such as phosphorylation-dephosphorylation cascades.
Contact: James Putney, (919) 541-1420, Laboratory of Cellular Molecular Pharmacology, Maildrop 19-01.

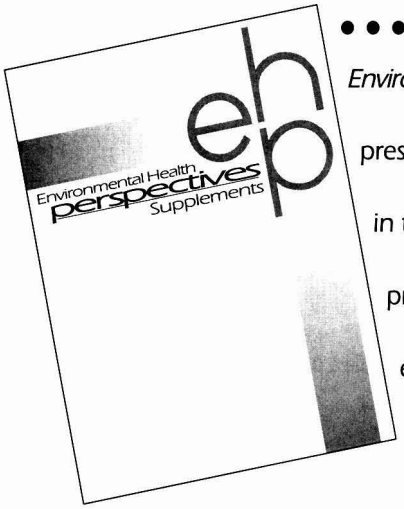
**Molecular Biology of Renal Transport (HNV118)**
Renal organic anion and cation secretion mediate elimination of toxic chemicals. Current projects use cultured epithelium, membrane vesicles and imaging to examine control of secretion and coordination of intracellular and membrane events during secretion. Expression cloning of secretory transport proteins has begun. A molecular biologist desired.
Contact: John B. Pritchard, (919) 541-4054, Laboratory of Cellular and Molecular Pharmacology, Maildrop 19-01.

**Molecular Biomarkers of Risk (HNV119)**
Molecular epidemiologic studies of gene-environment interaction. Development and application of methods for detecting somatic mutation and germline polymorphism in genes that modulate exposure, DNA damage and disease in human population studies. Candidates should have molecular biology experience.
Contact: Douglas A. Bell, (919) 541-7686, Laboratory of Biochemical Risk analysis, Maildrop C3-03.

**Ion Channel Physiology and Modulation (HNV120)**
Ligand-gated (serotonin 5-$HT_3$ and glutamate) and voltage-gated calcium channels are studied in neurons and cell lines, as well as channels expressed in mammalian cells or *Xenopus* oocytes. Structure-function aspects of theses channels are investigated, as well as how intracellular signal transduction pathways modulate the physiological properties of these channels. Applicants must have electrophysical (preferably patch-clamp) experience. Experience in molecular biological techniques would be a great asset.
Contact Jerrel L. Yakel, (919) 541-1407, Laboratory of Cellular and Molecular Pharmacology, Maildrop 19-04.

# Subscription Information

*Environmental Health Perspectives* offers cutting-edge research articles and news of the environment. To receive one year of *EHP*, fill out the upper form on the facing page.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# Editorial Policy

*Environmental Health Perspectives* is intended to be a forum for the discussion of issues in environmental health, and several formats have been devised for that purpose. In addition, several formats are available for the publication of scientific articles and scientific discussion. All scientific articles are subject to peer review. The primary criteria for publication are environmental significance and scientific quality.

Environmental science is made up of many fields, and therefore we are prepared to consider scientific progress in all of them. Cross-fertilization and serendipity have proven to be extremely important processes in the advance of science in general, and this must hold true for the science of environmental health. We will consider for publication articles ranging from the most basic molecular biology to environmental engineering. We particularly encourage those researchers concerned with mechanisms of toxic action and new approaches for detecting and/or remedying environmental damage.

Opinions and ideas based on scientific observation and argument are welcome. While the expression of opinions may lead to debate and disagreement, such reactions are healthy and can lead to new research and discoveries. Presentations of ideas and opinions will be promoted, but our policy will be to strive for objectivity and balance.

In addition to scientific articles and discussion, we publish news of the environment. We will consider factual articles about issues that affect the environment and human health. We summarize legislative and regulatory developments, grant information from NIEHS and other granting agencies, new research areas, environmental problems, technological advances, and information about the National Toxicology Program and other important programs. Presentations of news strives for objectivity and balance and is based on the strength of scientific evidence.

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

## Scientific Research

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

Research Articles are original manuscripts reporting scientific research and discovery in the broad field of environmental health. Research articles may come from any field of scientific research, from the most basic molecular biology and biochemistry to atmospheric physics, ecology, and engineering. The criteria for publication are weighted toward scientific quality and environmental significance. The work will be assessed according to its originality, scientific merit, and experimental design; the manuscript will be evaluated based on its conciseness, clarity, and presentation. We also attempt to address certain ethical problems during the review process. We require assurances that all human and animal subjects have been treated humanely and with due regard for the alleviation of suffering. Manuscript review also considers scientific integrity as part of the process.

Review Articles are narrowly focused articles that emphasize recent developments in a particular field of research. Lengthy historical perspectives are not appropriate.

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.

Meeting Reports are short summaries of conferences, symposia, or workshops in which the scientific objectives and achievements of a meeting are described.

## Opinions, Ideas, Perspectives

The journal is a forum for the expression of ideas and opinions. Opinions and ideas should be carefully considered and based on scientific principles. Several formats are offered:

Editorial statements are published by our editors, members of our editorial boards, and occasional guest editors. These statements are intended to focus attention on important or neglected areas of environmental health, offer opinions and ideas, and stimulate discussion.

Commentaries are up-to-date articles that may present commentaries offering perspective and insight on a particular topic.

Correspondence is encouraged. Opinions, perspectives, and insight are welcome. Comments on articles published in *Environmental Health Perspectives* are also welcome, but criticism will always be balanced by the opportunity for defense and clarification. Letters to the Editor cannot exceed 1200 words.

## Environews

The news section provides up-to-date information on important issues in environmental health covering a variety of areas including policy, legislative, and regulatory actions; innovative technological and conceptual research advances; conference and meeting summaries; and emerging environmental problems. The news section consists of several components:

Forum articles are brief reports on matters of potential environmental health significance such as chemical spills and contamination episodes. Brief reviews of recent scientific advances are also included.

NIEHS News summarizes significant activities or accomplishments at NIEHS and the National Toxicology Program.

Focus articles are substantive news items about important issues in environmental health. Examples include reports on risk assessment, risk management dilemmas, women's health initiatives, environmental equity, relevance of animal models to toxicity testing, and structure–activity approaches to toxicity evaluation.

Spheres of Influence is a legal/regulatory column that presents reports on significant events and decisions involving the executive branch, Congress, and regulatory agencies. Examples include new directions of White House policies, impact of Clean Air Act legislation, and coverage of congressional hearings on environmental health issues.

Innovations presents emerging opportunities in environmental health based on new discoveries or approaches in biology, chemistry, engineering, or information sciences. Examples include the use of transgenic animals in toxicity testing, new advances in molecular biology, development of more rapid and efficient methods for clean-up of hazardous wastes, and methods for early detection of environmental damage and environmentally mediated diseases.

Announcements includes a calendar of upcoming events such as conferences, workshops, and public hearings. Appropriate listings are made for industrial, academic, regulatory, and legal activities. This section also includes listings of fellowship and grant announcements and positions available.

## Environmental Health Perspectives Supplements

During the last 20 years, we have focused on the development of a series of monographs that have generally arisen from symposium or conference proceedings. We continue to publish monographs, but they now appear as supplements to the main journal. Six to eight supplements are published per year. Four to six of these consist of conference, workshop, or symposium proceedings, and two issues are dedicated to the publication of solicited and unsolicited comprehensive reviews on environmental health. All articles published in the supplements, regardless of their source, are peer reviewed.

Each supplement resulting from a conference, symposium, or workshop should address a specific problem, an area of concern, a research problem, or a particular scientific issue. Supplements will, in general, be dedicated to scientific issues and not programmatic themes. It is intended that each collection of manuscripts form a landmark statement for a particular subject. Each supplement must be an up-to-date, balanced source of reference material for researchers, teachers, legislators, and the informed public. Publication of conference proceedings in *Environmental Health Perspectives Supplements* requires the submission of a proposal as described in Instructions to Authors.

Supplement Articles from conferences are generally the result of research investigations, reviews, or a combination of both; however, brief reports and commentaries are also appropriate.

Perspective Reviews are targeted to the one or two specific issues of *Environmental Health Perspectives Supplements* set aside for the publication of reviews in environmental health sciences. Perspective reviews are in-depth, comprehensive review articles that address developments in specific scientific areas. Perspective reviews must not be simply a compilation of the literature. Perspective reviews should be scholarly, landmark statements offering a complete and balanced perspective as well as insight into the environmental significance of the research.

# Instructions to Authors

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

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

## Organization of Manuscripts

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

*Title Page*: List title, authors (first or second names spelled out in full), full address of the institution where the work was done, and affiliation of each author. Indicate author to whom galley proofs and reprints should be sent (include complete address for express mail service, telephone and FAX numbers).

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

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

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

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

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

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

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

*Journal Article:*

1. Canfield RE, O'Connor JF, Birken S, Kirchevsky A, Wilcox AJ. Development of an assay for a biomarker of pregnancy in early fetal loss. Environ Health Perspect 74:57–66 (1987).

*Book Chapter:*

2. Lohman AHM, Lammers AC. On the structure and fiber connections to olfactory centers in mammals. In: Progress in brain research: sensory mechanisms, vol 23 (Zotterman Y, ed). New York:Elsevier, 1967;65–82.

*Book:*

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

*Editor as Author:*

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

*Conference Proceedings:*

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

*Government Report:*

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

*Other Publications:*

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

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

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

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

*Tables*: Each table must be on a separate page. Tables should be numbered with Arabic numerals. General footnotes to tables should be indicated by lowercase superscript letters beginning with a for each table. Footnotes indicating statistical significance should be identified by *, **, #, ##. Type footnotes directly after the table. Complex tables should be submitted as glossy photographs.

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

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

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

Research Advances are concise articles intended to address only the most recent developments in a scientific field. Lengthy historical perspectives are not appropriate. Begin with the title page and continue as described for research articles. References, abbreviations, figures, and tables should be handled as described for research arti-

cles. Clarity of presentation is of primary importance and the use of color figures is encouraged. Include a photograph (black and white or color) of the author together with a brief biography. If multiple authors or groups are involved, up to three biographies with photographs may be included.

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

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

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

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

## ENVIRONMENTAL HEALTH PERSPECTIVES SUPPLEMENTS

SUPPLEMENT MANUSCRIPTS result from conferences, symposia, or workshops and may take several forms. 1) Manuscripts reporting original research should be formatted as described for Research Articles, 2) opinions and discussion about a particular topic should be formatted as described for Commentaries, 3) manuscripts reviewing a topic or reporting a combination of review and original research should be formatted as described below for Perspective Reviews.

PERSPECTIVE REVIEWS are in-depth, comprehensive reviews of a specific area. They should begin with a title and second page as described for research articles. Introduction and presentation of information should be continuous with specific items and discussion indentified by using subheadings. Abstracts, references, abbreviations, figures, and tables should also be handled as described for research articles.

PROPOSALS for the publication of conference, symposium, and workshop proceedings will be considered; however, space is limited. We turn away many excellent proposals simply because we do not have space to publish them.

All proposals are reviewed and examined with a number of specific questions in mind. In developing a proposal, consider the following: Proposals are assessed according to their originality and scientific merit. Is the supplement needed? Is the subject matter timely and potentially useful to workers in the field? What is the environmental significance of the topic being addressed? Is the proposed supplement a complete representation of the field? Are there other aspects that should be included? Does the proposal contain sufficient information for evaluation? Is the presentation clear? Can the organizers integrate the participants into a cohesive unit? Are the contributors appropriate for the topic listed and do they have scientific credibility?

The source of funding is also considered. Scientific objectivity is extremely important, and it must be clear that organizers are not being used to present a bias favored by the funding body. Contributions from an interested party to a conference need not disqualify a proposal, but it is appropriate that the major source of funding be from a disinterested source or that organizational safeguards be set in place to minimize the intrusion of institutional bias.

All proposals must be submitted at least six months in advance of the conference. In the publication of conference proceedings, timeliness is essential. Because it takes at least six months to publication, no proposal will be considered after the conference has been held.

## SUBMISSION OF MANUSCRIPTS AND PROPOSALS

Submit all manuscripts and proposals in quadruplicate to:

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

In your covering letter please provide assurances that the manuscript is not being considered for publication elsewhere and that all animals used in the research have been treated humanely according to institutional guidelines, with due consideration to the alleviation of distress and discomfort. If the research involved human subjects then a statement must be made to the effect that participation by those subjects did not occur until after informed consent was obtained.

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

Finally, a statement must be made indicating that all authors have read the manuscript and are in agreement that the work is ready for submission to a journal and that they accept the responsibility for the manuscript's contents.

Inquiries may be made by calling (919) 541-3406 or by FAX at (919) 541-0273.

## SUBMISSION OF NEWS INFORMATION

Environmental Health Perspectives welcomes items of interest for inclusion in the Environews, Calendar of Events, and Announcements sections of the journal. All items are published subject to the approval of the Editors-in-Chief. All submission for these sections should be sent to the attention of:

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

Items submitted for inclusion in the Forum section must not exceed 400 words. Items may be edited for style or content, and by-lines are not attached to these articles. If possible, items should be submitted on computer disk using WordPerfect or Microsoft Word, in straight text without formatting.

Items received for the Calendar of Events will be published in as timely a manner as possible, on a space-permitting basis. Submissions should include all relevant information about the subject, date, time, place, information contact, and sponsoring organization of the event.

Position announcements will be limited to scientific and environmental health positions and will be run on a space-permitting basis. Although we seek to publish all appropriate announcements, the timeliness of publication cannot be guaranteed.

Public information advertisements will be run free-of-cost as space becomes available. All ads are run subject to their appropriateness to the editorial format of the journal. Submissions of advertisements should include full-page, half-page, and quarter-page formats if available. Ads should be camera-ready, black and white positives.

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

*World Health Organization*

*International Agency for Research on Cancer (IARC)*

IARC has an opening in its headquarters in Lyon, France, for the post of

# *Chief of the Unit of Carcinogen Identification and Evaluation*

The main activity of this Unit is the production of the publication series IARC Monographs on the Identification and Evaluation of Carcinogenic Risks to Humans. The Monographs programme has provided reviews and evaluations of the evidence of carcinogenicity of about 800 chemicals mixtures, exposure circumstances and biological agents. Three volumes of Monographs are published annually; each volume includes documents prepared by a Working Group of scientific experts.

Applicants should have an MD, PhD, or equivalent in a field relevant to the Programme, a strong theoretical background and significant experience in one or preferably several of the areas of cancer epidemiology, cancer bioassays, toxicology and mechanisms of carcinogenesis, a solid understanding of all these areas and of their integration in the processes of cancer hazard identification and risks assessment. Good communication skills, ability to supervise staff, and ability to coordinate working group meetings are essential. Training in epidemiology, biostatistics, scientific publishing and experience in a national or international programme on carcinogenic hazards would be desirable.

The incumbent is expected to plan, lead and coordinate the execution of the Unit's programmes which includes selecting topics for monographs, providing authoritative advice to the Director, IARC, on the selection and assignments of experts, serving as secretary to the monographs meetings and being responsible for the production of high-quality monographs. He/she is also expected to stimulate development and to initiate intramural and extramural research activities.

The initial appointment will be for two years, the first being probationary.
The annual salary level is $53,611 US dollars tax-free at single rate and $57,806 US dollars for a staff member with dependents plus a cost of living element, which is currently 61% of the above figures.

Those interested should write, enclosing a curriculum vitae, to:

*Personnel Office*
*IARC*
*150, Cours Albert Thomas*
*F-69372 Lyon Cedex 08 France*
*FAX: (33) 72 73 83 35*

Applications from women are encouraged.

# In This Issue

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### Commentary



## Environews



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### Review



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